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GIBBERELLIN A_9: A POSSIBLE BIOCHEMICAL MARKER IN EARLY SELECTION AT THE FAMILY AND CLONAL LEVELS OF RADIATA PINE AND COASTAL DOUGLAS-FIR

BY

RUICHUAN ZHANG

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

DEPARTMENT OF RENEWABLE RESOURCES

EDMONTON, ALBERTA

FALL, 1996

ISBN 0-612-18138-3

University of Alberta

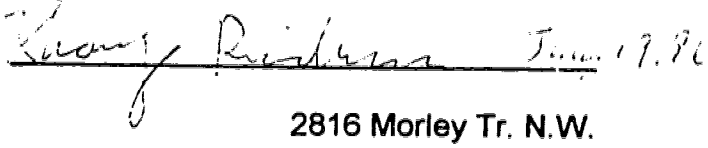
Library Release Form

Name of Author: Ruichuan Zhang

Title of Thesis: Gibberellin A_9: A Possible Biochemical Marker in Early Selection at the Family and Clonal Levels of Radiata Pine and Coastal Douglas-fir

Degree: Doctor of Philosophy

Year this Degree Granted: 1996



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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled GIBBERELLIN A_9: A POSSIBLE BIOCHEMICAL MARKER IN EARLY SELECTION AT THE FAMILY AND CLONAL LEVELS OF RADIATA PINE AND COASTAL DOUGLAS-FIR submitted by Ruichuan Zhang in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Gibberellins (GAs) $A_{4/7/9/15}$ and $GA_{4/9}$ glucosyl esters (GE) were characterized from Radiata pine (*Pinus radiata* D. Don) shoots and needles and $GA_{4/9/15/34}$ and $GA_{4/9}GE$ were characterized from coastal Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco var. *Menziesii*) embryos and needles by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM).

The major needle GA component is GA_9 and its metabolism may be rate-limiting. Thus, GA_9 levels, quantified by GC-MS-SIM, were chosen as a genetic trait to examine the relationship between hormone level and growth at the family and within-family (genotype) levels.

Families varied considerably in the frequency of genotypes whose growth was highly correlated with GA_9 level. For some families genotype growth was highly and significantly correlated with GA_9 levels (especially half-sib Radiata pine). For other families GA_9 levels showed only modest to nil correlations with genotype growth (especially full-sib Coastal Douglas-fir). However, when assessed across all families GA_9 levels were positively and highly significantly correlated with genotype growth for both species. These and other results suggest that GAs are causal for shoot growth of individual conifer genotypes and that needle GA_9 level reflects differences in growth capacity across a population of genotypes.

The relationship of GA_9 level to growth capacity between families is more complex. Family mean GA_9 level is poorly related to family mean for seedling growth except for age one month primary needles, or for fascicle needles from the very slowest-growing genotypes within each family. The relationship of GA_9 level

to family field performance was similarly poor.

However, a "second order relationship" was developed using slopes derived by regression analysis of GA_9 level with early genotype growth within each family. These "GA_9 slope" values were then compared with family field performance (stem diameter), and that comparison yielded statistically significant correlations for almost all Radiata pine half-sib families (e.g. after retrospective use of an outlier program) and for all Coastal Douglas-fir full-sib families.

For Radiata pine, seedling height, height growth, stem diameter and stem dry weight were positively correlated with family field performance and after retrospective use of an outlier program the correlations were significant (for 14/16 families). Outlier families for Radiata pine could also be identified "early" by applying an outlier program using needle GA_9 levels and seedling stem diameters. After two outlier families were removed "early", the comparisons for stem diameter and stem volume to field performance for the remaining 14 families became statistically significant. For Coastal Douglas-fir full-sibs, among several seedling growth parameters, only seedling stem diameter was significantly correlated with family field performance.

For predictive purposes, only seedling stem diameter and stem volume would be useful for Radiata pine (e.g. in roguing out the bottom 8 of 16 families, only two mistakes were made out of 16 possible). For Coastal Douglas-fir, seedling stem diameter or the slopes of GA_9 : various early growth parameters were good predictors of performance (e.g. only 2 or 3 mistakes were made out of 16 possible). Thus, the best predictor of field performance is seedling stem diameter for both species.

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere thanks to Drs. Richard P. Pharis and Bruce P. Dancik, my supervisors, for their support, guidance, help and encouragement during the course of this study.

I would also like to thank my committee members for their advice and counsel during the period of my study.

I would also like to thank Dr. Rolland Burdon of Forest Research Institute, Rototua, NZ for provision of Radiata pine seed and field performance results.

Thanks are also due to Dr. Carolyn Carpenter of Weyerhaeuser Technology Center, Tacoma, WA, USA for providing me with the Douglas-fir tissues, and their stem dry weights and heights, and field growth data.

I would also like to thank all the members in Dr. Pharis's laboratory for their help during the course of this work.

Most of all, I wish to thank my wife Yaying Wang for her endless patience and continued support over these years.

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ABBREVIATIONS

2,4-D:	2,4-dichlorophenoxyacetic acid
ABA:	abscisic acid
ACC:	1-aminocyclopropane-1-carboxylic acid
BA:	6-benzylaminopurine
BSA:	bovine serum albumin
BSTFA:	bis-trimethyl-silyltrifluoroacetamide
Cks:	cytokinins
dbh:	diameter at breast height
DF:	coastal Douglas-fir
dw:	dry weight
ELISA:	enzyme-linked-immunoassay
EtOAc:	ethyl acetate
GA:	gibberellic acid
GA_4GE:	GA_4 glucosyl ester
GCA:	additive genetic variance
GC-MS-SIM:	gas chromatography-mass spectrometry-selected ions monitoring
h:	hour
HOAc:	acetic acid
HPLC:	high performance liquid chromatography
Ht:	height
IAA:	indole-3-acetic acid

IFFY:	immunoaffinity column
K:	kinetin
KRI:	Kovat's retention index
MeOH:	Methanol
MSD:	mass spectrometry detector
NAA:	naphthaleneacetic acid
NI:	not integrated
NS:	non-significant
OP:	open pollinated
PEPC:	phosphoenolpyruvate carboxylase
PBS:	phosphate saline buffer
RAPD:	random amplified polymorphic DNA
RFLP:	restriction fragment length polymorphism
RP:	Radiata pine
Rt:	retention time
RuBPC:	ribulose-1,5-bisphosphate carboxylase
RuBPO:	ribulose-1,5-bisphosphate oxygenase
SCA:	non-additive genetic variance
SE:	standard error

CHAPTER 1 INTRODUCTION

As a renewable natural resource, forests are attracting increasing attention because of their important roles in maintaining a healthy environment and economy for human society. However, natural forests are declining throughout the world, especially in the developing countries. For example, it was estimated that the annual loss of forest was more than 2,500,000 ha in Brazil and more than 600,000 ha in Indonesia between 1981 and 1985 (Kahn, 1995). As the world population increases almost exponentially, reforestation and afforestation are more likely to be done on agriculturally marginal lands. Thus, genetically superior, fast-growing trees are urgently needed in order to meet an increasing demanding of wood products and to ameliorate the current trend of environmental deterioration. Forest trees can be selected and bred to improve their resistance to diseases and insects, as well as their adaptability to adverse environments (Zobel and Talbert, 1984). Additionally, it has been shown that fast-growing families can be selected which are also more disease resistant (Rajora et al., 1994).

There are mainly two ways of regenerating trees: by seed or micropropagation. Micropropagation can be accomplished via tissue culture, somatically-derived embryos and by rooting of cuttings or micropropagules from seedlings that were originally derived from phenotypically or genetically superior mother trees. At the operational level, reforestation for large areas is currently being accomplished by seeds collected from phenotypically superior parent trees

in the wild or collected from genetically improved trees in seed orchards. At least one company, Tasman Forests, is also regenerating Radiata pine plantations by micropropagation (R. P. Pharis, personal communication). Thus, identifying and breeding genetically superior forest trees will become essential for efficient reforestation and afforestation with "improved" families and genotypes. Ideally, the trees that exhibit superior growth characters should also be trees that are more adaptable to specific environments [e.g. a Radiata pine genotype was found which was obviously superior to surrounding trees in a boron-deficient environment (Zobel and Talbert, 1984)]. Reforestation or afforestation can thus be made by using seeds collected in half-sib or full-sib seed orchards from phenotypically superior parent trees, or through vegetative propagation of their progenies. Their genetic superiority can then be tested by progeny testing. That is, to determine genetic superiority of the families, progeny from crosses are planted in the field in a properly designed test trial. The progeny testing consists of two parts: mating design and field experimental design. When the offspring reaches 1/3 to 1/2 rotation age, the genetic value of the selected trees is determined. Usually, cuttings or seedlings from these crosses have also been used to establish advanced-generation seed orchards so as to produce genetically improved seeds for reforestation and afforestation. These seed orchards are then "rogued" of families shown to have poor growth, form (GF) and disease resistance characteristics as the various field progeny trials results are obtained (e.g. at 1/3 to 1/2 rotation age [Lambeth, 1980; Cotterill and Dean, 1988; McKeand, 1988]).

Thus, it may take a decade or more to get large amounts of genetically improved seeds for reforestation use.

Breeding forest trees is quite different from breeding annual agricultural crops due to the long generation cycle and their large size. For most economically important conifers, the juvenile stage lasts 10 or more years, and during that period they do not normally flower [e.g. Coastal Douglas-fir usually will not flower until 10-years of age (Fowells, 1965.a)]. In addition, field progeny testing takes an even longer time [e.g. up to one-half of rotation age (Zobel and Talbert, 1984; Lowe and van Buijtenen, 1989)] before reliable genetic and phenotypic evaluations can be made. For example, lodgepole pine (*Pinus contorta*) is one of the most economically important conifers in Alberta. Its juvenile phase lasts 15 to 20 years in heavily stocked stands (Fowells, 1965.b) and the lower limit of its rotation age is about 70 years (Smithers, 1961).

To overcome the obstacles of the long juvenile period and the need for extended field progeny testing, research has been concentrated in two main areas: inducing early and abundant flowering, and selecting genetically superior trees at an early age. Precocious and enhanced flowering can now be accomplished through use of gibberellins (GAs), often together with other cultural treatments such as fertilization and girdling (Pharis et al., 1987). However, early selection is still at the active research stage. Hence, this subject is the topic of this thesis.

Generally, early selection is referred to as any selection of family or genotype made before half of rotation age (Jiang, 1987). There are generally two

types of early selection: (i) comparison of growth data measured from the same trees at different ages and (ii) a comparison of growth data measured from different, but genetically related trees at different ages. The second type is known as a retrospective test. For instance, seeds from half-sib families were planted in the field and 10 years later, seeds from the same families are planted in a greenhouse and grown under either optimal growing conditions, or simulated field conditions. The growth data are then collected from both 10-year-old trees in the field and very young trees in the greenhouse. A statistical analysis can then be performed to test the family-mean correlations between the two sets of growth data, usually using height, diameter of the stem, shoot biomass, etc.

The advantages of being able to select families or genotypes early include the savings of time and the cost of field progeny trials. Selection at an early stage can greatly shorten the breeding cycle, and because culling can be made at an early age, selection intensity can be increased, or the scale of the field genetic tests can be reduced.

Early selection is often termed indirect selection because the desired trait (size of tree bole in the field) is selected by choosing another early trait that is correlated with it (Falconer, 1989). For example, attempting to predict the growth performance of mature trees by selecting seedling traits of height, diameter, etc., (Williams et al., 1987; Sulzer et al., 1993). Thus, the success of early selection is dependent on the age-age correlation between the traits measured from young and old trees and on greater heritability of the early traits (Wu, 1993).

At present, no single trait from young trees can be used to accurately predict growth performance of mature trees in general (e.g. there is no single trait across species, or across a range of sites). Many traits have been tested and they can be divided into three groups: morphological traits, physiological traits, and biochemical traits.

1.1 Morphological Traits:

Height is probably the most widely used seedling trait in early selection research. For example, height was tested in early selection in black spruce (*Picea mariana* (Mill.) B. S. P.) (Williams et al., 1987). Here, a retrospective test was carried out with 162 seedlings from 9 full-sib families. Heights and diameters were measured from 3- to 6-month-old greenhouse-grown seedlings. Both seedling heights and stem volumes on the family mean basis were significantly correlated with the height of 13-year-old field grown trees. A similar result was reported by Sulzer et al. (1993). In their experiment 695 seedlings of black spruce from 36 half-sib families were used. They found that family mean heights of 3-year-old greenhouse-grown seedlings were positively correlated with the family mean heights of 10-year-old trees which were growing in the field in New Brunswick with a r=value of 0.491. In another report, clonally propagated full-sib black spruce propagules were tested for age-age correlations (Mullin and Park, 1994). These came from seedlings of 40 full-sib families of black spruce that had been grown in a greenhouse. Six seedlings (genotypes) were selected from each family for clonal

replication by means of rooted cuttings. The correlations between the heights of 25-week-old ortets and families in the greenhouse and heights of 5- or 10-year-old vegetatively propagated ramets in the field were not statistically significant. In another genetic test, seedlings of 64 open-pollinated slash pine (*Pinus elliottii* Engelm. var. *elliottii*) families were raised under low and high nitrogen treatments (Smith et al., 1993a). Under the low nitrogen treatment the family mean heights of 13- and 16-month old seedlings were significantly correlated with both 5- and 15-year breeding values (breeding value is the average performance of the progeny of an individual when it is mated to a number of other individuals in the population) for field stem volume growth. However, by age 18 months no significant correlation could be found between the family mean height of 18-month seedlings (either N treatment) and 15-year breeding value. Surprisingly, under the high nitrogen treatment, total branch number for the seedlings was also significantly correlated with both 5- and 15-year breeding values for field volume growth. Of the many other traits monitored, only total stem units (all sterile and fertile cataphylls, branches, and lateral buds were tallied as "stem units") of one-year-old seedlings was significantly correlated with both the 5- and 15-year breeding values for both nitrogen treatments (Smith et al., 1993b). Similar results were reported for loblolly pine (*Pinus taeda* L.) (Li et al., 1991). Seedlings of 23 open-pollinated (OP) loblolly pine families were raised in a greenhouse under low and high nitrogen treatments for 35 weeks. In low nitrogen the correlation between number of seedling stem units at age 35 weeks and 12-year height was positive and significant.

Stem diameters of Radiata pine in the field were measured at ages 5, 10, and 17 years in a genetic test of 410 open-pollinated families (King and Burdon, 1991). The stem diameters of 5-year-old trees were significantly correlated with those of 10- and 17-year-old trees, the latter being near rotation age.

In a progeny trial of maritime pine, using 100 open-poilinated families, the frequency of the occurrence of secondary leader growth at the family level was positively correlated with annual height increment at a young age (< 10 years). However, the correlation with height increment of trees at an older age (> 14 years) became negative for most families (Magnussen and Kremer, 1994).

Total seedling dry weight is another trait frequently tested for use in early selection. In a study with black spruce, seeds from seven full-sib families were germinated and grown under moist or dry conditions in a nursery for two growing seasons (Tan et al., 1995). The family ranks for total dry weight of seedlings grown under dry conditions were found to be significantly correlated with family ranks in height of 16-year-old trees growing in the field under dry conditions.

In a retrospective study with slash pine, seeds were collected from 64 OP families. Dry weight of seed components (seed coat, gametophyte, embryo, and total seed) was correlated with 5-year and 15-year breeding values for stem volume (Surles et al., 1993). Among these seed components, embryo weight had the strongest correlation with 5-year and 15-year breeding values. Also, Robinson and van Buijtenen (1979) found a significant family mean correlation between total seed weight and field volume up to age 15 years ($r = 0.30$).

Thus, although there are many successful reports using morphological traits for early selection, the results overall are not consistent and the correlations differed appreciably from traits to traits, environment to environment and species to species. Perhaps, these variable results can best be understood if one realizes that tree growth and development are the overall result of many physiological processes, that in turn result from the interactions between hereditary potential and environmental conditions (Kozlowski et al., 1991). To be able to predict forest tree growth at the family and genotype levels at an early age, it is thus necessary to better understand tree growth at the physiological level.

1.2 Physiological Traits

The measurement of "physiological processes" is another class of indicator traits that has been used for early selection of genetically superior trees. As noted above, plant growth and development is the net result of many physiological activities. Among the many physiological processes, photosynthesis is the most frequently studied. Ceulemans et al. (1987) reported that net photosynthesis on attached leaves of 1-year-old *Populus* hybrids was not significantly correlated with height of 5-year-old trees in the field. In an early selection trial with Norway spruce (*Picea abies* L. Karst.) the photosynthesis of 3-year-old container-grown (field) seedlings was not significantly correlated with height, but was significantly (negatively) correlated with the diameter of 13-year-old field trees, (Larsen and Wellendorf, 1990). A similar result was also reported for black spruce (Sulzer,

1993), e.g. no significant correlation between photosynthesis of 3-year-old seedlings and height of 10-year-old field trees. On the other hand, Isebrands et al. (1988) found that leaf photosynthetic capacity of *Populus* was positively correlated with shoot growth and that whole tree photosynthesis was correlated with actual biomass measurement.

Ceulemans et al., (1987) also reported that no significant correlation was found between height of 5-year-old *Populus* clone in the field and leaf chlorophyll content of 1-year-old plants. Nor were there significant correlations between height of these 5-year-old *Populus* clones and, for 1-year-old plants activities of RuBPC, RuBPO, and PEPC, the main enzymes in the assimilation of CO_2,. Finally, the respiration rate of these 1-year-old plants was also not correlated with the height of the 5-year-old clones (Ceulemans et al., 1987).

However, in another study, Larsen and Wellendorf (1990) reported that the dark respiration rate of 3-year-old container-grown Norway spruce exhibited a negative but significant correlation with the diameter of 13-year-old trees in field, and that the transpiration rate was also negatively and significantly correlated with both height and diameter of 13-year-old trees.

To study the physiological basis for heterosis in jack pine (*Pinus banksiana* Lamb.), seedlings of six outcrossed families and three selfed families were grown in a nursery. As expected, seedlings of outcrossed families exhibited greater growth and net photosynthesis than did selfed seedlings (Blake and Yeatman, 1989).

In areas where soil water is limiting there are several physiological traits that can be used as a selection criterion. A drought-adaptation study with 6-month-old seedlings from *Eucalyptus camaldulensis* Dehnh., *E. tereticornis* Smith, *E. viminalis* Labill., and *E. grandis* Hill ex Maiden revealed that osmotic adjustment, which is related to water-stress tolerance, differed significantly among these species (Lemcoff et al., 1994). To investigate the physiological basis of drought tolerance in black spruce, 6-month-old seedlings from both faster- and slower-growing families growing on a dry site were used. No growth difference was found on moist sites, but the faster- and slower-growing families were well defined on the dry site (Tan et al., 1992). The progenies of the faster-growing families were able to lower osmotic potential and they also maintained a more normal turgor pressure under moderate osmotic stress than did progenies of the slower-growing family.

Since most conifer species are naturally distributed in the northern hemisphere, adaptability for cold weather is critical for their growth. In a genetic test with Norway spruce it was found that increased frost resistance of 3-year-old seedlings was negatively and significantly correlated with both height and diameter of 13-year-old field trees (Larsen and Wellendorf, 1990).

Smith and Blanchard (1984) reported that cambial electrical resistance was related to the number of cells per radial file of vascular cambium in balsam fir [*Abies balsamea* (L.) Mill.]. Electrical resistance of the cambial region was thus tested as a measure of tree vigour in Norway spruce (Lindberg and Johansson, 1989). A significant negative correlation was found between cambium electrical resistance

and annual ring width. However, one explanation for the low cambial electrical resistance in the vascular cambium of fast-growing trees is that as the electrode penetrates the cambium, the thicker cambium merely releases more ions into the apoplast (Blanchard et al., 1983).

Thus, as with morphological traits, the results of physiological traits have not been consistent between species. Even for the same species the correlations varied with different families, environmental conditions, and development stages.

1.3 Biochemical Traits

One explanation for heterosis or inherently rapid growth is that multiple forms of the same enzyme increase an organism's tolerance to environmental variation. Because it takes many years before forest trees become economically valuable, they have to face more environmental variation. One possible measure of their adaptability to environmental fluctuation is their heterozygosity. This can be estimated by isozyme analysis. It is thus hypothesized that increased heterozygosity of forest trees is correlated with their growth rate. The relationship between heterozygosity and growth rate has been examined in both animals and plants. While results from studies of forest trees are not conclusive, they will be discussed briefly.

A relationship between protein heterozygosity and mean annual width increment (estimated from tree cores) was reported in quaking aspen, *Populus tremuloides*, (Jelinski, 1993). Enzymes were extracted from dormant vegetative

buds. To estimate the heterozygosity, 14 polymorphic loci were monitored using starch gel electrophoresis. After adjustment for slope position, exposure, elevation and age, a positive correlation was found between mean annual stem width increment and heterozygosity.

To study the relationship between heterosis and heterozygosity in knobcone pine (*Pinus attenuata*), 103 inbred, and 80 outcrossed genotypes were sampled from 11 families originating from six populations. Isozymes were extracted from seed megagametophytes and 24 polymorphic loci were recorded by starch-gel electrophoresis to estimate the heterozygosity. Within the inbreds, heterozygosity was positively correlated with diameter growth at breast and knee heights at age 10. However, no correlation was found between heterozygosity and stem diameter growth for the crossbreds when a few outlyers were eliminated (Strauss, 1986).

Protein heterozygosity is also associated with growth rate in Radiata pine (Strauss and Libby, 1987). In this study about eight trees were sampled from 10 stands in each of three natural populations. Six ramets per clone survived after 14 years. The clonal heterozygosities were estimated at 27 polymorphic loci by electrophoresis of allozymes extracted from megagametophytes that were dissected from seven seeds (one tree per clone). A depressed height growth rate was associated with a low level of clonal heterozygosity. Also, the rate of height growth was negatively correlated with heterozygosity at a high level of clonal heterozygosity.

To study the relationship of growth to heterozygosity in pitch pine (*Pinus*

rigida Mill.), cones were collected from eight natural populations of different ages (Ledig et al., 1983). The number of trees from each population ranged from 28 to 57. Six to eight megagametophytes were used for each tree. Heterozygosity was estimated based on isozyme analysis at 21 loci. The results revealed that the regression coefficient relating growth to heterozygosity was strongly correlated with age. That is, for the oldest stand heterozygosity and basal area increment was significantly and positively correlated. But, the correlation became negative for the youngest stand. The authors suggested that the superiority of increased heterozygosity was expressed better in old trees because they had experienced more fluctuation in environmental conditions than had the younger trees.

Additionally, Aradhya and Phillips (1995) reported that no significant correlation was found between allozyme heterozygosity and juvenile growth traits in *Eucalyptus*.

It is not surprising to find that the correlation of heterozygosity with growth performance changed under differing environmental conditions and at different developmental stages of the trees (Ledig et al., 1983), since enzymes are the expression of a gene. Gene expression is regulated at different levels, such as transcription and translation. The genome of a given tree is stable during its life time, but the expression of a given gene may be controlled by "environmental conditions" as the tree ages. Thus, heterozygosity estimated by isozyme analysis only represents the polymorphism of certain genes under given environmental conditions. To reveal polymorphism at the DNA level, using RFLP or RAPD

analysis may be superior to isozyme analysis.

1.4 The Possibility of Using Plant Hormones as Genetic Markers for Early Selection

Forest tree growth is both longitudinal and radial. The longitudinal growth results from activity of apical and sub-apical meristems contained in the buds. The radial growth results from activity of the vascular cambium, the meristematic tissue located between the wood and bark. Both longitudinal and radial growth follow the same cycle: division of meristematic cells followed by cell enlargement and differentiation (Kozlowski et al., 1991).

The growth of forest trees is dependant upon their genotype, the natural growth environment and the interaction between genotype and environment. The interaction between the genotype and environment may be mediated by plant hormones (Cornish and Zeevaart, 1985; López-Juez et al., 1995; Yang and Hoffman, 1984). For example, when plants experience water deficit, the amount of ABA increases dramatically and this induces stomata to close, which in turn reduces further water loss. At present, five major plant hormone classes are recognized: GAs, auxins, ABA, cytokinins, and ethylene. They affect tree growth in different ways and to different degrees.

Gibberellins are one of the most important factors in controlling shoot growth in higher plants and this conclusion is supported by many experiments with single gene mutations, the application of exogenous GAs, and quantification of

endogenous GAs from various species.

Thus, it has been demonstrated that for single gene dwarf mutants of pea and maize, where length of the internode is much shorter than that of wild type genotype, GA biosynthesis is blocked and application of specific GAs can restore normal shoot growth for the dwarf mutants (Graebe, 1987; Ross et al., 1993). Shoot growth promotion by applied GAs has also been reported for many woody species (Pharis, 1976; Pharis and Kuo, 1977; Ross et al., 1983; Junttila, 1991; Little and Pharis, 1994). Application of GA_3 to debudded shoots of *Robinia pseudacacia* (Digby and Wareing, 1966) and *Quercus robur* (Zakrzewski, 1983) can promote cambial activity without inducing vessel differentiation and GA_4 application increases radial enlargement of tracheids in Radiata pine seedlings (Pharis et al., 1981). In *Pinus sylvestris* seedlings, a soil drench of $GA_{4/7}$ gave increased $GA_{4/7/9}$ and IAA levels in the cambial region while increasing tracheid production and stem elongation (Wang et al., 1992). For cambial region tissues of 3-year-old *Eucalyptus globulus*, the elongation of secondary xylem fibres was positively correlated with higher levels of endogenous GA_1 and GA_{20} (Ridoutt et al., 1995a). Furthermore, injection of trinexapac-ethyl, an inhibitor of GA biosynthesis, into the stems of *Eucalyptus globulus* trees reduced GA_1 and GA_{20} levels, as well as reduced average fibre length and fusiform cambial cell length (Ridoutt et al., 1995b).

In one experiment with black spruce, weekly application of $GA_{4/7}$ as a soil drench significantly promoted height growth of slow-growing families (including two selfed families), but no significant effect was found for fast-growing families

(Williams et al., 1987). The authors suggested that the endogenous level of GA is limiting in the slow-growing families, whereas the endogenous GA level is sufficient in the fast-growing families. A positive correlation between family GA_9 levels in needle tissue and family performance in field progeny tests was reported in a limited test for full-sib Radiata pine and levels of $GA_9 + GA_4 + GA_7 + GA_{20}$ were significantly correlated with family performance in lodgepole pine (Zhang, 1990). Height growth in *Abies balsamea* can be significantly accelerated by the application of GA_3 (Little and Loach, 1975) and a significant positive effect was observed for height growth of *Pinus strobus* after application of GA_3 (Jensen and Dochinger, 1972). Exogenous GA_3 also increased height growth rate in longleaf pine, *Pinus palustris*, (Kossuth, 1981), and the growth rate of coastal Douglas-fir seedlings grown under cold soil conditions could be stimulated by the application of GA_3 (Lavender et al., 1973). Applied AMO-1618, a potent early GA biosynthesis inhibitor, suppressed height growth in *Cupressus arizonica* seedlings and this inhibition of shoot growth could be counteracted by an application of GA_3 (Kuo and Pharis, 1975). More recently (Junttila et al., 1991), application of prohexadione (BX-112), a late stage GA biosynthesis inhibitor, retarded shoot elongation in seedlings of *Salix pentandra* grown under a long photoperiod that is normally highly promotive for shoot elongation. Applied GA_1 successfully overcame this inhibition.

Auxin is another major plant hormone class. In addition to many other physiological functions, such as apical dominance, induction of adventitious roots and inhibition of leaf and fruit abscission, IAA is also involved in controlling shoot

growth in higher plants.

It has been demonstrated for many species that inhibiting IAA transport to the cambial region by debudding, defoliation or girdling gives an arrested cambial growth and that this inhibition could be eliminated by application of IAA (Little and Savidge, 1987, and references therein). In debudded cuttings of *Pinus sylvestris,* exogenous IAA increased internal IAA level and stimulated tracheid production (Sundberg and Little, 1990). However, in another study with *Pinus sylvestris,* tracheid production was not directly related to IAA concentration in the cambial region (Sundberg et al., 1993). Also Browning et al. (1992) found that application of (2RS, 3RS)-paclobutrazol (an early stage GA biosynthesis inhibitor) to the shoot tip of pear cv. Doyenne du Comice inhibited shoot growth without affecting IAA levels in the shoot apex. Inconsistent results have been obtained in studies investigating the relationship between the level of endogenous IAA and seasonal periodicity of cambial activity. In some reports IAA level was higher during the growing season than during the dormant period (Little and Wareing, 1981; Sundberg et al., 1987; Savidge, 1991). However, this correlation was not consistent. Sundberg at al. (1990) found that while the amount of IAA was highest when the cambium was active, the IAA levels in the cambial region was lowest during the tracheid production period at the stem of *Pinus sylvestris.*

Endogenous cytokinins have been detected in different tissues of many woody species (see Little and Pharis, 1995). However, experiments with several woody plant species suggest that the function of CKs in cambial growth is not clear

(Little and Pharis, 1995). Application of BA alone or together with IAA stimulated the production of xylem, phloem, and ray tissue in *Picea sitchensis* (Philipson and Coutts, 1980), and ray formation in *Pinus halepensis* was promoted by the application of K with IAA, NAA, or 2,4-D (Fahn and Zamaki, 1970). However, no stimulation effect on cambial growth was observed when *Pinus sylvestris* shoots were treated with K, or K together with IAA (Wodzicki and Zakaczkowski, 1974; Zajaczkowski,1973). Exogenous BA or K did not promote tracheid production in *Pinus sylvestris* (Hejnowicz and Tomaszewski, 1969).

Abscisic acid is generally recognized as an inhibitor of plant growth. In *Abies balsamea* cuttings, tracheid production was reported to be reduced by application of ABA (Little, 1975; Little and Eidt, 1970). Webber et al. (1979) reported that the ABA levels were higher during the dormant period than during high cambial activity in Douglas-fir shoots. Injection of ABA into Radiata pine seedlings decreased the extent of tracheid radial enlargement (Jenkins, 1974), and in another report Pharis et al. (1981) observed that when applied in mid-late summer, ABA inhibited tracheid radial width in Radiata pine, but that in other months, ABA, and especially ABA + GA_4, promoted tracheid radial growth.

Ethylene, the only gaseous plant hormone (ignoring methyl jasmonate), is also involved in cambial growth, though its function is not fully understood. Savidge (1983) reported that the production of ACC, an ethylene precursor, was associated with compression wood formation in *Pinus contorta*. Eklund (1991) identified ethylene from the cambial region of *Picea abies*. In Radiata pine application of an

ethylene-generating compound, Ethrel, promoted both xylem and phloem tissue production (Barker, 1979). Eklund (1990) reported that the cambial region of *Picea abies* and *Abies balsamea* cuttings released more ethylene during the active growth period than during dormancy (Eklund and Little, 1995). In contrast, evolution of ethylene from *Pinus taeda* seedling stems was found to be negatively correlated with their diameter growth (Telewski, 1990).

Thus, based on published information it appears that GAs are the class of hormones that most consistently promote intact shoot growth of a wide variety of tree species (as well as other higher plants), and this promotion occurs for both the vertical and radial dimensions. Hence, this thesis will concentrate on the GA class of hormones, and I will briefly introduce the extensive literature implicating GAs as causal factors in shoot growth.

Gibberellins are known to stimulate plant shoot elongation, but only GA_1 or other C-3b-hydroxylated GAs (e.g. GA_3, GA_4, GA_7, GA_{85}) are likely to be the "effectors" *per se*. The best evidence for this conclusion was reached in a series of experiments with dwarf maize (see MacMillan and Phinney, 1987). Phenotypically, the internodes of dwarf maize (*Zea mays*) are much shorter than internodes of their normal counterparts. Identification of endogenous GAs from the maize shoot indicated that GA biosynthesis occurs mainly by the early 13-hydroxylation pathway. Quantification of endogenous GAs from the shoot tissue of *dwarf*-1 (one of several dwarf maize mutants) revealed that only a trace amount of GA_1 was found in spite of an abundant GA_{20} level, GA_{20} being the immediate

recursor of GA_1 in the early 13-hydroxylation pathway. Feeding [^{13}C]GA_{20} to both normal wild type and *dwarf*-1 seedlings resulted in [^{13}C]GA_1 formation in the normal maize but not in the *dwarf*-1 seedlings. Application of GA_1 could, however, cause shoot growth to resume, yielding a normal height for the *dwarf*-1 genotype. However, application of the GA_1 precursor, GA_{20}, had no significant effect. Hence, the authors concluded that GA_1 is the sole "effector" in shoot elongation, and the short internode in this dwarf maize variety is indeed due to a deficit of GA_1 since *dwarf*-1 is unable to convert GA_{20} to GA_1. This and parallel work with pea and rice further proved that GA_1, GA_3 or GA_4 (all C-3β-hydroxylated GAs) were causal for shoot elongation (Ross et al., 1993; Takahashi and Kobayashi, 1991).

Further studies have indicated that GAs not only control shoot elongation, but also overall shoot growth vigour. The involvement of GAs in heterosis of maize was demonstrated in several experiments. Rood et al. (1983) reported that parental inbreds were quite responsive to the application of GA_3, but the response of heterotic hybrids to GA_3 was slight and variable in term of shoot height and dry weight increment. With more definitive analysis methods (e.g. GC-MS-SIM), endogenous GAs were identified and quantified from apical meristematic shoot cylinders of both hybrid and inbred *Zea mays* (Rood et al., 1988). The results revealed that GA_1 (the "effector" in shoot elongation) and GA_{19} (a precursor of GA_1 via GA_{20} in this species) levels were significantly higher in hybrid genotypes than in the parental inbred genotypes. Further study indicated that the log of endogenous $GA_{1/19}$ concentration in the shoot tissue was significantly correlated

with height, leaf area, relative growth rate and height increment of inbred and hybrid maize genotypes (Rood et al., 1990).

At present, 96 endogenous GAs from plants, fungi, and bacteria have been identified and given trivial numbers (Mander, 1992 personal communication). Based on their natural occurrence, different degrees of oxidation, studies with GA-deficit mutants and radioactive metabolism experiments, GAs can be categorized into at least four main biosynthetic pathways: early non-hydroxylation, early 3β-hydroxylation, early 13-hydroxylation, and early 3β, 13-hydroxylation (Fig. 1.1) (Graebe, 1987). This does not, however, mean that additional pathways, such as early 15β- and early 12-hydroxylation and possibly even early C-2 or C-11 hydroxylation pathways, do not exist. These biosynthetic pathways can be species and/or organ specific (Graebe, 1987; Sponsel, 1995). In higher plants, the endogenous GAs appear to be mainly biosynthesized in tissues which are actively engaged in growth and development, like shoot and root tips, young leaves and seeds (Sponsel, 1995). Often, many GAs can be found in the same tissue (e.g. seeds) from a given species. But, most of these GAs are precursors to, or metabolites of, the biologically active ones (Graebe, 1987; Sponsel, 1995).

In woody plants, endogenous GAs have been found in different tissues from many species (see Little and Pharis, 1994). For woody angiosperms, the early 13-hydroxylation pathway seems to be the major GA pathway of vegetative tissue. Endogenous GA_{19} and GA_{53} were found in the roots and branches of *Juglans regia* (Dathe et al., 1982). Endogenous GA_1, GA_8, GA_{19}, GA_{20}, GA_{29}, and GA_{44} were

identified in the shoots of navel orange, *Citrus unshi*, (Poling and Mair, 1988). Zanewich and Rood (1994) reported that endogenous GA_1, GA_8, and GA_{20} were identified from expanding vegetative buds of river alder (*Alnus tenuifolia*), that GA_1, GA_8, GA_{19}, and GA_{20} were present in flushing buds of *Populus tremuloides*, and that GA_1, GA_8, GA_{19}, GA_{20}, and GA_{29} were endogenous in white birch (*Betula pendula*) buds. In roots of elongating *Salix pentandra* seedlings, GA_1, GA_8, GA_{19}, GA_{20} GA_{29}, and GA_{56} were found to be endogenous (Olsen et al., 1994). Gibberellins A_1, A_4, A_8, A_9, A_{19}, A_{44} and A_{81} were characterized from the cambial region of *Eucalyptus globulus* (Hasan et al., 1994; Ridoutt et al., 1995).

Metabolic studies have also been accomplished. Deuterated GA_9 injected into mature leaves of *Salix pentandra* seedlings was metabolized mainly to $[^2H_2]GA_{20}$, with the presence of $[^2H_2]GA_1$ and $[^2H_2]GA_{29}$ noted for both short and treated leaves (Junttila et al., 1992). In a subsequent experiment, $[^2H_2]GA_4$ was injected into a mature leaf of *Salix pentandra* and one of the main metabolites was $[^2H_2]GA_1$ in the leaf and stem (Junttila, 1993).

For conifers, the early non-hydroxylation pathway appears to predominate (Fig. 1.2). In Norway spruce [*Picea abies* (L.) Karst] GA_1, GA_3, GA_4, and GA_9 were identified using GC-MS (Orden et al., 1987). The identified endogenous GAs in the shoots of coastal Douglas-fir are GA_1, GA_3, GA_4, GA_7 and GA_9 (Doumas et al., 1992). In Radiata pine GA_4, GA_7, GA_9, and GA_{15} were detected by GC-MS (Zhang, 1990). Endogenous GA_1, GA_3, GA_4 and GA_7 were found in Sitka spruce (*Picea sitchensis* [Bong.] Carr.) (Moritz et al., 1989a). Moritz et al. (1989b) reported that

$[^2H_2]GA_9$ was converted to $[^2H_2]GA_4$, $[^2H_2]GA_{34}$, and $[^2H_2]GA_1$, while $[^2H_2]GA_4$ was metabolized to $[^2H_2]GA_1$ and $[^2H]GA_{34}$ in elongating shoots of Sitka spruce. Tritiated GA_2 and $[^3H]GA_{34}$ were identified as metabolites of $[^3H]GA_4$ in vegetative shoots of Douglas-fir (Wample et al., 1975) and Norway spruce (Dunberg et al., 1983).

Thus, a non-hydroxylated GA metabolic pathway in *Pinus* and other conifer species is proposed (Fig. 1.2) based on known endogenous GAs, feeding experiments (bold line), and known pathways from other plant species (solid line), or from the fungus *Gibberellia fujikuroi* (dashed line).

Among the endogenous GAs identified from conifers, the level of $GA_{4/9}$, and especially GA_9, are consistently high (Zhang, unpublished results). In the non-hydroxylation pathway GA_9 is the first C-19 GA (after loss of C-20 from GA_{24}), from which all other C-19 GAs are derived. Its very high levels imply that metabolism of GA_9 to other "downstream" GAs may thus be rate-limiting. Thus, for both Radiata pine and coastal Douglas-fir, the GA_9 content of needle tissue was used as a biochemical trait in this study as was done with GA_{19} in studying heterosis in maize (Rood et al., 1988). Further, in my study, GA_9 levels in needle tissue, as a phenotypic and biochemical trait, were measured, and compared with genotypic performance within a family, as well as with among family performance for each of Radiata pine and coastal Douglas-fir.

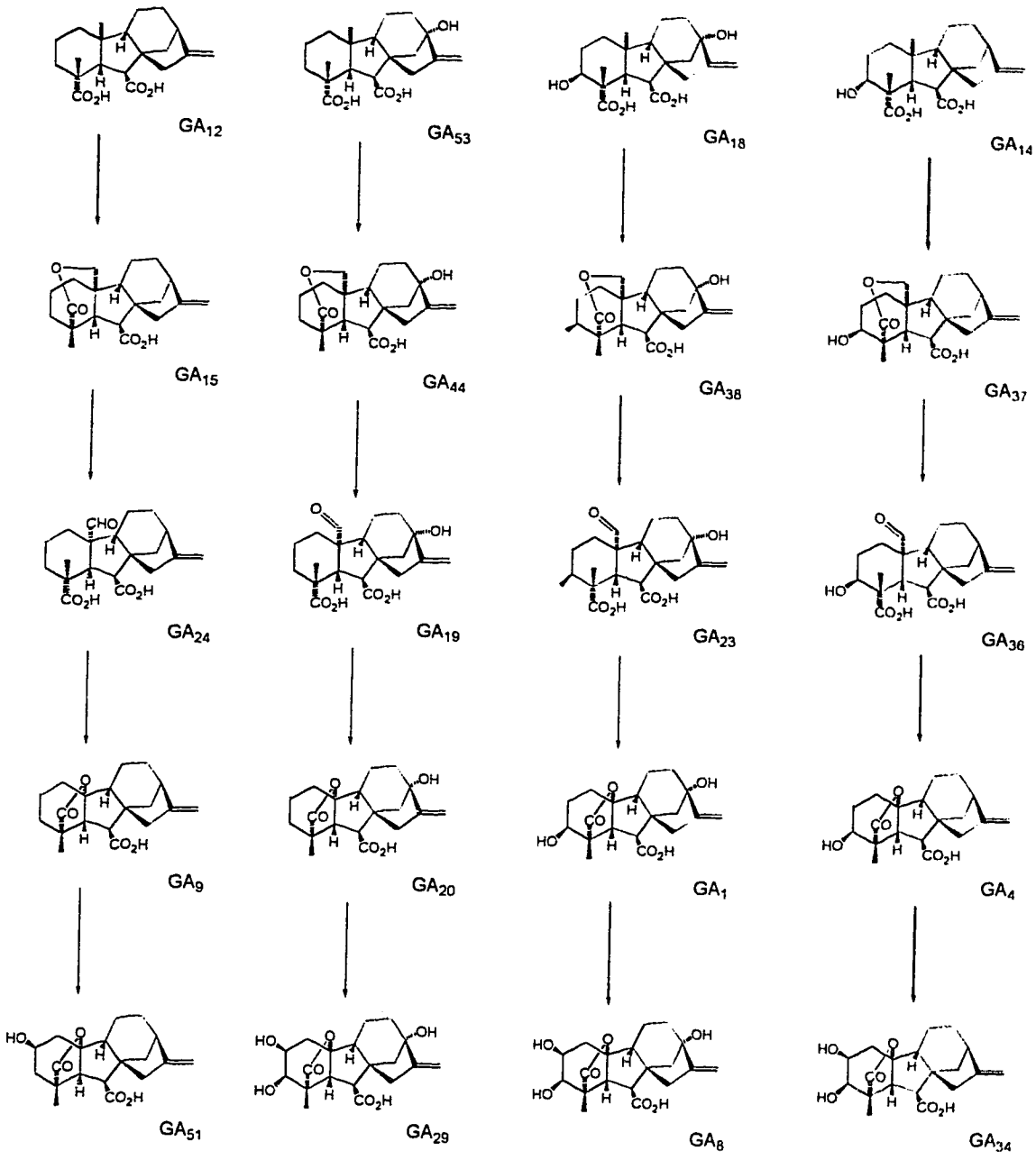


Figure 1.1 Several general GA biosynthesis pathways. From left to right: early non-hydroxylation, early 13α-hydroxylation, early 3β, 13α-hydroxylation, and early 3β-hydroxylation (adapted from Graebe, 1987).

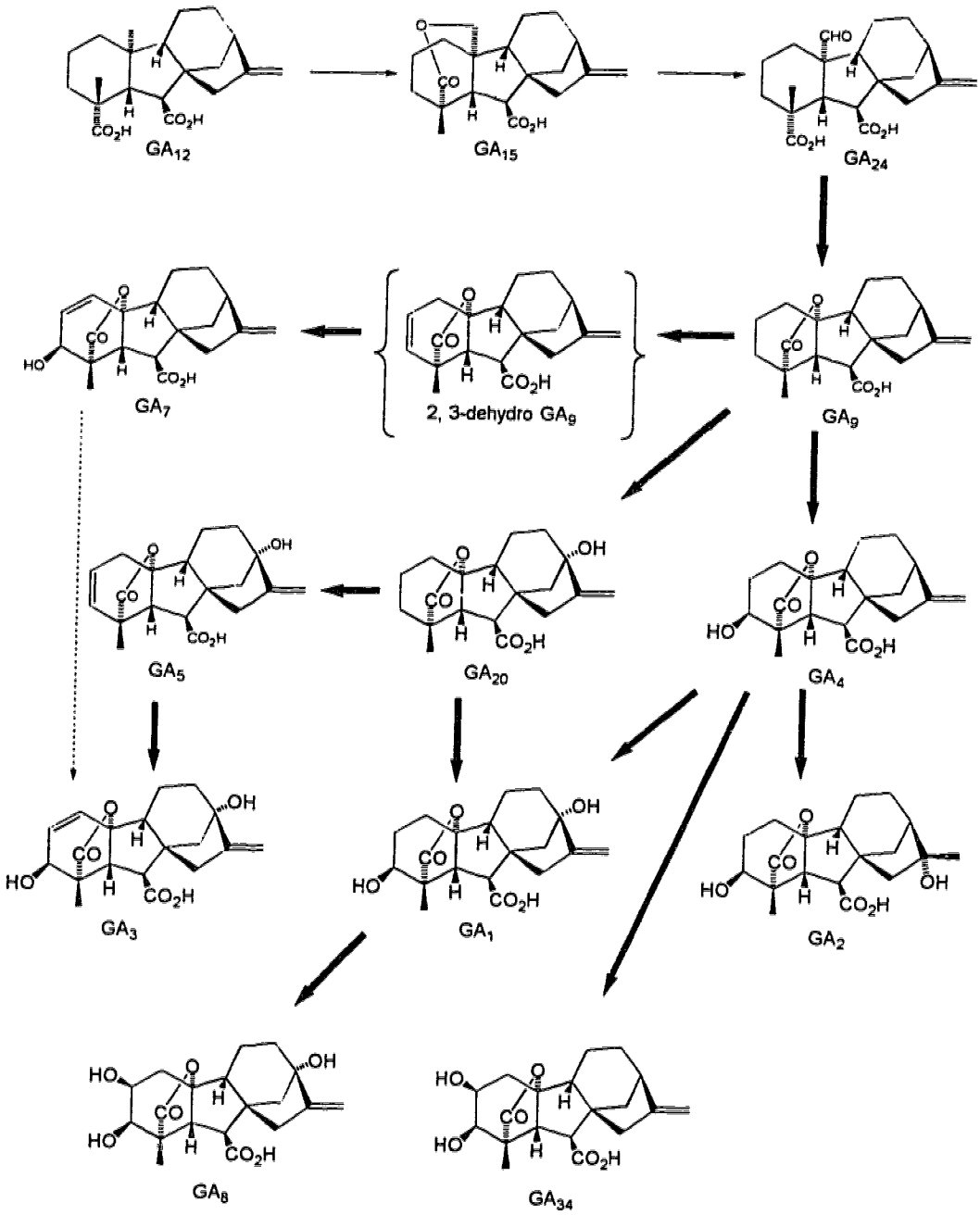


Figure 1.2 Proposed early non-hydroxylation gibberellin biosynthesis pathway in conifers: solid arrows represent experimental results from other plant species; bold arrows are based on feeding experiments with conifers or other plants; the dashed arrow is based on a feeding experiment with fungus (Gibberellin fujikuroi) (see text for details).

CHAPTER 2 ANALYSIS OF GIBBERELLINS - an overview

As natural organic compounds, GAs are present in a variety organisms from bacteria to higher plants. Among the five major plant hormones (GAs, auxin, ABA, cytokinins and ethylene), the GA level is usually the lowest, especially in vegetative tissue. For example, GA_9 level is about 10 - 20 ng/g dw, while ABA level is about 1000 ng/g dw in needle tissue of Radiata pine. However, it can be quite high in apices (e.g. *Lolium*, see Pharis et al., 1987). The analysis method is thus crucial for accurate and precise estimation of GAs.

There are three main difficulties in the analysis of endogenous GAs in higher plants and these were pointed out by Hedden (1987). First, the level of GAs is usually very low, in the ng/g dw range. Second, there are many interfering natural organic compounds, and the more than 96 structurally similar GAs make it difficult to identify a given GA. Finally, GAs do not have any characteristic physical property that is easy to measure, such as fluorescence or distinctive UV absorption. This limits the accurate and precise analysis of GAs to only a few methods.

Generally speaking, the analysis of GAs in plant tissue can be divided into the following stages: extraction, purification, separation and identification and/or quantification.

2.1 Extraction

At this stage, a suitable solvent must be used to efficiently extract GAs from plant tissue. Ideally, the solvent will dissolve only GAs and like substances, thereby making further purification easier. In practice, several aqueous-organic solvents have been used which include methanol, aqueous methanol and acetone. Currently there are two extraction methods commonly used. The plant tissue is ground with 80% aqueous methanol. After filtration the residue is re-extracted three more times with 80% methanol. Alternatively, the plant tissue can be extracted with 80% methanol twice only, each extraction lasting 1 h after grinding. The combined filtrate is then purified. Alternatively, the methanol in the combined filtrate can be removed *in vacuo* at 35°C and the remaining aqueous phase is then purified by other methods.

2.2 Purification

The GAs are purified and concentrated by a variety of methods which range from solvent partitioning to varied forms of HPLC. These methods have different sample capacity and purification mechanisms. Depending on the GAs of interest and the type of plant tissue, different combinations of methods, each with different purification mechanisms, can often be optimized to achieve the desired results.

Partitioning

Partitioning between immiscible solvents is suitable for processing large amounts of sample. I thus only used partitioning for initial identification of endogenous conifer GAs. Partitioning is usually used immediately after extraction at an early stage of purification. Partitioning was most often used in early years of GA analysis. As more sensitive and selective instruments have been introduced, the amount of plant tissue required for analysis has become smaller. Thus, partitioning is generally used now only for identifying unknown GAs which require large amounts of tissue initially, with a high degree of purification for subsequent physical-chemical analysis.

Since GAs as a group are weak acids, whose pKa ≈ 4.0 (Hedden, 1987), under alkaline conditions they are relatively soluble in water. Under acidic conditions they are mainly soluble in less-polar organic solvents. Based on this behaviour GAs will partition quite differently between relatively non-polar organic and aqueous phases at each of high or low pH. Usually pH 3.0 is used for extraction into the organic phase, after purification at pH 8.0 to remove many less polar impurities. In practice, the organic solvent for extraction is usually 80% aqueous methanol, the MeOH being removed *in vacuo* at 35°C. The remaining aqueous phase is adjusted to pH ≈ 3.0 with acetic acid or HCl. The aqueous phase is then partitioned against ethyl acetate (pH ≈ 3.0), usually three to four times. The combined ethyl acetate phase is then adjusted to pH ≈ 8.0 with NH_4OH, then partitioned against the new aqueous phase (water) four times.

Four partitionings are usually sufficient to transfer GAs from ethyl acetate to aqueous solutions at high pH, and vice versa at low pH, as was demonstrated earlier (Durley and Pharis, 1972). For example, at pH 8.0, if ethyl acetate and an aqueous solvent are partitioned once (1:1 = v:v), at least 80% of the GAs remains in the aqueous phase and 20% partition to the ethyl acetate. After four partitions only 0.16% of the original GAs are left in the ethyl acetate. Based on very conservative estimations, partition coefficients of a range of GA structures were determined by Durley and Pharis (1972).

In aqueous solution at extremes of pH and elevated temperatures, some GAs tend to undergo rearrangements and degradation (MacMillan and Pryce, 1973; Kirkwood et al., 1980; Grove et al., 1960; Pryce, 1973). Because of this it is suggested that the pH range should be kept between 2.5 to 8.5 and that the temperature should be no more than 40°C for processing in organic solvents and at least -20°C for storage.

Chromatographic Methods

The final purification of GAs is mainly accomplished by application of a variety of chromatographic methods. By the definition of the International Union of Pure and Applied Chemistry, chromatography is "A method used primarily for the separation of the components of a sample, in which the components are distributed between two phases, one of which is stationary while the other moves. The stationary phase may be a solid, or a liquid supported on a solid , or distributed as

a film, etc.; in these definitions 'chromatographic bed' is used as a general term to denote any of the different forms in which the stationary phase may be used. The mobile phase may be gaseous or liquid." (IUPAC, 1974).

There are four main types of chromatography based on the type of stationary phase and chromatographic principle. In adsorption chromatography the components of a sample are distributed between a solid adsorbent and the mobile phase. In partition chromatography, the components of a sample are partitioned between a liquid stationary phase and the mobile phase. In ion exchange chromatography, the components of a sample compete between an ion exchange resin stationary phase and liquid mobile phase. In gel permeation chromatography, the components of a sample are distributed between a polymer matrix and liquid mobile phase. Based on the mobile phase, the chromatography can be classified as either liquid chromatography or gas chromatography.

C_{18} Reversed-phase Preparative Column Chromatography

In some GA purification procedures a large capacity column is used instead of solvent partitioning. One such column consists of a 2.5 × 15 cm syringe barrel packed with 3 g of C_{18} reversed-phase material. This material is silica gel to which octadecylsilane (ODS) is chemically bonded to form a stationary phase. Thus, this column retains less-polar compounds while allowing polar compounds to pass easily through it. The column can be used to remove most pigments and non-polar impurities from the extract. In a procedure devised by Koshioka et al. (1983) the

column is washed first with 40 ml of methanol, then conditioned with 40 ml of 80% aqueous methanol. The extract dissolved in 80% methanol was loaded onto the column and forced through it followed by 20 ml of 80% methanol. The 60 ml of 80% methanol eluate will contain most free GAs while many pigments and non-polar impurities are left on the column. This column has been used routinely in purifying endogenous GAs from *Pisum sativum* L. (Koshioka et al., 1983) and Radiata pine (Zhang, 1990).

Sep-Pak C_{18} Column

This is a convenient reversed-phase cartridge (Waters Associates Inc.) for processing small amounts of sample. The stationary phase and the purification mechanism are the same as that of the C_{18} preparative column. However, the procedure for using this cartridge differs somewhat from the above methods, and is described by Hedden (1987). The cartridge is first washed with 5 ml of methanol. Then it is conditioned by 5 ml of 5% acetic acid. The sample is loaded onto the cartridge in 0.1 M phosphate buffer at pH 2.5. The loaded cartridge is then washed by 5 ml of 5% acetic acid followed with 5 ml of water to remove polar impurities. The GAs are then recovered by elution with 80% aqueous methanol while the non-polar impurities are left on the cartridge.

Polyvinylpolypyrrolidone (PVPP) or Polyvinylpyrrolidone (PVP)

Sample weight can be significantly reduced by use of this material, due to

its efficient removal of phenolic compounds which are abundant in conifer tissue (Glenn et al., 1972). The material can be used either as a stationary phase in a column or directly mixed as a slurry with an aqueous sample followed by filtration. This method has been used to purify extract from Sitka spruce (Moritz et al., 1990), Douglas-fir (Doumas et al., 1992), and *Salix pentandra* (Olsen et al., 1994). However, it was not used in the present study due to a concern over removal of putative GA:phenolic adducts (Nutbeam and Briggs, 1982).

Ion-Exchange Column Chromatography

Because GAs are weak natural organic acids, anion-exchange chromatography is another potentially useful method for purification. In this method the stationary phase is the anion-exchanger, such as DEAE-Sephadex A-25, QAE-Sephadex A-25, or Dowex 1 × 1-100. The mobile phase includes water, methanol and buffer. Purification is achieved through a process where active ions of the stationary phase are replaced by GAs in a neutral or mildly alkaline solution. Then, after impurities are washed away, the GAs are recovered by elution with a stronger acidic solution. A method for using the DEAE-Sephadex A-25 column is described by Crozier and Durley (1983). A QAE Sephadex A25 column has been used to purify extracts from apical buds and cambial region scrapings of *Eucalyptus* (Hasan et al., 1994). An ion-exchange column packed with Dowex 1 × 1-100 in the formate form was used to purify extract from immature seeds of *Pisum sativum* (Sponsel and MacMillan, 1978). Because of the high sample capacity Dowex 1×1-100 column

can be used at an early stage of purification to substantially reduce sample weight.

Gel Chromatography

Another chromatographic method used for purification of free GAs is based on their molecular size (e.g. 300-400 amu). In gel chromatography purification of GAs the stationary phase is a porous polymer matrix whose pores are completely filled with the mobile phase, whereas the mobile phase is usually tetrahydrofuran (Hedden, 1987). However, this method was not used in my work because separation of polar GAs from GA glucosyl conjugates is imperfect (R. P. Pharis unpublished results and personal communication).

SiO_2 Partition Column Chromatography

The stationary phase of the SiO_2 partition column is silica gel covered with a layer of water. The mobile phase is an organic solvent that is immiscible in water, usually ethyl acetate in n-hexane at different compositions, saturated with 0.5 M formic acid. Here, the mobile phase flows through the column, and various solutes are partitioned between the aqueous layer and the mobile phase according to their inherent partition coefficients. Solutes emerge from the column in order of polarity, the more polar ones last. Thus, this column can efficiently remove polar impurities from certain samples (Koshioka et al., 1983), although the silica gel acts partially as an adsorption matrix, and some non-polar substances are also retained. This column was used only in preparative purification in the present study to purify and

separate most free GAs from GA-glucosyl conjugates. Here, Woelm SiO_2 (32 - 100 μm) was deactivated by equilibration with 20% water by weight. Five g of the deactivated SiO_2 was packed in a 1.5 cm i.d. column. The sample was absorbed onto 1 g of celite, which was then loaded onto the column. The free GAs were eluted as a group with 80 ml of mobile solvent [0.5 M formic acid saturated solution of n-hexane and EtOAc (5:95 = v/v)].

Immunoaffinity Chromatography

Applications of immunology to plant physiology have steadily increased in the past 12 years. One active area has been to develop an antibody for analysis of plant hormones that are usually present in plant tissues in trace amount. Theoretically, this approach has great potential due to the specific affinity between antigen and antibody. With regard to analysis of GAs, polyclonal and monoclonal antibodies have been developed. Because of their small molecular size, GAs do not induce significant immunological reactions (e.g. do not produce usable antibodies). Hence, the GA is linked to a protein such as bovine serum albumin (BSA) which is then used to raise antibody. The specificity of the antibody is thus dependent on the way by which the GA is linked to the protein. The linkage between the GA and the protein is usually via the C-3β or C-13α hydroxyl groups, or the C-7 carboxyl group, thus allowing the rest of the GA molecule to contribute to the specificity of the GA:BSA conjugate. Because there are so many structurally similar GAs and so many unknown natural compounds in an extract, it is inevitable

that one will, in using immunoassay, encounter interference from other compounds and cross-reactivity from other GA:BSA conjugates. Thus, while methods for direct quantification of GAs by a variety of immunoassays, such as radioimmunoassay (Nakajima et al., 1991) and enzyme-linked immunoassay [ELISA] (Atzorn and Weiler, 1983) have been developed, because of these problems, complete separation of GAs prior to derivatization is necessary. Unfortunately this makes the immunoassay much less useful, especially since GC-MS-SIM can give more reliable results at this stage of purification and separation. As a matter of fact, any immunoassay must be validated with GC-MS-SIM before it can be employed routinely.

An immunoaffinity column (IAC) has also been developed for purification of GAs. In theory, the IAC column has great potential for its simplified purification procedures, and this was tested for use in my study for purification of GA_9. The stationary phase of the IAC column is antibody covalently bonded to a gel matrix, usually Sepharose. The purification procedure can be illustrated by the following example. Cotyledons of *Pisum sativum* were extracted with 80% methanol and the methanol was removed *in vacuo* (Smith and MacMillan, 1989). The aqueous sample was then loaded onto a 6 × 0.3 cm immunoaffinity column. After washing out unbound impurities with 0.1 M potassium phosphate, the GAs were recovered by elution with water at pH 6.5. This aqueous phase was then extracted for GAs with EtOAc at pH 3.0. My attempt to research the IAC method so it might become a rapid method for purification of large numbers of conifer needle extracts for GA_9

is detailed later.

HPLC - the Final Separation Step Prior to Identification and Quantification

The wide variety of chromatography methods described above can all be classified as liquid chromatography. Limitations of this kind of chromatography for GAs are low separation efficiency and limited usage, since there are more than 96 structurally similar free GAs. Hence, these preparative columns are mainly used to purify free GAs as a group, or to separate free GAs from their conjugates. To overcome these limitations HPLC was utilized which was developed in the 1960's (see Horvath et al., 1967).

The efficiency of HPLC columns is based on the rate of mass transfer and equilibration of solute molecules between mobile and stationary phases. This rate can be improved by packing columns with small sized particles (Giddings, 1965).

At present there are four types of columns used in HPLC for analysis of GAs: normal phase, reversed phase, Nucleosil $N(CH_3)_2$, and size-exclusion. Among them, reversed phase C_{18} and Nucleosil $N(CH_3)_2$ are more popular and the Rts of many GAs on these two HPLC columns have been documented (Pearce et al., 1994 and references cited therein).

1. Reversed Phase C_{18} HPLC

This type of HPLC is the most widely used in purification and separation of GAs. The packing materials are a variety of silica gels whose active hydroxyl

groups on the particle surface are bonded by hydrocarbons of different chain length, such as C_{18} or C_8. Because the stationary phase is chemically bonded to the silica, it is very stable. Thus, gradient elution with different solvents can be used without fear of stripping off the stationary phase, and the column can usually be used for one to several years. The predominant mobile phase is aqueous methanol or acetonitrile, which contains a small volume of acetic acid to prevent ionization of the GAs. Generally, the sequence of eluted GAs from the column is in the order of decreasing polarity. For example, among $GA_{1/4/8,}$ the more polar GA_8 comes out first, then GA_1 and finally GA_4 in accordance with the number of hydroxyl groups present. The Rts of GAs on μBondapak C_{18} reversed-phase HPLC has been reported by Koshioka et al. (1983), on Supelcosil LC 18 by Jensen (1986), and on Ultrasphere ODS by Lin et al. (1991). HPLC with μBondapak C_{18} was used extensively in my study.

2. Nucleosil $N(CH_3)_2$ HPLC

Nucleosil $N(CH_3)_2$ HPLC is another favoured technique in analysis of GAs. The stationary phase is a dimethylamino [$N(CH_3)_2$] group chemically bonded to silica gel. The mobile phase is methanol with 0.05-0.1% acetic acid. The Rt of GAs on this column is very different from that of the above ODS (C_{18}) columns. Thus, these two columns are often used sequentially [C_{18} HPLC, then Nucleosil $N(CH_3)_2$ HPLC] in purification and separation of GAs. Such an approach not only purifies and separates the GAs, but also provides valuable information for their subsequent

identification by more definitive means, such as GC-MS. Yamaguchi et al., (1982) and Pearce et al., (1994) report a variety of GA Rts on Nucleosil $N(CH_3)_2$ HPLC.

3. Normal Phase HPLC

Application of normal phase HPLC in analysis of GAs was pioneered by Reeve et al. (1976). The stationary phase on the column is 0.5 M formic acid coated onto a Partisil 20 silica gel support and the mobile phase is n-hexane-ethyl acetate. The main drawback of this column is that the stationary phase is gradually stripped off. This column was not used in my study and is not discussed further.

2.3 Identification and Quantification

After extensive purification and preliminary separation on a variety of chromatographic columns, the sample is usually pure enough for identification and quantification. However, because there are so many structurally similar GAs, it is often difficult to identify any given GA, even when quite pure. Thus, it is necessary to separate structurally similar GAs before identification is attempted. Such complete separation is best accomplished after derivatization by capillary column GC, currently the most powerful separation method, although packed columns can also be used. While the capacity of a capillary GC column is limited, its separation efficiency is very much higher than other chromatographic methods and the number of effective theoretical plates can reach 3000 per meter. The mobile phase for GC

can be a variety of gases, such as He, N_2, or H_2. Currently, many laboratories (MacMillan, J.; Pharis, R.P.; Phinney, B.O.; Takahashi, N.) are using fused-silica capillary columns with non-polar dimethylpolysiloxane silicone coatings such as OV-1 (DB-1, BP-1, HP-1) in GA analysis. For identification, the eluate from a GC column must be detected, and two formerly methods were the flame ionization detector (FID) and electron capture detector (ECD). However, because these techniques are not definitive, whereas GC-MS is (see later), they are no longer in common use. Thus, the choice of the detector is the mass spectrometer (MS), and my research has utilized GC-MS extensively.

The most commonly used MS in GA analysis is a quadrapole. In quadrapole MS four round rods are arranged in a quadrant, with opposite rods electrically connected. An oscillation field is formed between the rods when they are charged with a direct-current at radiofrequency voltages. When an ion moves into this quadrupole field it will oscillate between the electrodes. Only ions of a particular m/e have oscillations which are stable at a given voltage can pass the quadrupoles to the electron multiplier. Ions of other m/e values will move out of the quadrupole field due to unstable oscillations. Scanning is achieved by varying the magnitudes of the current and voltage; however, by keeping the ratio constant, a linear mass spectrum is produced. The resolution of a quadrupole mass spectrometer can be up to 1 in 2000, with mass range to 1000 amu. The advantages of this mass spectrometer are the fast scan times, down to 1 ms, linear spectra and easy interfacing for electronic control.

Application of GC-MS in GA analysis was pioneered by MacMillan and co-workers (1967). A detailed summary of GC-MS analysis of GAs can be found in Gaskin and MacMillan (1991). Free GAs are not in themselves sufficiently volatile for GC and therefore require derivatization prior to analysis. Generally, the carboxyl groups of GAs are methylated with diazomethane, which is a yellow gas dissolved in ether solution (Schlenk and Gellerman, 1960). The hydroxyl groups of GAs are usually then converted to trimethylsilyl ethers with BSTFA (bis-trimethyl-silyltrifluoroacetamide) or MSTFA (N-methyl-O trimethyl-silyltrifluoroacetamide). This derivatization not only increases the volatility of GAs, thus, making it possible to analyze them on GC, but also gives characteristic fragments and stronger molecular ions on MS for identification and quantification.

The identification of GAs can be divided into two classes: the identification of a known GA and the identification of an unknown GA. To identify a known GA, the chromatographic property and spectrum on GC - MS of the GAs are compared with that of an authentic GA. The identification of an unknown GA is much more complex, and is usually accomplished as follows. Based on spectroscopic and chemical properties of the unknown GA, a structure is proposed. Then, one or more candidate GAs are chemically synthesized. Finally, a comparison is made between the unknown GA and the synthetic GA based on the GC Rt (e.g. KRI, a GA Rt index relative to a hydrocarbon mixture) and mass spectrum (see below). The derivatized GAs are thus injected into the capillary column on a GC whose outlet directly leads to the ion source of the MS. There are two modes in MS analysis:

selected ion monitoring (SIM) and full scan. The SIM mode gives better sensitivity but less information, and is most often used in quantification. The full scan mode produces more information, but has less sensitivity, and is best used for identification. Thus, the identification of GAs can be deduced by comparing the sample Rt (KRI) and spectrum with that of authentic GA.

The quantification of GAs using SIM is based on the area ratio of sample ions of the GA to that of stable isotope-labelled GAs. To minimize the effect of experimental conditions on Rt, the Kovat's retention index (KRI) was established (Van den Dool and Kratz, 1963). The KRI is a relative Rt index, and a KRI of any GA can be obtained by co-injecting the sample with a series of straight-chain saturated hydrocarbons on GC-MS. The KRI can be calculated by the following equation:

$$KRI = 100\frac{R_x - R_n}{R_{n-1} - R_n} + 100n$$

Where R_x = the Rt of the sample.

R_n = the Rt of hydrocarbon with n carbon atoms.

R_{n+1} = the Rt of hydrocarbon with n+1 carbon atoms.

Quantification of GAs

Currently the most reliable method for quantifying endogenous GAs is by adding isotopically-labelled GAs as internal standards to the extract of the plant

tissue. After purification, the sample containing the internal standard is subjected to GC-MS analysis. The amount of endogenous GA in the plant tissue is then estimated, based on the m/e ion area ratio between the endogenous GA and that of the internal standard (stable isotope labelled) GA. Since the isotopically-labelled GAs are not perfectly labelled, and the abundances of natural isotopes in the sample and internal standard are variables, the peak areas of the GC-MS chromatogram are not used directly in the calculations. Instead, two approaches have been developed to overcome this problem. First, a calibration curve is established with standard compounds in which the ratios of peak areas are plotted across a range of molar ratios against molar ratios for endogenous GAs and the isotopically-labelled standards (Hedden, 1987). Then, using the intensities of the M^+ ion cluster or an appropriate fragment ion cluster, an isotope dilution method can be employed to estimate endogenous GAs (Gaskin and MacMillan, 1991). The contribution of each of the endogenous and isotopically-labelled GAs is estimated by measurement of ion intensities from the complete mass spectrum cluster.

CHAPTER 3 METHODS USED FOR THE IDENTIFICATION OF ENDOGENOUS GAs IN RADIATA PINE AND COASTAL DOUGLAS-FIR

To accurately estimate endogenous GAs in plant tissue the following two conditions must be met: the identity of the endogenous GAs in the tissue must be determined unequivocally, and the corresponding stable isotope-labelled GAs must be utilized. Other approaches are possible but provide less accuracy.

Previous results (Zhang, 1990) suggested that the biosynthetic pathway in Radiata pine is the early non-hydroxylation pathway. Thus, corresponding deuterated GAs and deuterated $GA_{4/7/9}GE$ were used as internal standards to assist in the identification of endogenous GAs in needle and shoot tissues of Radiata pine and needle tissue of coastal Douglas-fir.

3.1 Identification and Quantification of GAs

Extraction

About 1 g of needle tissue from 4-month-old radiata pine was put into a mortar with a small amount of acid-washed sand to assist in grinding. About 30 ml of liquid N_2 was used to freeze the needle tissue. As soon as the N_2 evaporated the frozen needle tissue was ground into a fine powder with a pestle. Ten ml of 80% MeOH (H_2O:MeOH = 20:80, v/v) (all solvents used were glass distilled except when specified otherwise) was added to the mortar to extract the tissue. After a few min

stirring, the solution was filtered through 3 pieces of Whatman No. 1 filter paper on a Büchner funnel. Ten ng each of [17, 17- $^{2}H_{2}$]$GA_{1/3/4/7/8/9/12/15/20/24/34}$ (their protio counterparts are the main GAs in the early non-hydroxylation pathway) and 100 ng each of [17, 17-$^{2}H_{2}$]$GA_{4/7/9}$GE were added to the first 10 ml of 80% MeOH extract along with 8,000 Bq each of [1,2(n)-^{3}H]GA_{1} (purchased from Amersham, 37.7 Ci/mmol), [1,2(n)-^{3}H]GA_{4} (Amersham, 32.2 Ci/mmol) and [1,2(n)-^{3}H]GA_{9} (Yokota et al., 1976) as internal standards for monitoring GAs and estimating recovery during purification.

C_{18} Preparative Column (C_{18}-PC)

A syringe barrel (inside diameter 2 cm) was used to make a C_{18}-PC with 3 g of C_{18} preparative reversed-phase material (Waters Associates) as stationary phase. Elution was as noted earlier under negative pressure using a Büchner funnel. Non-polar substances including most of plant pigments were retained on the column. Free GAs and $GA_{4/7/9}$GE were eluted out in the 80% MeOH. The 60 ml of 80% MeOH eluate was then transferred into a 250 ml flask and taken to dryness on a rotary evaporator *in vacuo* at 35°C. This 80% MeOH residue was stored at -20°C for further purification.

SiO_2 Partition Column (SiO_2-PC)

Five g of Woelm SiO_2 (32-100 mesh) was deactivated by equilibration with 20% water by weight. The deactivated SiO_2 was packed in a 1.5 cm i.d. column to

make a SiO_2-PC. The 80% MeOH residue from C_{18}-PC was dissolved in 1 ml of MeOH, then 1 ml of water, followed by another 1 ml of MeOH repetitively. Each 1 ml of solute was transferred onto 1 g of celite. The celite loaded with sample was taken to dryness under a warm air blower. This procedure was repeated until all the residue was transferred onto celite. After taking to dryness the celite was loaded onto the SiO_2-PC. The column was first washed with 90 ml of EtOAc:hexane (95:5, v/v; saturated with 0.5 M formic acid). The column was then washed with 100 ml of MeOH. The EtOAc:hexane eluate contained free GAs and the MeOH wash contained $GA_{4/7/9}GE$. Both fractions were taken to dryness *in vacuo* and stored at -20°C.

C_{18}-HPLC

The free GAs fraction from SiO_2-PC was further purified and separated by HPLC. The HPLC was a Waters Associates liquid chromatography apparatus with two model M-45 pumps, a model 680 automated gradient controller, and a model 7125 Rheodyne injector. The solvents were, pump A: 10% MeOH in 1% acetic acid [H_2O:MeOH:acetic acid = 89:10:1, (v/v)], pump B: 100% MeOH. The MeOH for HPLC was purchased as HPLC grade solvent. The other solvents for HPLC were filtered through 0.45 μm (HATF for H_2O) and 0.5 μm (FHUP for MeOH) pore size filters, respectively. The HPLC solvents were allowed to equilibrate, and then degassed before using. A Waters Associates reversed phase C_{18} Radial-PAK μ-Bondapak column (8 mm × 10 cm) was used with a 55-100% linear gradient

program at a flow rate of 1.5 ml/min. The manually implemented 55-100% linear gradient program was 0-20 min (pump A, 50%; pump B, 50%), 20-25 min (pump A, 50%-0; pump B, 50-100%), 25-40 min (pump B, 100%). Before injecting a sample the column was washed with MeOH for 30 min and then conditioned at 55% MeOH for 30 min. The free GAs residue from SiO_2-PC was dissolved in 55% MeOH, then filtered by a syringe connected to a 0.45 µm pore size filter tip. The filtrate was then injected into the HPLC. Forty, one min fractions were collected with a Gilson Model 202 autocollector and a 1/50 aliquot was taken from each fraction to locate the $[^3H]GA_{1/4/9}$ containing fractions. Based on the Rts of $[^3H]GA_{1/4/9}$ and published GA Rts on C_{18}-HPLC (see Pearce et al., 1994 and references therein) the 40 fractions were combined into 4 groups. The first, the GA_1 group, began just before the Rt of $[^3H]GA_8$ on the C_{18} HPLC and ended at the fraction immediately after $[^3H]GA_1$. This group contained $GA_{1/3/8}$. The second group contained fractions beginning after the GA_1 group and ending immediately after the $[^3H]GA_4$ Rt. This group contained $GA_{4/7/20/34}$. The third group included fractions beginning after the GA_4 group and ending immediately after $[^3H]GA_9$. This group contained $GA_{9/15}$. The last group included the remaining fractions and contained $GA_{12/24}$. These 4 groups were taken to dryness *in vacuo* and then stored at -20°C for further purification and separation.

Nucleosil $N(CH_3)_2$ HPLC

Each of the residues of the 4 groups from C_{18} HPLC was dissolved in 99.9%

MeOH in 0.1% acetic acid [MeOH:acetic acid = 999:1, (v/v)] and then filtered by a syringe connected to a 0.20 µm pore size filter tip. The filtrate was injected into the Waters Associates HPLC equipped with an Alltech Associates normal phase Nucleosil $N(CH_3)_2$ column (4.6 mm × 15 cm). The elution solvent was 99.9% MeOH in 0.1% HOAc running at 1 ml/min on isocratic mode. Sixty, one min fractions were collected and a 1/50 aliquot was taken from each fraction to locate the $[^3H]GA_{1/4/9}$ fractions. The HPLC fractions were taken to dryness *in vacuo* and then stored at -20 °C.

GC-MS-SIM

According to the Rts of $[^3H]GA_{1/4/9}$ and the published GA Rts on both C_{18} HPLC and Nucleosil $N(CH_3)_2$ HPLC (Pearce et al., 1994, and references therein) the residues of Nucleosil $N(CH_3)_2$ HPLC fractions were assigned possible identities. Each GA-containing grouped fraction was transferred into a reactivial in MeOH and taken to dryness under a flow of N_2. The carboxyl group of the GA molecule was methylated by dissolving the sample in 10 µl of MeOH followed by 90 µl of ethereal CH_2N_2. After stirring for one min the mixture was left at room temperature for 15 min. If a precipitate formed during the reaction it was removed by centrifugation. The solvent was then removed under a flow of N_2 at room temperature. For GAs with hydroxyl groups, silylation after methylation was accomplished by dissolving the methylated sample in 50 µl of GC-MS grade pyridine, followed by 50 µl of BSTFA with 1% TMCS (Pierce Chemical Co.) and mixing throughly. The reactivial

was flushed with N_2, then left at 70 °C for 30 min. The sample was then taken to dryness under N_2.

The identification and quantification of GAs was carried out on GC-MS in SIM mode. The derivatized sample was dissolved in 10 µl of HPLC grade n-hexane and directly injected onto a capillary column installed in a Hewlett-Packard 5890 GC with a capillary direct interface to a HP 5970 MSD. The capillary column was a 0.25 µm film thickness, 0.25 mm internal diameter, 15 m DB1-15 N column (J & W Scientific, Inc.). The capillary head pressure was 4 psi with a He carrier gas flow rate of 1.1 ml/min. The GC temperature program was as follows: 0.1 min at 60 °C, then go up to 200 °C at 20 °C per min then go up to 250 °C at 4 °C per min and last go up to 300 °C at 25 °C per min, stay at 300 °C for 5 min before returning to 60 °C. The interface temperature was maintained at 300 °C and the MSD was operated with the electron multiplier at 1800 V. Three m/z ions from each deuterated GA and endogenous GA were monitored. The dwell time was 10 seconds. The data were processed using the HP G1034C MS ChemStation Software. Based on the Rts and relative abundance of m/z ions monitored, endogenous GAs were identified and amounts estimated.

3.2 Identification and Quantification of $GA_{4/7/9}GE$

To identify GA-GEs in the same plant tissue 8,000 Bq of $[^3H]GA_{1/4/9}$ were added to the MeOH wash from the SiO_2 partition column. This sample was then

purified and separated on the C_{18}-HPLC with the 55% MeOH program described as above. The fractions containing $GA_{4/7/9}$GEs were located based on the Rts of $[^3H]GA_{4/7/9}$ and the estimated Rts of $GA_{4/7/9}$GEs (Koshioka et al., 1983). They were then combined into a $GA_{4/7}$GE group and a GA_9GE group. Each group was further purified and separated from free GAs on Nucleosil $N(CH_3)_2$ HPLC. The first 8 min eluate from Nucleosil $N(CH_3)_2$ HPLC were combined to make up the GA-GE sample. The GA-GE sample was then hydrolysed with cellulase in a procedure adapted from Schliemann and Schneider (1979). The hydrolysis condition was optimized by experiments testing three enzymes at different enzyme concentration and pH combinations.

The putative GA-GE sample from conifer tissue was dissolved in pH 5.0 buffer made with 0.1 M citric acid and 0.2 M disodium phosphate. The cellulase ('onozuka' RS, Japan) was suspended in the same buffer and added to the sample solution to a final concentration of about 1 mg/ml. The solution was then incubated at 37 °C for 24 h with shaking. After incubation the solution was adjusted to pH 3.0 with HCl and then partitioned against EtOAc (formic acid saturated) four times. The combined EtOAc fraction was subsequently taken to dryness and spiked with 4,000 Bq of $[^3H]GA_{4/9}$. It was then purified and separated on C_{18}-HPLC and Nucleosil $N(CH_3)_2$ HPLC respectively, as described above. The $GA_{4/7/9}$ fractions from Nucleosil $N(CH_3)_2$ HPLC were methylated and silylated accordingly. The identification and quantifications were accomplished on GC-MS in SIM mode.

3.3 Results

GC-MS-SIM data suggested that GA_4, GA_9, GA_4GE and GA_9GE were endogenous in the needle tissue of Radiata pine (see also Zhang 1990) and that GA_4, GA_9, GA_{15}, GA_{34}, GA_4GE and GA_9GE were present in the needle tissue of coastal Douglas-fir. Based on the ratio between m/z ions of GAs and deuterated GAs, GA_9 was the major endogenous GA in both of the Radiata pine and coastal Douglas-fir. The Rts and the relative abundances of m/z ions monitored are shown in Tables 5.1 to 5.3 for Radiata pine and coastal Douglas-fir respectively.

CHAPTER 4 METHODS DEVELOPED FOR THE QUANTIFICATION OF ENDOGENOUS GA_9 IN THE NEEDLE TISSUE OF RADIATA PINE AND COASTAL DOUGLAS-FIR

4.1 Quantification of Endogenous GA_9 in the Needle Tissue of Radiata Pine

4.1.1 Plant Materials

Radiata pine seeds originating from half-sibling seed orchards in New Zealand were utilized (provided by Dr. Rolland Burdon). Seeds were sown in plastic pots containing a 1:1 (v/v) mixture of perlite (Aust. Perlite Co. P) and vermiculite (Neuchatel Pty Ltd) at the end of May 1991. The seedlings were grown in the glasshouse of the University of Alberta in Edmonton at 25°C (day) and 20°C (night) under a 16 h photoperiod. There were 16 families and well in excess of 25 seedlings per families. Seedlings were arranged in a 4 × 4 complete randomized block of non-contiguous family plot, i.e. there were single-tree plots, one seedling per family per replication, 25 replications in total. The seedlings were watered twice a day, morning and afternoon, with tap water. The 1-month-old seedlings were harvested on June 24, 1991 during a "thinning process". The seedlings were gently pulled from the pots and those thinned seedlings which were typical of the single seedling left in the pot were harvested using the same procedure noted below for 4-month-old seedlings. After this first harvest each family was left with 25 seedlings. These residual 400 seedlings were harvested on September 24, 1991.

The 4-month-old seedlings were cut at the soil surface and the shoot with needles was immersed in liquid N_2 immediately after cutting. The frozen tissue was then put into a paper bag, stapled, and kept in a cooler containing dry ice. After transportation to the University of Calgary on dry ice, the tissues were freeze-dried for 10 days, then removed for weighing. The 1-month-old tissues were weighed as two parts: hypocotyl and primary needles and stem above the cotyledonary needles. The 4-month-old seedling tissues were segregated into 5 parts: the bottom stem (the woody stem below first branch, e.g. the cotyledonary needle point), the upper stem (the woody stem above and including first branch), the needle tissue along the main stem, the lateral branch and the needle tissue along the lateral branch. Their weights were recorded separately. The weighed tissues were then put in Ziplock bags with some blue silica gel desiccant, sealed and stored at -20 °C until analysis.

4.1.2 Extraction, Purification and Analysis of GA_9

Extraction

After warming to room temperature while sealed, the tissue was removed from its sealed package. The 1-month-old seedlings from each family were grouped based on their hypocotyl dry weight. The 4-month-old seedlings within each family were grouped according to their upper stem dry weight. Then needle tissue was selected from each seedling within an alike group for quantification of endogenous GA_9. Usually, needle tissue from 5 seedlings per family was combined

to make one sample for analysis. The needle tissue was put into a mortar, a small amount of sand added (to assist in grinding the tissue) and then about 30 ml of liquid N_2 was added to freeze the tissue. Immediately after the liquid N evaporated, the tissue was ground into fine powder with a pestle. The ground tissue was then extracted with 10 ml of 80% MeOH (H_2O:MeOH = 20:80 v/v) (all solvents used were glass distilled except when specified otherwise). Ten ng of deuterated GA_9 was added to the solution as an internal standard for quantification [deuterated GA_9 was synthesized using the method of Lombardo et al. (1982) by Prof. L.N. Mander, Research School of Chemistry, Australian National University, Canberra]. The extracting solution was stirred for a few min and then filtered with 3 pieces of Whatman No. 1 filter paper on a Büchner funnel. The extraction was repeated 3 more times, each time with 10 ml of 80% aqueous MeOH. After extracting and filtering the tissue 4 times, the filtrate became a transparent green.

C_{18}-PC

The first step of purification was achieved using a C_{18} preparative column (C_{18} - PC) [i.e. Waters Assoc. C_{18} preparative reversed-phase material was used to make a small column (ca. 3 times the original sample dw) in a syringe barrel (inside diameter 2 cm)]. The newly made C_{18}-PC was washed with 40 ml of MeOH and then conditioned with 40 ml of 80% aqueous MeOH. The 40 ml of 80% MeOH filtrate was then forced through the conditioned column under negative pressure (suction) using a Büchner funnel and the column was washed with another 20 ml

of 80% MeOH. That GA_9 eluted in the 80% MeOH eluate was determined using [^{3}H]GA_9. Most of the plant pigments and non-polar substances were retained on the column. The 80% MeOH eluate was transferred into a 250 ml flask and taken to dryness on a rotary evaporator *in vacuo* at 35°C. This 80% MeOH residue was stored at -20 °C for further purification.

C_{18} HPLC

The HPLC was a Waters Associates liquid chromatography apparatus with two model M-45 pumps, a model 680 automated gradient controller, and a model 7125 Rheodyne injector. The solvents were, pump A: 10% MeOH in 1% acetic acid [H_2O:MeOH:acetic acid = 89:10:1, (v/v)], pump B: 100% MeOH. The MeOH for HPLC was purchased as HPLC grade solvent. The other solvents for HPLC were filtered through 0.45µm (HATF for H_2O) and 0.5 µm (FHUP for MeOH) pore size filters, respectively. The HPLC solvents were allowed to equilibrate, and then degassed before using. A Waters Associates reversed phase C_{18} Radial-PAK µ-Bondapak column (8 mm × 10 cm) was used with a 55-100% MeOH gradient at a flow rate of 1.5 ml/min. The manually implemented 55-100% linear gradient program was 0-20 min (pump A, 50%; pump B, 50%), 20-25 min (pump A, 50%-0; pump B, 50-100%), 25-60 min (pump B, 100%), 60-65 min (pump A, 0-50%; pump B, 100-50%). Before injecting a sample the column was washed with MeOH for 30 min and then conditioned at 55% MeOH for 30 min. The GA_9-containing sample (dry residue) was dissolved in 55% MeOH , then filtered by a syringe connected to

a 0.45μm pore size filter tip. The filtrate was then injected into the HPLC. Thirty, one min fractions were collected with a Gilson Model 202 autocollector and 1/100 aliquots were taken for locating [^{3}H]GA_9 internal standard. Those fractions containing the [^{3}H]GA_9 fractions were then grouped and taken to dryness *in vacuo*.

Nucleosil $N(CH_3)_2$ HPLC

The residue of GA_9-containing fractions from C_{18} HPLC was dissolved in 99.9% MeOH in 0.1% acetic acid [MeOH:acetic acid = 999:1 (v/v)] and then filtered through a syringe connected to a 0.20μm pore size filter tip. The filtrate was injected into the Waters Associates HPLC equipped with an Alltech Associates normal phase Nucleosil $N(CH_3)_2$ column (4.6 mm × 15 cm). The elution solvent was 99.9% MeOH in 0.1% HOAc running at 1ml/min in isocratic mode. Thirty, one min fractions were collected. A 1/50 aliquot was taken from each fraction for locating [^{3}H]GA_9 and estimating recovery. The fractions containing [^{3}H]GA_9 were combined and taken to dryness *in vacuo*.

GC-MS-SIM

The [^{3}H]GA_9 fraction was then methylated with ethereal CH_2N_2 as illustrated by the following equation: $GA_9 + CH_2N_2 \rightarrow GA_9Me + N_2$

To accomplish this, the sample was dissolved in 10μl of MeOH. Then 90 μl of ethereal CH_2N_2 was added. After stirring for one min the mixture was left at room temperature for 15 min. The solvent was then removed under a flow of N_2 at room

temperature. The methylated sample was dissolved in 10µl of HPLC grade n-hexane and directly injected onto a capillary column installed in a Hewlett-Packard 5890 GC with a capillary direct interface to a HP 5970 MSD. The capillary column was a 0.25µm film thickness, 0.25 mm i.d., 15 m DB1-15N column (J & W Scientific, Inc.). The capillary head pressure was 4 psi with a He carrier gas flow rate of 1.1 ml/min. The GC temperature program was as follows: 0.1 min at 60 °C, then up to 200 °C at 20 °C per min followed by a gradient to 250 °C at 4 °C per min. The final gradient was 250 °C to 300 °C at 25 °C per min, the latter temperature being held for 5 min before returning to 60 °C. Thus, a single GC-MS run for a GA_9-containing fraction took about 30 min, including time to return to initial conditions. This allowed 16 to 20 fractions to be assayed for GA_9 in one day. The interface temperature was maintained at 300 °C and the MSD was operated with the electron multiplier at 1800 V. The m/z ions monitored for both $[^2H_2]GA_9$ and GA_9 were 332, 330, 300, 298, 272, 270. The dwell time was 10 sec. The data were processed using the HP G1034C MS ChemStation Software.

4.1.3 Quantification of Endogenous GA_9

The amount of endogenous GA_9 was estimated using the molecular m/z ions, 332 and 330, if the intensity of the molecular ions are very low the base m/z ions, 300 and 298 are used, for $[^2H_2]GA_9$ and GA_9 respectively, using an isotope dilution analysis (Fujioka et al., 1988; modified by D. Pearce, unpublished):

$$ngGA = \frac{S}{100}\left(\frac{BCE}{D-FC} - A\right)$$

A = % of unlabelled molecules in the internal standard.

B = % of labelled molecules in the internal standard.

C = measured intensity of M^+ of unlabelled GA.

D = measured intensity of M^+ of completely $[^2H_2]$-labelled GA.

E = is a factor calculated from the relative intensities of ions in the M^+ cluster of unlabelled GA and the amount of partly $[^2H_2]$-labelled GA relative to fully $[^2H_2]$-labelled GA in the internal standard. This calculation thus accounts for the partly $[^2H_2]$-labelled species that is not measured.

F = is the intensity of the m/z ion equivalent to M^+ of the fully $[^2H_2]$-labelled GA in the M^+ cluster of the unlabelled GA, relative to the intensity of M^+.

S = ng of internal standard added.

4.2 Quantification of Endogenous GA_9 in the Needle Tissue of Coastal Douglas-fir

4.2.1 Plant Materials

Coastal Douglas-fir seedlings for endogenous hormone assessment (and specifically for GA_9 assessment) were grown by Weyerhaeuser Co. Technology Center in temperature-controlled polyhouses (16.6 °C night and 23.9 °C day) under

natural sunlight plus supplemental incandescent bulb light (photoperiod maintained at 16 h) from July 22, 1992 to Oct. 23, 1992 at Federal Way, WA, USA. Seeds were stratified for 60 days at 3 °C, and sown on July 22, 1992 in cells (cell dia. 1.5" and depth 8.25"). There were 98 cells per tray with tray length 24" and width 12". The seedlings were watered as necessary, with nutrients being provided by irrigation. Final harvests for freezing and freeze-drying were made on Oct. 23, 1993, at which time height was also measured. Interim heights were not measured.

Seed originated from 16 full sib families used earlier in a field progeny trial on three sites in western Washington. Families for this retrospective polyhouse early growth/hormone analysis trial were chosen by Weyerhaeuser to represent a range of "slow, middle, and fast" growing families based on 8-year field progeny trial performance across three sites for stem volume. The experimental design for the 16 family comparison within the polyhouse trial consisted of single-tree plots, i.e. one tree per family per replication, trees randomized within replications, 16 replications in total.

Stem dry weights were obtained for all seedlings from all families by Weyerhaeuser. Ten representative seedlings were randomly chosen from each family in such a way that the broad range of seedling stem dry weights within that family was uniformly represented from low stem dry weight to high stem dry weight.

Thus, 160 seedlings, 10 for each of 16 families, were shipped to the University of Calgary in a "blind" fashion, numbered sequentially for identification.

Subsequently, and in a "non-blind" fashion, the diameters of these seedlings

were measured with a caliper. At any one measurement point and date, each seedling was measured 3 times and the mean of the 3 measurements was used as the diameter for that seedling. Each seedling was measured at 4 different locations along the stem. That is, there were 4 diameters for each seedling. The stem locations measured were ca. 0.7, 1, and ca. 3 cm from the basal swelling. The ca. 3 cm measurements were made twice, once by me, and once by Mrs Loeke Janzen with me recording.

4.2.2 Testing of an Immunoaffinity Column (IAC) Purification Scheme for Rapid Isolation of GA_9 from Coastal Douglas-fir Seedlings Prior to GC-MS-SIM

The MAC 175 (an antibody with high binding specificity for GA_9) bound to Sepharose 4B was purchased from Prof. J. MacMillan. The IAC (3 × 0.5 cm) was packed with this MAC 175 matrix. The purification procedure was first tested with $[^3H]GA_9$ based only on the method described in Smith and MacMillan (1989). About 8,000 Bq of $[^3H]GA_9$ was dissolved in 500 µl of PBS at pH 7.2 and loaded onto the IAC. The column was first washed with 15 ml of PBS at a flow rate of about 0.2 ml/min. Fifteen ml of PBS-methanol (7:3 = v:v) was used to elute the $[^3H]GA_9$. All eluate was collected in 5 ml fractions which were checked for the presence of $[^3H]GA_9$. However, based on the recovery of radioactivity in the 30 fractions, it was obvious that the majority of $[^3H]GA_9$ still remained on the column. Thus, another procedure was tested. After loading the $[^3H]GA_9$ in PBS solution, the IAC column was washed with 15 ml of PBS. Water was then used to elute the $[^3H]GA_9$. The

entire spike of [^{3}H]GA_9 was thus eluted in about 40 ml of water.

After this preliminary test the possible use of IAC for rapid quantification of GA_9 in large numbers of samples of coastal Douglas-fir needle tissue was assessed. Unfortunately, a negative decision was reached based on time and cost. The initial reasons for wanting to use the IAC were its simplicity and speed, because minimum purification procedures were [theoretically] required and I had many samples to be purified. However, because the cost is high, about $400 for each IAC column, the flow rate is slow (a few h per sample), and the water eluate must be extracted by partitioning, I decided that use of IAC to purify needle tissue samples from the 160 different coastal Douglas-fir genotypes using IAC was neither time nor cost efficient.

4.2.3 The C_{18} Method

Instead, a more efficient method, in terms of time and cost, was devised to purify the 160 samples. This rapid method used a C_{18}-Sep-Pak column which can be purchased ready to use and at reasonable cost (less than $2 each). Also, because the column is only used once, there can be no contamination between samples. Although the capacity of the C_{18} Sep-Pak column is limited, the GA_9 concentration in coastal Douglas-fir is relatively high, about 50 to 100 ng/g dw. Thus, a small amount of needle tissue can be used.

Extraction

After warming to room temperature while sealed, the tissue was removed from its sealed package. About 200 mg dry weight of needle tissue (needles chosen were morphologically similar) was selected from each coastal Douglas-fir seedling for quantification of endogenous GA_9. The needle tissue was extracted with 80% MeOH as described previously.

C_{18}-PC

The coastal Douglas-fir extract solution was first purified with a C_{18} - PC in the same procedure as that for Radiata pine needle tissue.

C_{18}-Sep-Pak

The sample residue after the C_{18}-PC was then dissolved in 5 ml of 20% aqueous MeOH, to which 8,000 Bq of [1,2(n)-^{3}H]GA_9 (Yokota et al., 1976) was added as internal radioactive standard to monitor the endogenous GA_9 during purification. The C_{18}-Sep-Pak was preconditioned with 10 ml of MeOH, fcllowed by 10 ml of 20% aqueous MeOH. The sample was then loaded with 20% aqueous MeOH into the C_{18}-Sep-Pak and passed through it under negative (gentle suction) pressure. Ten 3 ml fractions were collected and 1/100 aliquots were taken from each fraction to locate the [^{3}H]GA_9-containing fractions, which were then grouped and taken to dryness *in vacuo*.

Nucleosil $N(CH_3)_2$ HPLC

The [^{3}H]GA_9 fraction from the C_{18} Sep-Pak was purified on a HPLC equipped with a Nucleosil $N(CH_3)_2$ column using the same procedure as that for Radiata pine needle tissue. Then, the [^{3}H]GA_9 fractions were combined and taken to dryness *in vacuo*. Thus, by using the C_{18} Sep-Pak procedure subsequent to the C_{18}-PC, use of a reversed phase C_{18} HPLC purification step could be eliminated.

GC-MS-SIM

The [^{3}H]GA_9 fraction from Nucleosil $N(CH_3)_2$ HPLC was methylated with ethereal CH_2N_2 as specified above for Radiata pine tissue. The methylated sample was then analysed on GC-MS-SIM and quantified as detailed for Radiata pine tissue.

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Rationale For Examining Gibberellins As Possible Causal Factors For Inherently Rapid Growth

From several earlier studies (Phinney et al., 1986; Rood et al., 1988; Rood et al., 1990), it was suggested that the efficiency of GA metabolism from precursor GAs to ar ...ector GA" is causally implicated in shoot growth of higher plants. For example, in single gene dwarf maize and pea mutants shoot elongation growth is retarded and levels of various "early" GAs in the biosynthetic pathway are much higher than for normal genotypes (Phinney, 1990; Reid, 1990). Conversely, levels of an "effector" GA, GA_1, are much lower in the dwarf mutants than for seedlings of normal genotypes (ibid). These results and further genetic and biochemical analysis indicate that the turnover from GAs precursor to GA_1 is dramatically reduced in various maize mutants due to a deficiency of specific GA biosynthetic enzymes (ibid). The levels of endogenous GAs have also been related to heterosis (hybrid vigour for shoot growth) in maize (Rood et al., 1988; Rood et al., 1990), sorghum (Rood et al., 1992) and poplar (Rood and Pharis, 1988; Bate et al., 1988). In inbred maize parents, which have a very much reduced shoot growth relative to their hybrid crosses, GA_1 and GA_{19} levels are lower than in their hybrid crosses, and the slow-growing parental inbreds are disproportionately more responsive to application of exogenous GA_3 (Rood et al., 1983). There are analogous examples

in conifers. For example, GA_9 levels (GA_9 is a precursor to GA_4, GA_{20}, and likely GA_7 and GA_3) in mature needles of Radiata pine seedlings were higher in faster-growing than in slower-growing full-sib families in a five family comparison (Zhang, 1990). Also seedlings of slower-growing black spruce and lodgepole pine families respond disproportionately better to applied $GA_{4/7}$ than do the faster-growing families (Williams et al., 1987; I. Dymock, R. P. Pharis, F. C. Yeh and B. P. Dancik, unpublished, respectively).

Thus, in this investigation I have identified a number of native GAs and quantified one GA (GA_9) as a possible causal factor for early seedling growth, early family performance, and later field performance of two conifer species, Radiata pine and coastal Douglas-fir. Based on previous studies (Stiebeling et al., 1985; Zhang, 1990; Doumas et al., 1992; Wang et al., 1995) and present work (see below), the early non-hydroxylation pathway of GA biosynthesis appears to be the only pathway utilized in conifers (Fig. 1.2). Further, GA_9 is at the centre position of this pathway (Fig. 1.2) and is present as the major endogenous GA in these two conifers (Zhang, 1990; Zhang et al., unpublished results). Based on previous results (Zhang, 1990) the biosynthetic step from GA_9 to other GAs is likely a rate-limiting step in conifers. Therefore, it was GA_9 levels which were tested as a potential genetic marker in the present work, which is retrospective in nature.

Results

The GC-MS-SIM results indicate that GA_4, GA_9, GA_4GE and GA_9GE are endogenous to needle tissue of Radiata pine (see also Zhang 1990) and that GA_4, GA_9, GA_{15}, GA_{34}, GA_4GE and GA_9GE are endogenous to needle tissue of coastal Douglas-fir. Based on the ratio between m/z ions of GAs and deuterated GAs, GA_9 was the major endogenous GA in both Radiata pine and coastal Douglas-fir needles. The Rts and the relative abundances of m/z ions monitored are shown in Tables 5.1 to 5.3 for Radiata pine and coastal Douglas-fir respectively.

5.2 Radiata Pine

Use of "Outlier" Programs to Better Delineate Trends Exhibited by the Majority of Families

In the following sections, to better reveal trends exhibited by the majority of families, "early" and "retrospective" applications of an outlier program at 2.0 SE have been accomplished. This is done primarily for discussion purposes. The "early" application of an outlier program means that the outlier family was recognized by regression analysis using only early measurement parameters, e.g. needle GA_9 levels regressed with various seedling growth parameters. After the outlier(s) was removed, the remaining families could then be reassessed for their trends at the seedling stage. Additionally, the remaining families (after early outlier

Table 5.1 Capillary GC-MS-SIM of Authentic and Putative GAs for Radiata Pine

	Rt(min) or KRI	Percentage Abundance of Peak at m/z					
M^+ or m/z ions		418(M^+)	328	289	284	225	
Authentic GA_4MeTMSi	2537	332(25%)	367(27%)	346(26%)	1353(100%)	857(63%)	
Putative GA_4MeTMSi	2537	72(23%)	97(31%)	132(42%)	318(100%)	211(66%)	
M^+ or m/z ions		416(M^+)	384	356	298	282	222
Authentic GA_7MeTMSi	21.17	386(6%)	80(1%)	112(2%)	463(7%)	1635(26%)	6397(100%)
Putative GA_7MeTMSi	21.08	10(5%)	3(2%)	5(3%)	10(5%)	47(26%)	183(100%)
M^+ or m/z ions		330(M+)	298	270	243	226	
Authentic GA_9Me	2352	9(12%)	71(93%)	70(92%)	70(92%)	76(100%)	
Putative GA_9Me	2354	10(13%)	71(93%)	69(91%)	61(80%)	76(100%)	
M^+ or m/z ions		344(M+)	312	298	284	239	195
Authentic GA_{15}Me	16.03	2700(13%)	2511(12%)	1909(9%)	7941(39%)	20251(100%)	6507(32%)
Putative GA_{15}Me	15.97	14(11%)	20(16%)	13(10%)	49(39%)	127(100%)	47(37%)

Table 5.2 Capillary GC-MS-SIM of Authentic and Putative GAs for Coastal Douglas-fir

	Rt(min)	Percentage Abundance of Peak at m/z					
M^+ or m/z ions		420(M^+)	286	226	418(M^+)	284	224
$[^2H_2]GA_4MeTMSi$	15.8	31849	79574	47372	-	7539	3350
d+p $GA_4MeTMSi$	15.8	52353(21.3%)	245420(100%)	211387(86.1%)	4819	47150	35979
corrected $GA_4MeTMSi$					4819(20.2%)	23909(100%)	21034(88.0%)
M^+ or m/z ions		332(M^+)	300	272	330(M^+)	298	270
$[^2H_2]GA_9Me$	13.4	113620	742325	435149	-	-	-
d+p GA_9Me	13.4	13898(10.6%)	130512(100%)	101888(78.1%)	4225	37784	28332
corrected GA_9Me					4225(11.2%)	37784(100%)	28332(75.0%)
M^+ or m/z ions		346(M^+)	286	241	344(M^+)	284	239
$[^2H_2]GA_{15}Me$	17.9	59371	72725	119297	-	-	3154
d+p $GA_{15}Me$	17.9	23399(16.4%)	64193(45.0%)	142684(100%)	4114	9721	22509
corrected $GA_{15}Me$					4114(22.0%)	9721(51.9%)	18737(100%)
M^+ or m/z ions		508(M^+)	418	290	506(M^+)	416	288
$[^2H_2]GA_{34}MeTMSi$	18.7	671177	44036	53889	4965	-	12925
d+p $GA_{34}MeTMSi$	18.7	76243(100%)	4402(5.8%)	62052(81.4%)	65040	NI	41961
corrected $GA_{34}MeTMSi$					64476(100%)	-	27081(42.0%)

Table 5.3 Capillary GC-MS-SIM of Authentic and Putative $GA_{4/9}GE$ for Radiata pine and Coastal Douglas-fir

	Rt(min)	Percentage Abundance of Peak at m/z					
M^+ or m/z ions		420(M^+)	286	226	418(M^+)	284	224
$[^2H_2]GA_4MeTMSi$	14.1	31849	79574	47372	-	7539	3350
d+p $GA_4MeTMSi$(RP)	14.1	56343(24.8%)	227479(100%)	179305(78.8%)	41707	153256	94151
corrected $GA_4MeTMSi$					41707(31.7%)	131714(100%)	81474(61.9%)
M^+ or m/z ions		332(M^+)	300	272	330(M^+)	298	270
$[^2H_2]GA_9Me$	11.6	113620	742325	435149	-	-	-
d+p GA_9Me(RP)	11.6	35990(12.1%)	296804(100%)	153365(51.7%)	33856	297569	163850
corrected GA_9Me					33856(11.4%)	297569(100%)	163850(55.1%)
M^+ or m/z ions		420(M^+)	286	226	418(M^+)	284	224
$[^2H_2]GA_4MeTMSi$	16.1	31849	79574	47372	-	7539	3350
d+p $GA_4MeTMSi$(DF)	16.1	78063(19.1%)	409360(100%)	340373(83.1%)	4682	61691	50094
corrected $GA_4MeTMSi$					4682(20.4%)	22924(100%)	26030(113%)
M^+ or m/z ions		332(M^+)	300	272	330(M^+)	298	270
$[^2H_2]GA_9Me$	13.4	113620	742325	435149	-	-	-
d+p GA_9Me(DF)	13.4	17953(11.7%)	151441(100%)	105278(69.5%)	NI	36425	22626
corrected GA_9Me					-	36425(100%)	22626(62.1%)

removal) could also be compared (for GA_9 level, or for various seedling growth parameters) with family field performance(dbh). Finally, I also used retrospective application of an outlier program. Here, "retrospective" means that the outlier was identified after-the-fact by regression analysis in a comparison of the various seedling parameters to later field performance (e.g. dbh).

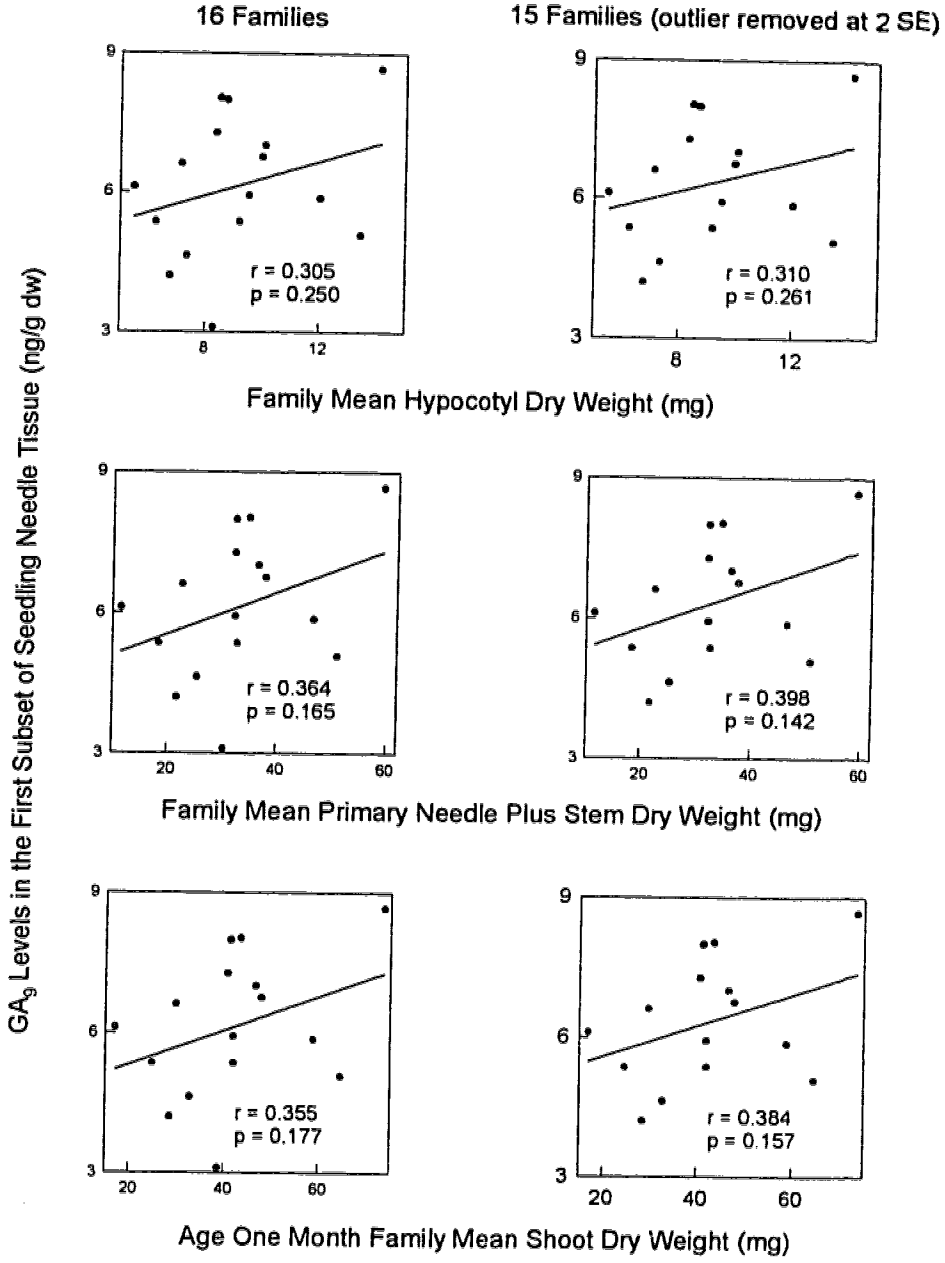
5.2.1 One-Month-Old Seedling Primary Needle GA_9 Levels in Relation to Early Seedling Growth

GA_9 and Biomass at the family level

The family means (of three replicate tissue sets) for GA_9 levels in the primary needle tissue of 1-month-old seedlings were first assessed by regression analysis for their relationship to family mean for (i) hypocotyls only, (ii) primary needles plus stem, and (iii) the sum of these (i.e. overall shoot dry weight). The trend (negative) was NS, even after outliers were removed at 2 SE (data not shown). However, when the family mean for GA_9 levels was replaced with GA_9 levels of only the first subset of seedlings chosen (this subset contained the largest seedlings), a positive but NS correlation of GA_9 with family mean for shoot biomass occurred with or without using an outlier program at 2 SE (Fig. 5.1). Similar results were also obtained when GA_9 levels for the first subset were correlated with shoot dry weight of this first subset of seedlings (data not shown).

Thus, although at the family level no significant correlation was found

Figure 5.1 Radiata pine F_2, one-month-old seedlings: GA_9 levels in the first subset of needle tissues compared with seedling biomass - between families



between primary needle GA_9 at age one month and biomass accumulation at age one month, the needle GA_9 levels from the largest seedlings (genotypes) did show a positive correlation with seedling biomass and with family mean biomass (with p-values ranging from 0.165 to 0.250). Hence, if age one month seedling biomass accumulation is related to age one month needle GA_9 level, the relationship is reasonably strong only for the largest seedlings (e.g. the fastest-growing genotypes) within a family.

Next, the family mean GA_9 levels in primary needles at age one month and subsequent growth were assessed, e.g. with various tissue dry weights of 4-month-old seedlings, such as stem or needle dry weight along the stem. While not significant they all show negative correlations (p-values ranging from 0.266 to 0.406 [data not shown]). Thus, high primary needle GA_9 (mean of three subsets) tended to be negatively correlated with subsequent rapid biomass accumulation at the family level. Application of an outlier program at 2.0 SE did not appreciably increase these correlations (data not shown).

The family mean GA_9 levels were also compared with upper stem dry weight, needle (along the upper stem) dry weight and total shoot dry weight (above ground parts) of 4-month-old seedlings by the one-tail Spearman rank correlation test. All correlation coefficients were negative with p-values ranging from 0.136 to 0.320 (Table 5.4).

Table 5.4 One-tail Spearman Rank Correlation Coefficients for Various Seedling Gibberellin A_9 Correlations with Family Means for Various Seedling Growth Measurements. Probabilities with Regard to Statistical Significance of these Correlation Coefficients are Given in Parentheses.

Seedling Growth Parameters (family mean)	fam1mGA_9	1m1rGA_9	fam4mGA_9
4mUpstem dw	-0.127(0.320)	0.300(0.130)	-0.041(0.440)
4mUpneedle dw	-0.292(0.136)	0.327(0.108)	0.031(0.455)
total shoot dw	-0.209(0.219)	0.266(0.159)	-0.041(0.440)
stem volume 1	-0.392(0.065)	-0.019(0.472)	-0.038(0.444)
stem volume 2	-0.328(0.107)	0.112(0.340)	-0.218(0.209)
stem volume 3	-0.334(0.103)	0.140(0.303)	-0.162(0.275)
mean3vol	-0.393(0.066)	0.029(0.457)	-0.100(0.356)
stem diameter 1	-0.367(0.081)	-0.025(0.463)	-0.166(0.270)
stem diameter 2	-0.282(0.145)	0.116(0.334)	-0.159(0.279)
stem diameter 3	-0.367(0.081)	0.174(0.260)	-0.323(0.111)
mean3dia	-0.465(0.035)*	-0.050(0.427)	-0.024(0.466)
height 1	-0.053(0.423)	0.393(0.066)	-0.197(0.232)
height 2	-0.072(0.395)	0.344(0.096)	-0.158(0.280)
height 3	-0.186(0.246)	0.331(0.105)	-0.097(0.360)

Table 5.4 continued

height 4	-0.206(0.222)	0.290(0.138)	-0.112(0.340)
height 5	-0.238(0.187)	0.243(0.182)	-0.024(0.466)
height 6	-0.266(0.159)	0.166(0.269)	0.000(0.500)
RGRH3H1	-0.040(0.442)	-0.328(0.107)	0.132(0.314)
RGRH3H2	-0.153(0.286)	-0.343(0.096)	-0.132(0.313)
RGRH4H1	0.035(0.449)	-0.331(0.105)	-0.184(0.247)
RGRH4H2	-0.165(0.271)	-0.414(0.056)	-0.060(0.413)
RGRH4H3	-0.131(0.314)	-0.484(0.029)*	0.109(0.344)
RGRH6H1	-0.208(0.219)	-0.515(0.021)*	0.173(0.260)
RGRH6H2	-0.126(0.321)	-0.442(0.043)*	0.076(0.390)
RGRH6H3	0.180(0.252)	-0.292(0.136)	-0.002(0.498)
RGRH6H4	0.176(0.257)	-0.217(0.210)	0.163(0.274)
RGRH6H5	-0.335(0.102)	-0.490(0.027)*	0.067(0.403)
RGRD3D2	-0.136(0.308)	-0.421(0.052)	-0.179(0.254)

Note: Early seedling RGR parameters for stem heights, diameters and volumes that are not shown had no correlations with significance values better than $p = 0.220$.

* These data are significant at $p \leq 0.05$.

Abbreviations: fam1mGA_9 = family mean primary needle GA_9 levels (1-month-old seedlings).

1m1rGA_9 = first subset (largest seedlings) primary needle GA_9 levels of (1-month-old seedlings).

fam4mGA_9 = family mean fascicle needle GA_9 levels (4-month-old seedlings).

mean3vol = mean of 3 stem volume measurements at ages 11, 13 and 15 weeks.

mean3dia = mean of 3 stem diameter measurements at ages 11, 13 and 15 weeks.

Height 1-6 = heights measured at ages 4, 6, 8, 10, 12 and 14 weeks.

RGR = relative growth rate for various height measurement intervals and for Diameter 2 versus Diameter 3 interval.

Height and Height Growth

The family mean GA_9 levels (i.e. mean of 3 subsets of seedlings) in primary needles of 1-month-old seedlings were assessed in relation to subsequent heights of the greenhouse-grown seedlings, measured from age 1 month to 4 months. The trends were negative and NS (data not shown) even after the application of an outlier program at 2.0 SE (data not shown). Nonetheless, these negative correlations increased as the seedlings became older (e.g. r = 0.06 to 0.08 for first three means at ages 4, 6 and 8 weeks, r = 0.13 to 0.4 for last two means at ages 12 and 14 weeks). That is, the strongest negative correlation of family mean age 1-month-old primary needle GA_9 was found with the final height of seedlings at age 4 months. Application of one-tail Spearman rank correlation test gave the same trend (Table 5.4). This is the opposite of the trend found for GA_9 levels from the "largest seedlings" subset (Table 5.4).

Next, family mean GA_9 levels at age one month were assessed in relation to subsequent seedling height growth. The correlation was negative but NS and no outlier was detected with use of an outlier program at 2 SE (data not shown).

Thus, subsequent seedling height growth at the family level tends to be inversely correlated with family mean GA_9 in primary needles at age one month. Hence, for height and especially height growth, high primary needle GA_9 (family mean) at age one month is an inverse indicator of family potential for subsequent early growth. That is, faster-growing families tend to have a lower family mean GA_9 level in their primary needles. Thus, while very early height and shoot biomass

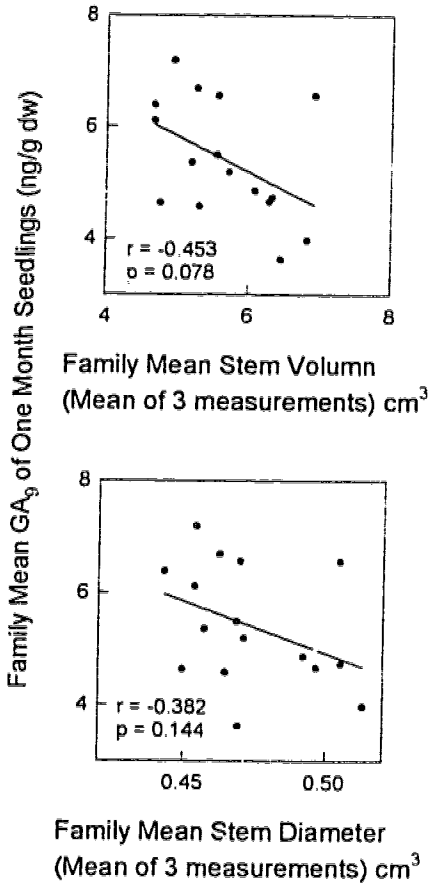
accumulation between families is best correlated with high primary needle GA_9 of the faster-growing genotypes (Fig. 5.1, Table 5.4), height and subsequent height growth at the family level is best reflected by low family mean GA_9 levels (data not shown). These results suggest that the rate of GA biosynthesis (or transport from the needles to the stem) may vary for different genotypes (faster-growing versus slower-growing) at different developmental stages, and for different growth parameters (height vs biomass).

Diameter and Diameter Growth

Family mean GA_9 levels in primary needles at age 1-month and subsequent seedling diameter and diameter growth were also assessed at the family level. They all showed negative correlations, with p-values ranging from 0.086 to 0.313 (data not shown). No outlier was found when an outlier program was used at 2 SE. Nor was a significant correlation found when the family mean GA_9 levels (mean of 3 subsets) were replaced with GA_9 levels in the first subset (largest seedlings) (data not shown). When age one month GA_9 levels were compared with subsequent seedling diameters using the one-tail Spearman rank correlation test, the correlation coefficients had p-values ranging from 0.081 to 0.145 (Table 5.4).

Similarly, family mean GA_9 levels in primary needles at age one month also tended to be negatively correlated with the mean of three diameter measurements (age 11, 13 and 15 weeks) (Fig. 5.2). When this comparison was subjected to the one-tail Spearman rank correlation test, the negative correlation became significant

Figure 5.2 Radiata pine F_2, family mean needle GA_9 levels of one-month-old seedlings compared with family mean stem volume and stem diameter (mean of ? measurements)



at $p \leq 0.05$ (Table 5.4). Family mean GA_9 levels at age one month were also assessed by regression analysis for their relationship with family mean stem volumes. Again, the correlation was negative with a p-value of 0.078 (Fig. 5.2). Application of an outlier program at 2 SE did not reveal any outlier for these two comparisons. Application of the one-tail Spearman rank correlation test yielded same result with a p-value of 0.132 (Table 5.4).

Thus, as with height and height growth, subsequent diameter, diameter growth and stem volume of 4-month-old seedlings is negatively correlated with the family mean for GA_9 levels in the primary needles. Hence, families with low GA_9 in their primary needles tend to have rapid subsequent seedling height, stem diameter and stem volume growth. Reasons could include more rapid metabolism of GA_9 to a C-3β hydroxylated "effector" GA (e.g. GA_4, GA_7, GA_1 or GA_3, Fig. 1.1), or perhaps to a more rapid transport of GA_9 or its metabolites to the growing stem.

5.2.2 GA_9 Levels in Fascicle Needles from Four-Month-Old Seedlings in Relation to Early Growth of the Seedlings

GA_9 and Biomass accumulation at the family level

First, family mean GA_9 levels in 4-month-old seedling needle tissues were assessed by both regression analysis and the one-tail Spearman rank correlation test for their relationship with various tissue dry weights at age four months. All correlations were negative and NS (data shown only for one-tail Spearman rank

correlation test; Table 5.4).

Then, GA_9 levels in needle tissues of 4-month-old seedlings, grouped from the 5 slowest genotypes per family, were assessed by regression analysis in relation to the mean dry weights for various tissues of these same genotypes. Here, "slowest-growing" is defined as the 5 out-of 25 seedlings with the lightest stem dry weights within each family. All correlations were negative and NS (data not shown). Application of an outlier program at 2 SE improved the correlations with p-values ranging from 0.087 to 0.137) (Fig. 5.3). These same GA_9 values (from the 5 slowest growing families) were then compared with mean family seedling biomass measurements using the one tail Spearman rank correlation test, and significance was gained ($p = 0.024$) for age four month total seedling dry weight (Table 5.5).

Finally, the GA_9 levels in 4-month-old seedling needle tissues from the 5 fastest genotypes were assessed in relation to the family mean tissue dry weights. No significant correlation was found even after use of the outlier program at 2 SE (data not shown).

Thus, as was seen for comparisons of family mean age 1-month GA_9 and various age 4-month shoot biomass measurements, age 4-month needle GA_9 levels are also negatively correlated with age 4-month seedling biomass accumulation. Again, then, at the seedling stage, the fastest-growing families tend to have the lowest GA_9 levels.

Figure 5.3 Radiata pine F_2, four-month-old seedlings: GA_9 levels in needle tissues taken from the 5 slowest genotypes compared with seedling biomass (outlier removed at 2 SE) - between families

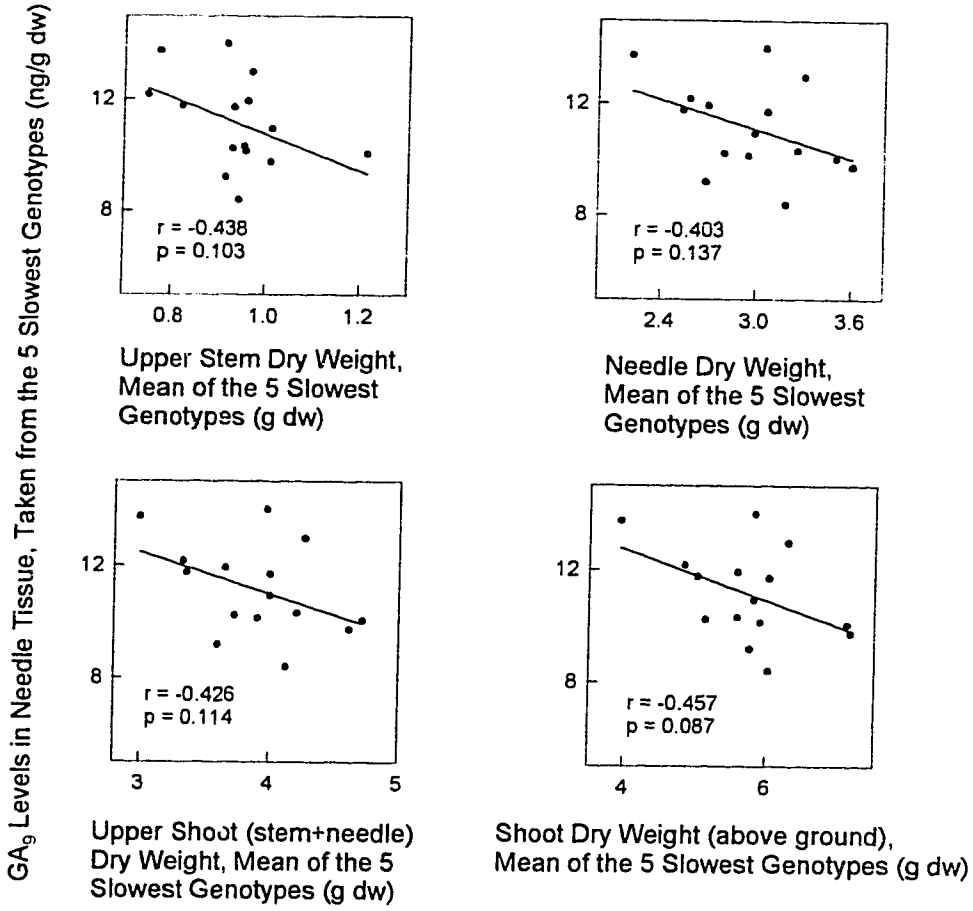


Table 5.5 One-tail Spearman Correlation Coefficients for Comparisons of Radiata Pine Fascicle Needle GA_9 Levels Sampled at Age 4 Months from the Slowest Growing Seedlings (e.g. from the 5 out of 25 seedlings with the lightest stem dry weights) with Various Seedling Growth Parameters (family mean)

	GA_9 Levels in Needles from 5 Slowest Growing Seedlings	
Family Mean	Correlation Coefficient (r)	Probability (p)
Upper Stem DW	-0.360	0.085
Needles Along Upper Stem DW	-0.333	0.104
Total Seedling DW	-0.503	0.024*
Stem Volume 1	-0.456	0.038*
Stem Volume 2	-0.591	0.008*
Stem Volume 3	-0.565	0.011*
Stem Diameter 1	-0.499	0.025*
Stem Diameter 2	-0.595	0.008*
Stem Diameter 3	-0.659	0.003*
Height 1	-0.312	0.120
Height 2	-0.380	0.073
Height 3	-0.430	0.048*
Height 4	-0.471	0.033*
Height 5	-0.438	0.045*
Height 6	-0.453	0.039*

*: These data are significant at $p \leq 0.05$.

Assessment of Needle GA_9 Levels from Four-Month-Old Seedlings in Relation to Seedling (Genotype) Performance within A Family

For each of the 16 families GA_9 levels in needle tissues of 4-month-old seedlings were assessed within each family, initially in relation to seedling upper stem dry weight within each family (Fig. 5.4). All 16 comparisons yielded positive correlations, with p-values ranging from 0.391 to 0.001 (Fig. 5.4).

The slopes (b1) of the regression lines for each of these 16 families were then used to develop a second order relationship of GA_9:stem dry weight within a family, relative to that family's performance in the field. This will be discussed later.

Thus, within all half-sib Radiata pine families, faster-growing seedlings (genotypes) have higher GA_9 levels. These results are consistent with results from hybrid maize, where fast-growing hybrid offspring had higher GA levels than their slow-growing inbred parents (Rood et al., 1988, 1990). This result is also consistent with the previous report that fast-growing genotypes within several full-sib families tend to have higher levels of GA_9 (Zhang, 1990 and unpublished).

Individual GA_9 Levels of Fascicle Needles in Relation to Early Growth of Genotypes Across All Families

As described in the Methods section, all GA_9 levels were estimated from needle tissues grouped from at least 5 genotypes. These individual GA_9 levels were then assessed across all families (i.e. family was ignored) for their relationship to the seedlings' mean stem dry weight or needle dry weight (i.e. mean of 5 or more

Figure 5.4 Radiata pine F_2, four-month-old seedlings: needle GA_9 levels compared with seedling upper stem dry weight - within families

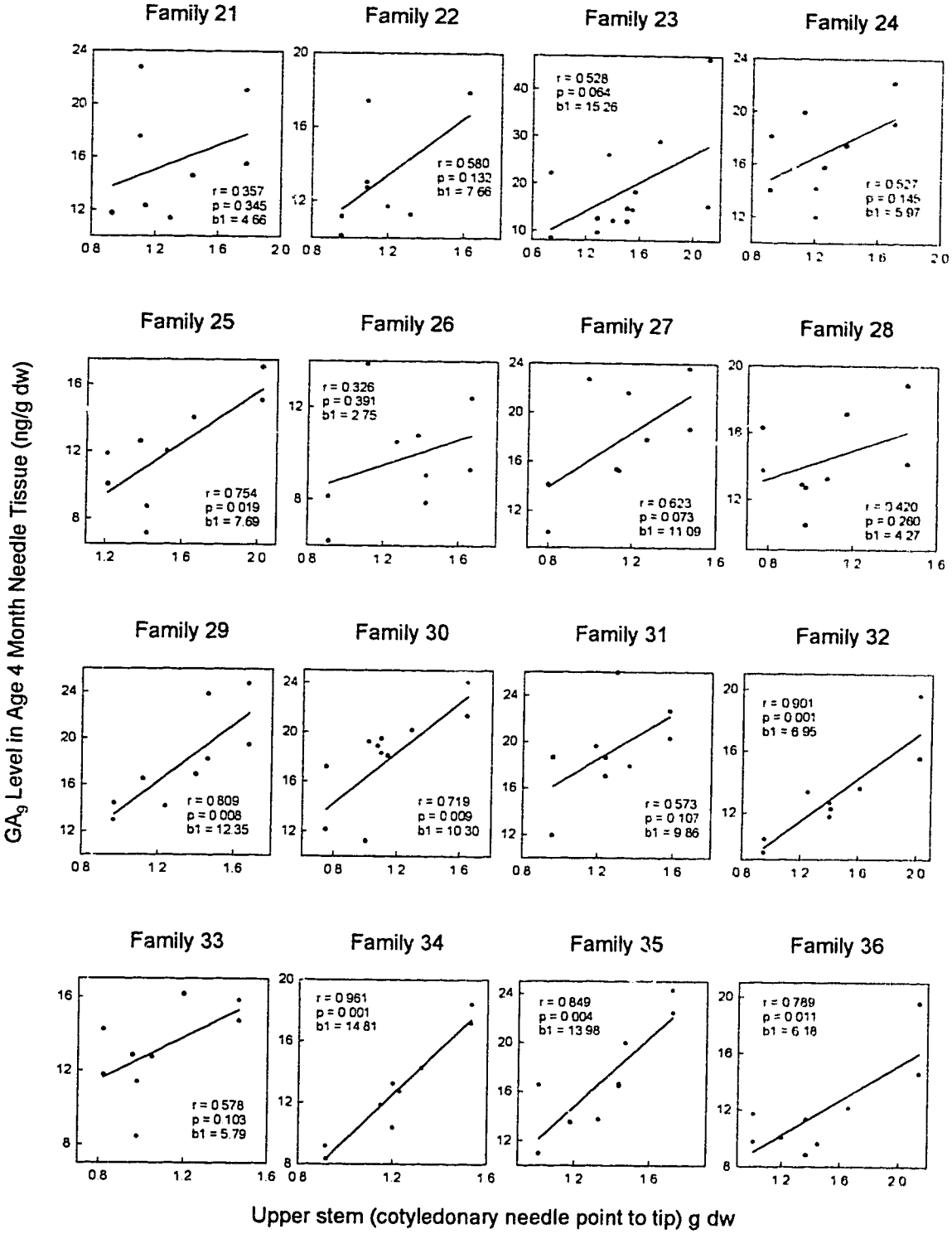
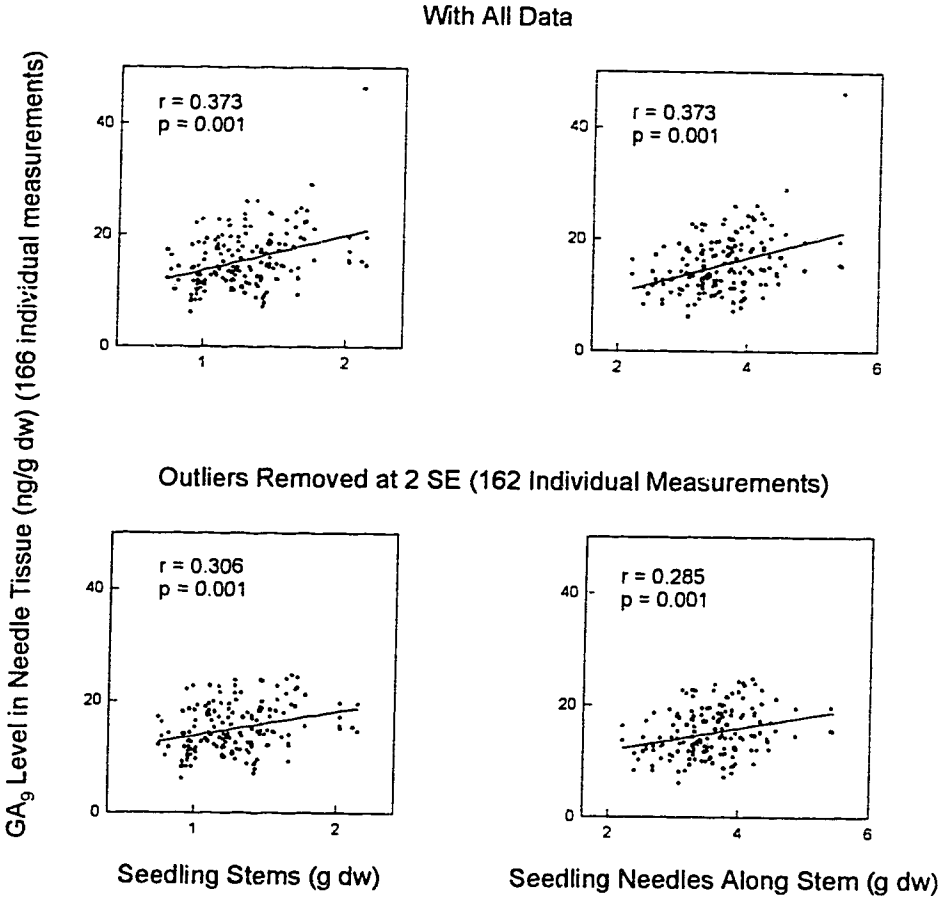


Figure 5.5 Radiata pine F_2, four-month-old seedlings: needle GA_9 levels compared with seedling biomass - family ignored



Note: GA_9 values are based on needle tissue samples grouped from at least 5 individual seedlings. Growth parameters are the means of those same 5 or more seedlings.

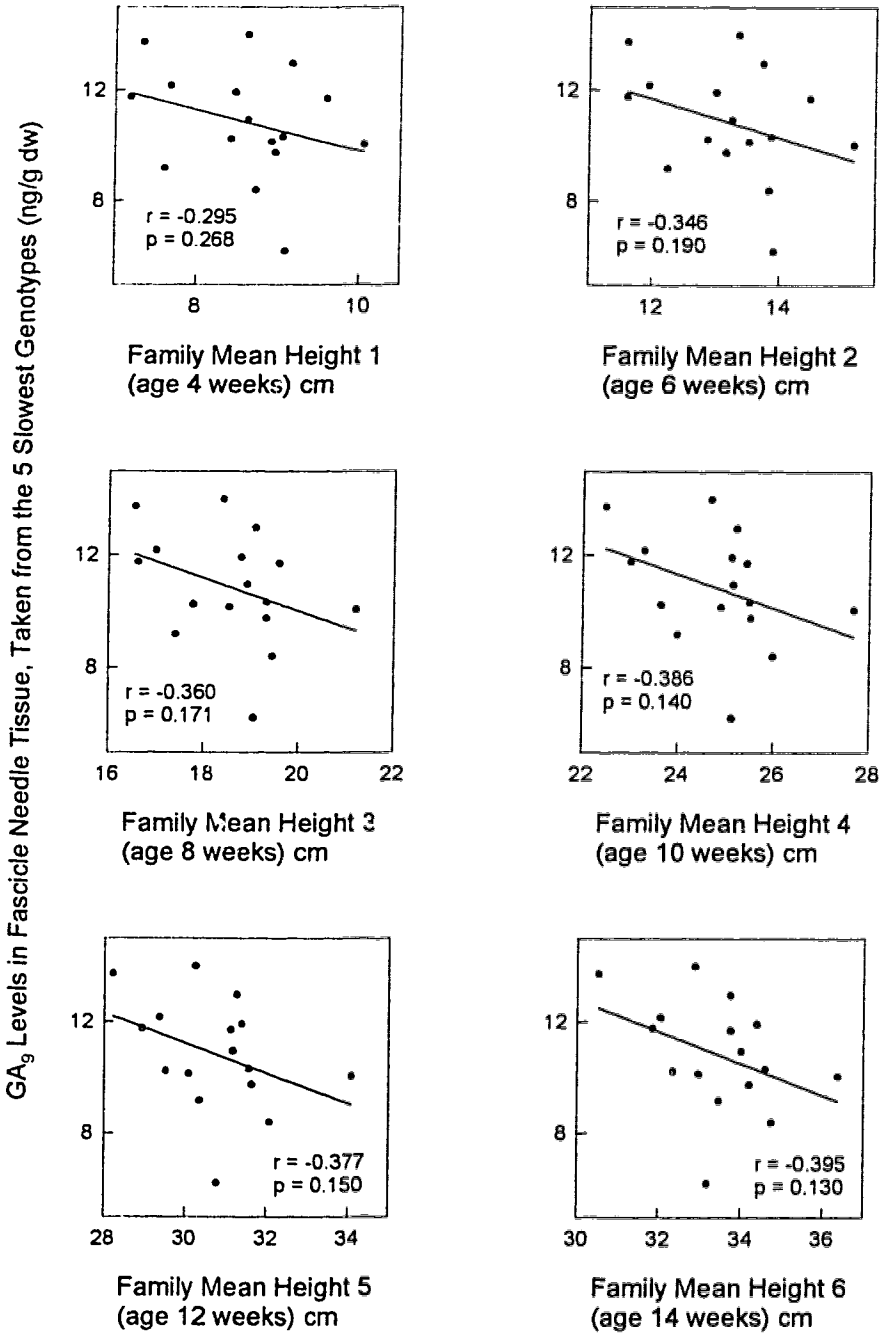
genotypes). The correlations were positive and highly significant ($p \leq 0.001$) (Fig. 5.5). Application of an outlier program at 2 SE did not change the correlations appreciably (Fig. 5.5). Thus, when family is ignored, GA_9 level is a highly significant correlate with larger seedlings having higher GA_9 levels. These results are consistent with a previous report that fast-growing Radiata pine genotypes tend to have higher GA_9 levels (Zhang, 1990). Also, fast-growing hybrid maize genotypes had higher GA_{19} plus GA_1 levels than their slow-growing inbred parental genotypes (Rood et al., 1983, 1988).

Height and Height Growth

Family means for GA_9 level in fascicle needles from 4-month-old seedlings were also assessed for their relationship with 4-month-old and earlier family mean heights. All correlations were negative and NS (data not shown) and use of the outlier program did not increase the correlations overall (data not shown). A similar exercise was accomplished for early seedling height growth, but no comparisons showed significant correlations even after use of the outlier program at 2 SE (data not shown). Use of the one-tail Spearman rank correlation test gave similar results (Table 5.4). Interestingly, the correlations of mean family GA_9 levels from age four month seedlings are highest with heights of older seedlings, as are also mean family primary needle GA_9 levels from age one month seedlings (Table 5.4).

Then the mean GA_9 levels of 4-month-old seedling needle tissue, grouped from the 5 slowest genotypes per family, were assessed for their relationship to

Figure 5.6 Radiata pine F_2, GA_9 levels in needle tissues taken from the 5 slowest genotypes of four-month-old seedlings compared with seedling heights at different ages - between families



family mean height for the greenhouse-grown seedlings. The correlations were all negative and NS (Fig. 5.6), but use of an outlier program at 2 SE improved 4 out of the 6 comparisons, one showing a significant correlation at $p \leq 0.05$ [final height (Ht 6, age 14 weeks)] (Fig. 5.7). Use of the one-tail Spearman rank correlation test gave similar strong and highly significant correlations of GA_9 with family mean for height (Table 5.5). Thus, the correlations were much better between 4-month-old needle GA_9 in the 5 slowest-growing seedlings and family mean heights at various earlier times, than were found for family mean GA_9, or for GA_9 in 1-month-old primary needles and subsequent seedling height.

The GA_9 levels in fascicle needles grouped from the 5 slowest-growing genotypes (seedlings) per family were then assessed by regression analysis for their relationship to height growth. No comparisons were significant (data not shown). However, after use of an outlier program at 2 SE all height growth:GA_9 correlations were appreciably improved, five becoming significant at $p \leq 0.05$ (Fig. 5.8). This same exercise was accomplished for the 5 fastest genotypes (seedlings) per family for each of height growth and actual height, but no significant correlations were found (data not shown).

The results for fascicle needle GA_9 levels and 4-month-old seedling height and height growth are consistent with the results for family mean correlations using 1-month-old seedling GA_9 (e.g. all height comparisons tend to be negatively correlated with GA_9 levels). Thus, seedlings in faster-growing families (re height) tend to have lower primary and fascicle needle GA_9 than seedlings in slower-

Figure 5.7 Radiata pine F_2, GA_9 levels in needle tissues taken from the 5 slowest genotypes of four-month-old seedlings compared with seedling heights at different ages (as per Fig. 5.6, only outlier removed at 2 SE) - between families

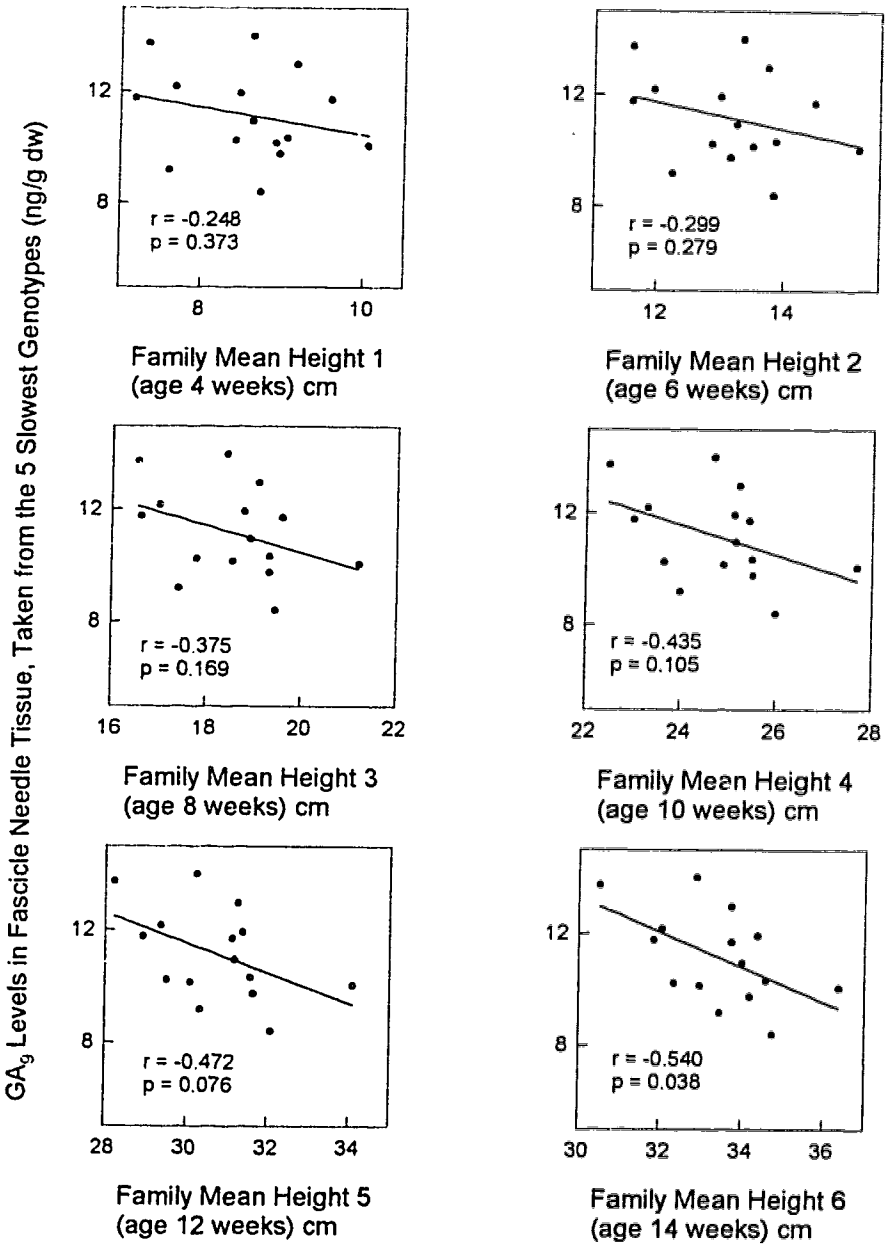
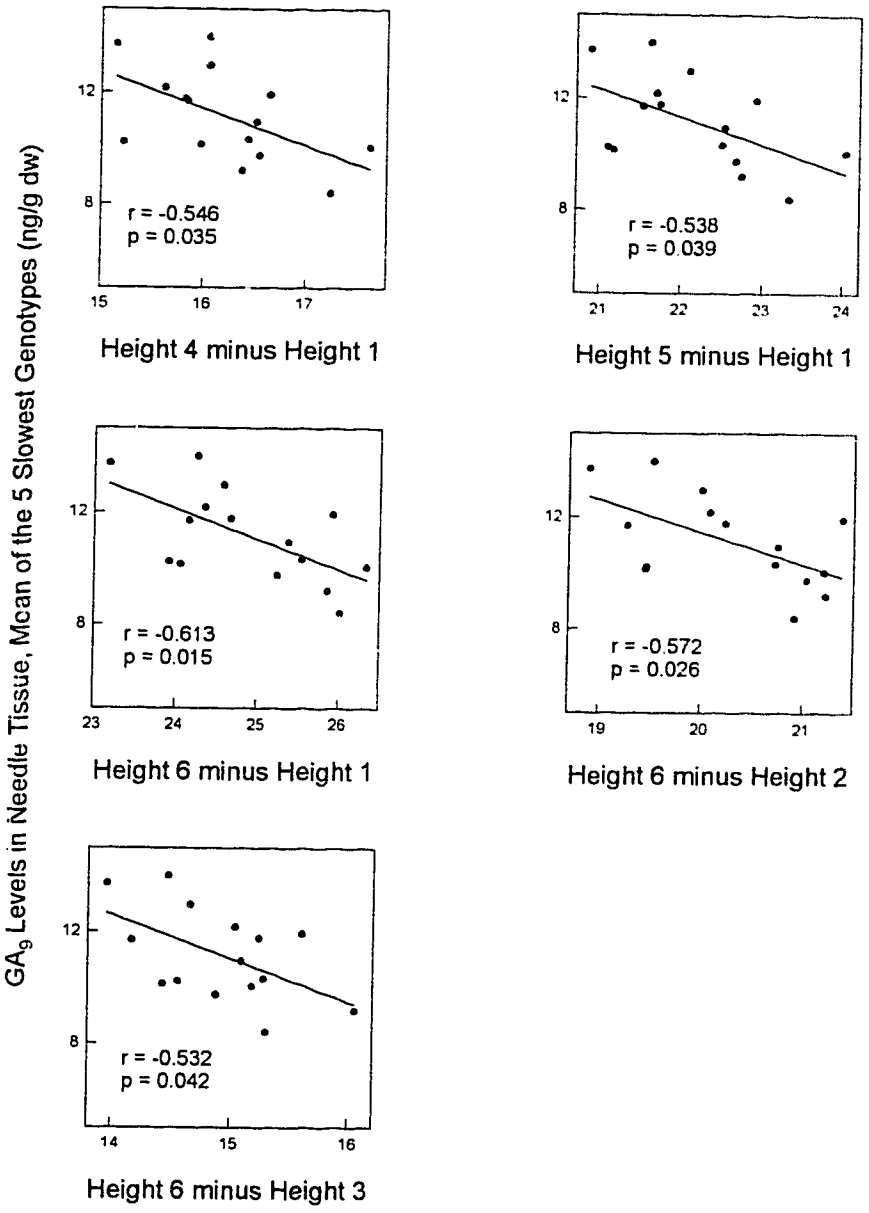


Figure 5.8 Radiata pine F_2, GA_9 levels in needle tissues taken from the 5 slowest genotypes of four-month-old seedlings compared with family mean seedling height growths at different ages (outlier removed at 2 SE) - between families



growing families. The low GA_9 levels in these faster-growing families could be the result of a faster metabolism of GA_9 to bioactive GAs such as GA_4 or GA_1.

Diameter and Diameter Growth

Family mean GA_9 levels in 4-month-old seedling needle tissues were assessed in relation to family mean for seedling diameters. All correlations were negative with p-values ranging from 0.198 to 0.329 (Fig. 5.9). When the comparisons were tested with the one-tail Spearman rank correlation test, the p-values of these negative correlations were 0.270, 0.279 and 0.111 (Table 5.4).

Then, the GA_9 levels in fascicle needles grouped from the 5 slowest-growing genotypes per family were assessed by regression analysis and by the one-tail Spearman rank correlation test in relation to family mean diameters of greenhouse-grown seedlings. Interestingly, all family mean diameter measurements showed negative and significant correlations with GA_9 levels in needles of these 5 slowest-growing seedlings (Fig. 5.10 and Table 5.5). Similar and significant correlations were seen for GA_9 levels and mean family stem volume (Table 5.6). Thus, as with height, it is GA_9 levels in fascicle needles of the very slowest seedlings which best predict mean family rank for these half-sib Radiata pine seedlings. However, mean family diameter growth for these seedlings was not significantly correlated with GA_9 levels (data not shown).

Finally, the GA_9 levels in fascicle needles, grouped from the 5 fastest genotypes per family, were assessed in relation to seedling diameter and diameter

Figure 5.9 Radiata pine F_2, family mean needle GA_9 levels of four-month-old seedlings compared with family mean seedling diameters at different ages - between families

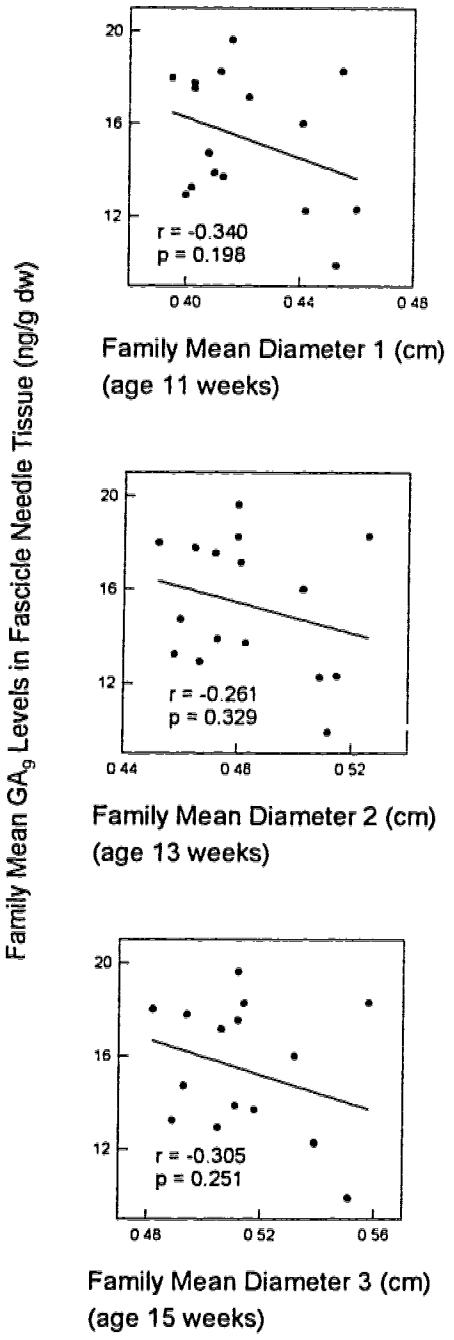


Figure 5.10 Radiata pine F_2, GA_9 levels in needle tissues taken from the 5 slowest genotypes of four-month-old seedlings compared with family mean seedling diameters at different ages - between families

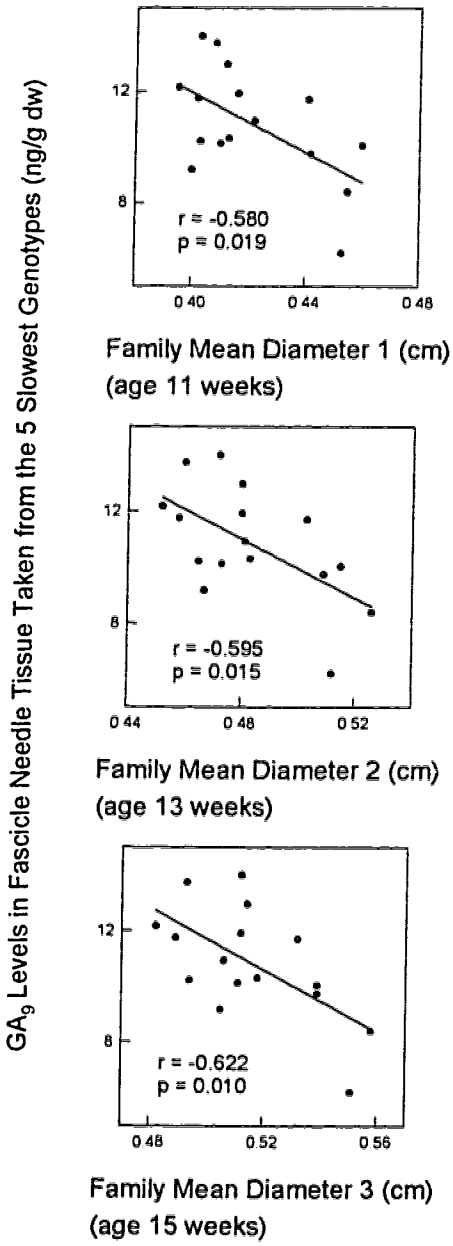


Table 5.6 One-tail Spearman Rank Correlation Coefficients for Various Seedling Gibberellin A_9 and Growth Parameters, Relative to Family Field Performance (dbh) on Each of 5 New Zealand Sites, and Across All 5 Sites for Radiata Pine. Probabilities with regard to Statistical Significance of these Correlation Coefficients are Given in Parentheses

Seedling Parameter	Taupo 85L	Thorp Rd	Rotoehu	Moerewa 2	Moerewa 1	NZ 5 Sites (Mean)
fam1mGA$_9$	-0.196(0.251)	-0.306(0.144)	-0.211(0.234)	-0.546(0.022)*	-0.427(0.064)	-0.333(0.104)
fam4mGA$_9$	0.020(0.473)	0.196(0.251)	-0.051(0.432)	0.015(0.479)	0.213(0.232)	0.097(0.360)
5s4mGA$_9$	-0.108(0.357)	-0.046(0.438)	-0.117(0.346)	-0.363(0.101)	-0.099(0.368)	-0.141(0.301)
1mtotalwt	0.459(0.049)*	0.235(0.209)	0.292(0.155)	0.262(0.183)	0.350(0.110)	0.335(0.102)
4mUpstem dw	0.311(0.139)	0.192(0.255)	0.351(0.109)	0.227(0.217)	0.492(0.037)*	0.422(0.052)
4mUpneedle dw	0.326(0.128)	0.361(0.102)	0.414(0.071)	0.295(0.153)	0.482(0.040)*	0.423(0.051)
4mtotalwt	0.381(0.090)	0.249(0.196)	0.387(0.086)	0.290(0.157)	0.440(0.058)	0.384(0.071)
4mtotalwt (2SE)	0.643(0.009)*	-	0.698(0.004)*	-	-	0.586(0.011)*
diameter1	0.672(0.004)*	0.444(0.056)	0.550(0.021)*	0.407(0.074)	0.663(0.005)*	0.573(0.010)*

Table 5.6 continued

diameter2	0.503(0.033)*	0.329(0.125)	0.421(0.067)	0.349(0.111)	0.495(0.036)*	0.458(0.037)*
diameter3	0.466(0.047)*	0.321(0.132)	0.382(0.089)	0.371(0.096)	0.423(0.066)	0.406(0.059)
mean3dia	0.563(0.018)*	0.467(0.046)*	0.406(0.075)	0.413(0.071)	0.607(0.011)*	0.500(0.024)*
height 3	0.374(0.094)	0.257(0.187)	0.381(0.090)	0.271(0.175)	0.427(0.064)	0.399(0.063)
height 4	0.358(0.104)	0.213(0.232)	0.363(0.101)	0.253(0.192)	0.446(0.055)	0.432(0.047)*
height 5	0.306(0.144)	0.257(0.187)	0.363(0.101)	0.257(0.187)	0.477(0.042)*	0.424(0.051)
height 6	0.301(0.148)	0.279(0.167)	0.354(0.107)	0.270(0.175)	0.481(0.041)*	0.459(0.037)*
mean3vol	0.459(0.049)*	0.354(0.107)	0.376(0.093)	0.301(0.148)	0.512(0.031)*	0.462(0.036)*
mean3vol(2SE)	-	-	-	-	0.681(0.005)*	0.600(0.009)*
ht3-ht1	0.367(0.098)	0.226(0.218)	0.354(0.107)	0.314(0.137)	0.543(0.022)*	0.447(0.041)*
ht4-ht1	0.220(0.225)	0.128(0.332)	0.238(0.207)	0.191(0.256)	0.477(0.042)*	0.316(0.120)
ht5-ht1	0.048(0.435)	0.035(0.452)	0.059(0.420)	0.130(0.329)	0.341(0.116)	0.133(0.312)
ht6-ht1	-0.007(0.491)	-0.046(0.438)	0.037(0.450)	0.130(0.329)	0.292(0.155)	0.118(0.332)

Table 5.6 continued

RGR(ht4-ht3)	-0.515(0.030)*	-0.325(0.129)	-0.515(0.030)*	-0.230(0.214)	-0.330(0.125)	-0.441(0.044)*
RGR(ht5-ht3)	-0.623(0.009)*	-0.391(0.083)	-0.514(0.030)*	-0.285(0.161)	-0.468(0.046)*	-0.477(0.031)*
RGR(ht6-ht3)	-0.423(0.066)	-0.398(0.079)	-0.474(0.044)*	-0.227(0.218)	-0.334(0.122)	-0.378(0.074)
RGR(vol3-vol1)	0.544(0.022)*	0.355(0.107)	0.421(0.067)	0.256(0.189)	0.612(0.010)*	0.408(0.058)

Abbreviation: fam1mGA_9 = family mean GA_9 levels in needle tissues of 1-month-old seedlings.

fam4mGA_9 = family mean GA_9 levels in needle tissues of 4-month-old seedlings.

5s4mGA_9 = GA_9 levels from 5 slowest-growing seedlings in each family.

1mtotalwt = family mean total shoot biomass dry weight of 1-month-old seedlings.

4mUpstem dw = family mean of upper stem dry weight from cotyledonary needle point to the tip of the shoot of 4-month-old seedlings.

4mUpneedle dw = family mean of needle dry weight along the upper stem of 4-month-old seedlings.

4mtotalwt = family mean total shoot dry weight of upper stem, needles along upper stem, and lateral branches of 4-month-old seedlings (above ground part).

4mtotalwt (2SE) = same as above but with one outlier family removed retrospectly at 2 SE by regression analysis of 4mtotalwt and field performance (dbh).

diameter1, 2, 3 = family mean stem diameters at ages 11, 13 and 15 weeks.

mean3dia = mean of the above 3 diameter measurements.

height 3, 4, 5, 6 = family mean seedling heights at ages 8, 10, 12 and 14 weeks.

mean3vol = mean of the 3 seedling stem volume measurements based on stem diameters and the last 3 height measurements (see above).

mean3vol (2SE) = same as above but with one outlier family removed early at 2 SE by regression analysis of the mean of the 3 seedling stem volume measurements with family mean needle GA_9 at age 1-month.

ht3-ht1 = height at age 8-weeks minus height at age 4-weeks.

.....

ht6-ht1 = height at age 14-weeks minus height at age 4-weeks.

RGR = relative growth rate.

* = significant at $p \leq 0.05$.

growth. The correlations were NS, but they were relatively high for seedling diameter (e.g. p-values of 0.104, 0.136 and 0.185; data not shown).

Thus, for early seedling height, height growth, diameter, diameter growth and stem volume, the trends for GA_9 level between families are the same as those shown for family mean GA_9 by 1-month-old seedlings. That is, the fast-growing families have or tend to have lower needle GA_9. In contrast, within families (Fig 5.4) or across all families (Fig. 5.5), the fast-growing genotypes (seedlings) have higher GA_9 levels.

Thus, a reduced level of GA_9 in fascicle needles is a good marker for identifying fast-growing families at the seedling stage, although significance is gained only if one uses the GA_9 levels from 5 slowest-growing genotypes (seedlings) within each family as markers (Figs 5.8 and 10 and Table 5.5). The reason why fast-growing families have a lower GA_9 level may be that they have a more rapid metabolism of GA_9 to a bioactive growth GA, such as GA_4 or GA_1. Such a trend would be consistent with the situation for certain of the dwarf pea and maize genotypes, where the slow-growing dwarf genotypes, which are severely blocked in their GA metabolism, contain more precursor GA, e.g. GA_{20}, and less growth-active GA_1 than normal genotypes (Phinney et al., 1986; Phinney and Spray, 1990; Ingram, et al., 1984; Reid, 1990).

The observation that within these half-sib families GA_9 levels are positively correlated with seedling growth, while among families GA_9 levels are negatively correlated with family growth (at the seedling stage), could suggest that when the

seedlings are genetically closely related, their GA_9 levels are more likely to reflect their growth in a positive manner. However, when the seedlings are not closely related (i.e. when comparing family means from different open-pollinated families) their GA_9 levels tend to be negatively correlated with growth. Along the same line, perhaps, because GA metabolism is likely regulated at multiple steps, optimal environmental conditions have more of an effect on GA metabolism for diversified genotypes (e.g. different families), than for more uniform genotypes (e.g. within any one family). Another possibility is that the within family component is mainly explained by non-additive genetic variance, while the among family component is mainly due to additive genetic variance. Heritability analysis suggests that GA_9 level has a significant non-additive component in nature (Dr. F. C. Yeh, personal communication).

5.2.3 Early Seedling Growth in Relation to Field Progeny Trial Performance

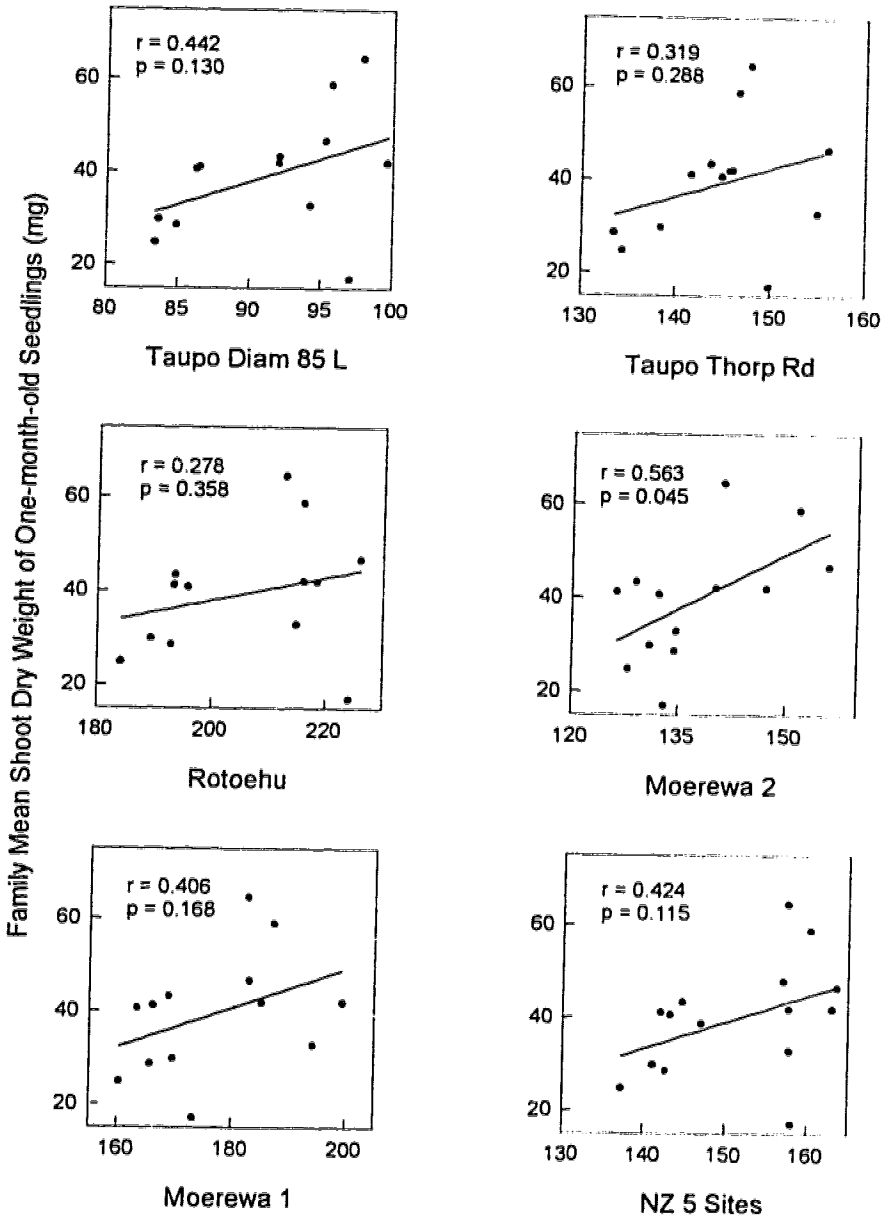
Age One-Month Seedlings

Biomass

Family means for hypocotyl dry weight and stem plus primary needle dry weight of 1-month-old seedlings were assessed in relation to field performance at ages 4.5 - 7.5 years (dbh). All correlations were positive, but NS (data not shown).

Then, family mean for total seedling dry weight (all above ground parts) at age one month was assessed for its relationship with field performance (dbh). As

Figure 5.11 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean shoot dry weight of one-month-old seedlings (outlier removed in retrospect at 2 SE)



above, the correlations were positive, but NS (data not shown). Retrospective application of the outlier program at 2 SE improved all correlations, with Moerewa 2 becoming significant at $p \leq 0.05$ (Fig. 5.11). Thus, for the majority of families (13/14 or 15/16), very early seedling growth (family mean seedling biomass) does reflect later field performance. Such results are consistent with those reported by Waxler and van Buijtenen (1981) for loblolly pine, where seedling shoot weights at age 24-weeks were significantly correlated with field performance in terms of average stem volume superiority, by Williams et al. (1987) where 6-month-old seedling biomass was significantly correlated with 12-year-old tree heights for black spruce, and by Mullin and Park (1994) where 25-week-old black spruce seedling biomass was significantly correlated with field heights at ages 5 and 10 years.

Age Four-Month-Old Seedlings

Biomass

First, family mean upper stem dry weights (cotyledonary needle point to tip of stem) of 4-month-old seedlings were assessed in relation to field performance (dbh). All correlations were positive, but NS (data not shown). Retrospective use of an outlier program at 2 SE removed one family, causing three correlations (Moerewa 1, Moerewa 2 and NZ 5 Sites) to become significant at $p \leq 0.05$ (Fig. 5.12).

Family mean needle dry weights (along the main stem) of 4-month-old seedlings were then assessed for their relationship with field performance (dbh).

Figure 5.12 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean upper stem dry weight of four-month-old seedlings (outlier removed in retrospect at 2 SE)

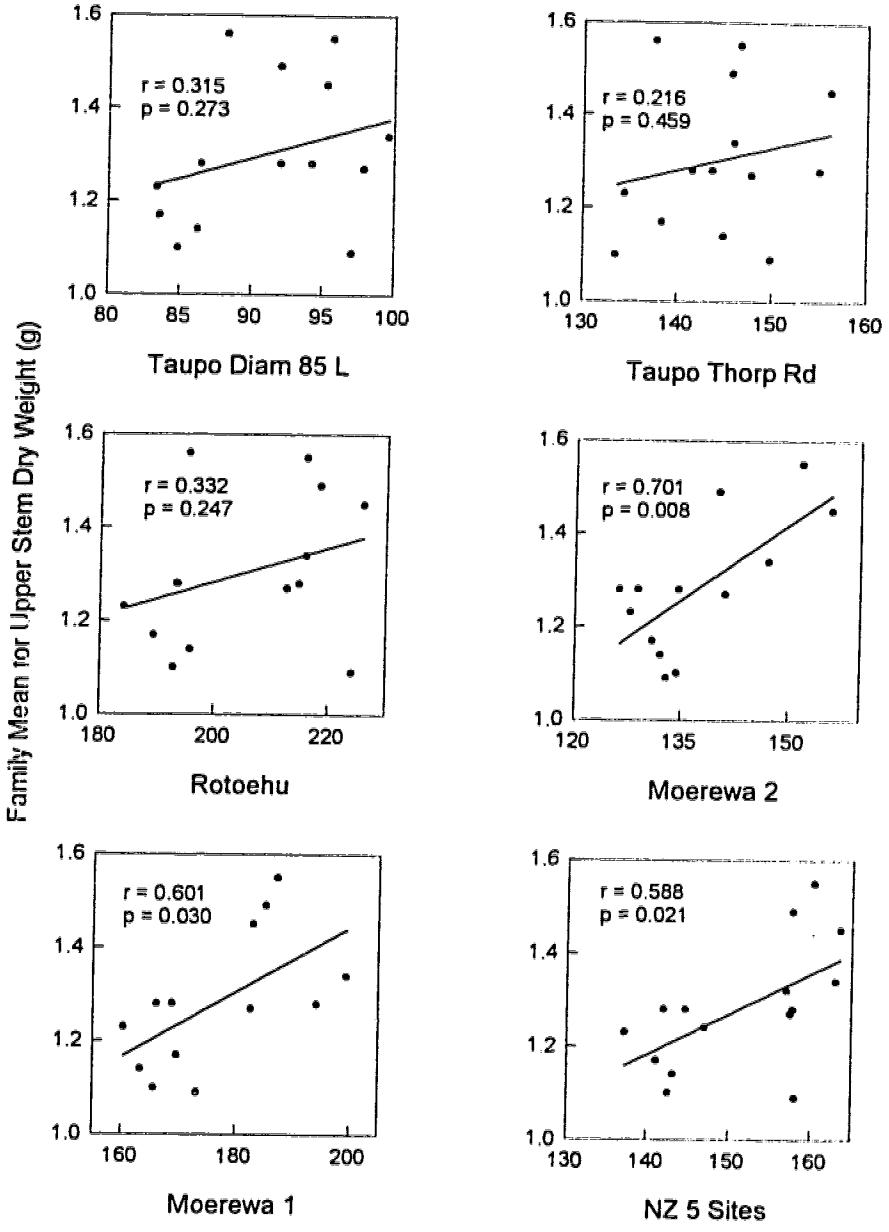
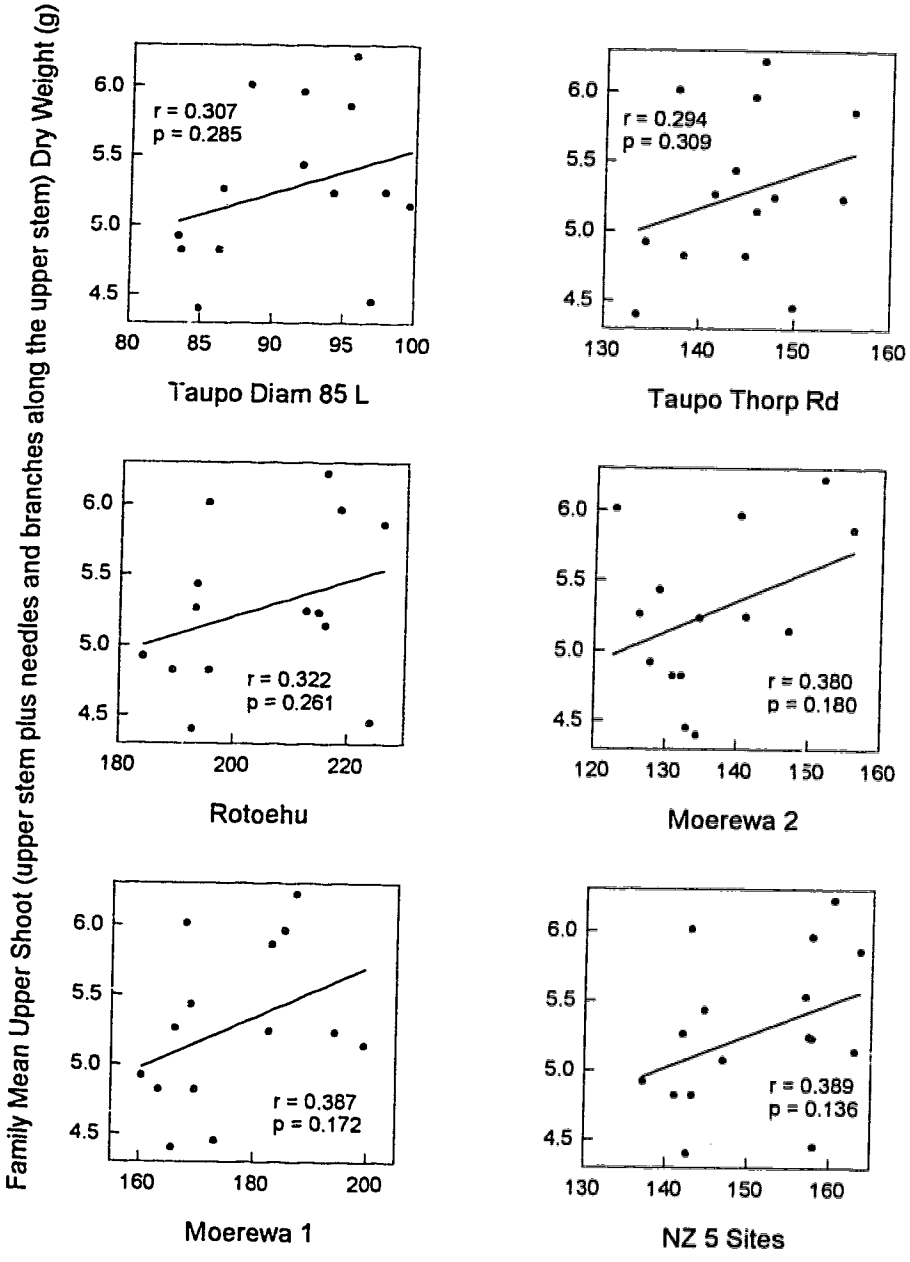


Figure 5.13 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean upper shoot dry weight of four-month-old seedlings



All correlations were positive but NS (data not shown).

Finally, family mean upper shoot dry weights of 4-month-old seedlings (i.e. upper stem plus needles and lateral branches along the upper stem) were assessed for their relationship with field performance (dbh). All correlations were positive, with p-values ranging from 0.309 to 0.136 (Fig. 5.13).

Thus, the correlations of 4-month-old seedling biomass with field performance showed a similar trend as that of 1-month-old seedling biomass; both gave positive, non-significant correlations unless an outlier program at 2.0 SE was utilized retrospectively.

Height and Height Growth

Heights of the greenhouse-grown seedlings were measured six times at 2-week intervals. Thus, family means for seedling height at age 4 and 6 weeks were assessed for their relationship to field performance (dbh). All correlations were positive, but NS (data not shown). After retrospective use of an outlier program at 2 SE, all correlations were improved for each of heights at week 4 and 6, e.g. for height at week 6 the p-values ranged from 0.070 to 0.246 (data not shown).

Family mean heights at ages 8, 10, 12 and 14 weeks were also assessed for their relationship with field performance (dbh). The correlations for each of these later heights were positive but not significant (data not shown). However, retrospective use of an outlier program at 2 SE caused comparisons on all but Rotoehu site to become significant at $p \leq 0.05$ for a majority of families (e.g. see

Figure 5.14 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean seedling heights at age 14 weeks (outlier removed in retrospect at 2 SE)

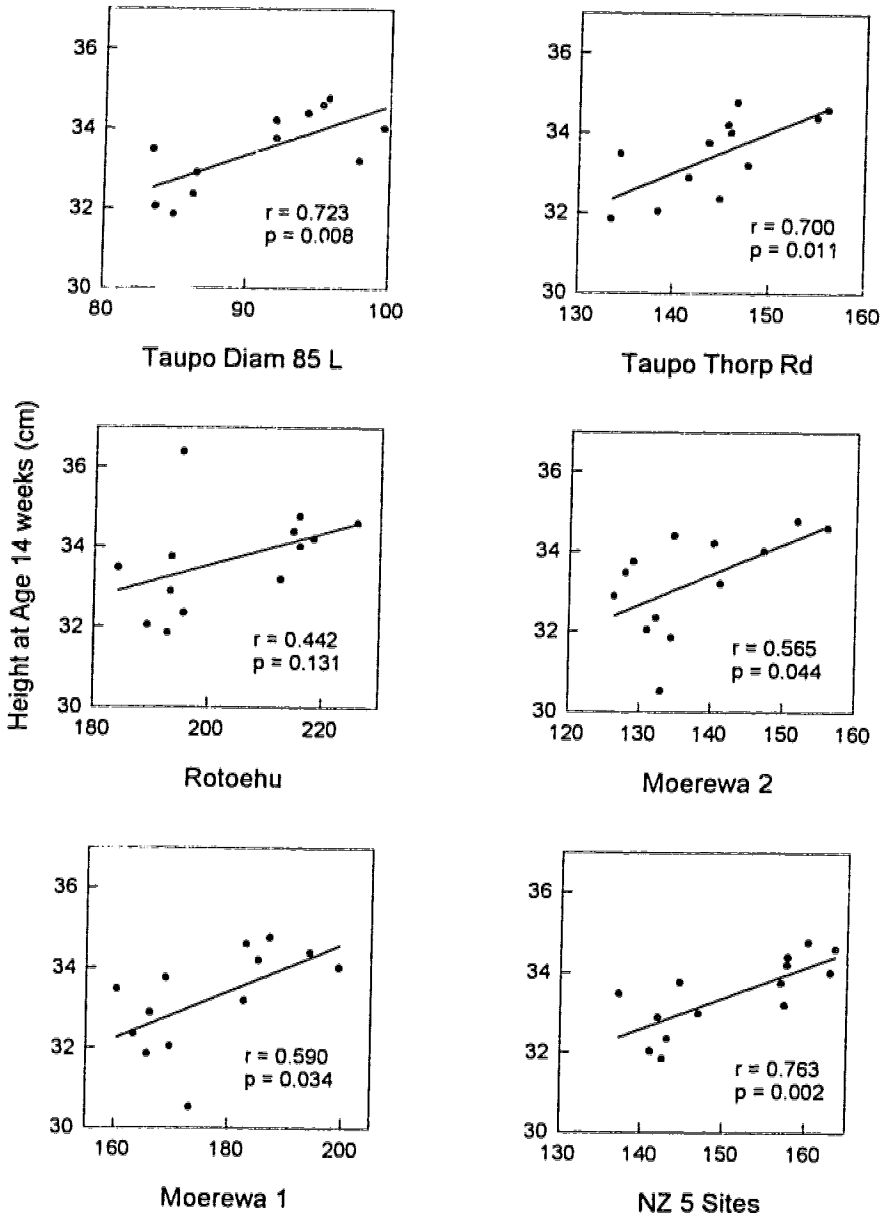


Figure 5.15 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean seedling height growth from ages 4 to 8 weeks (outlier removed in retrospect at 2 SE)

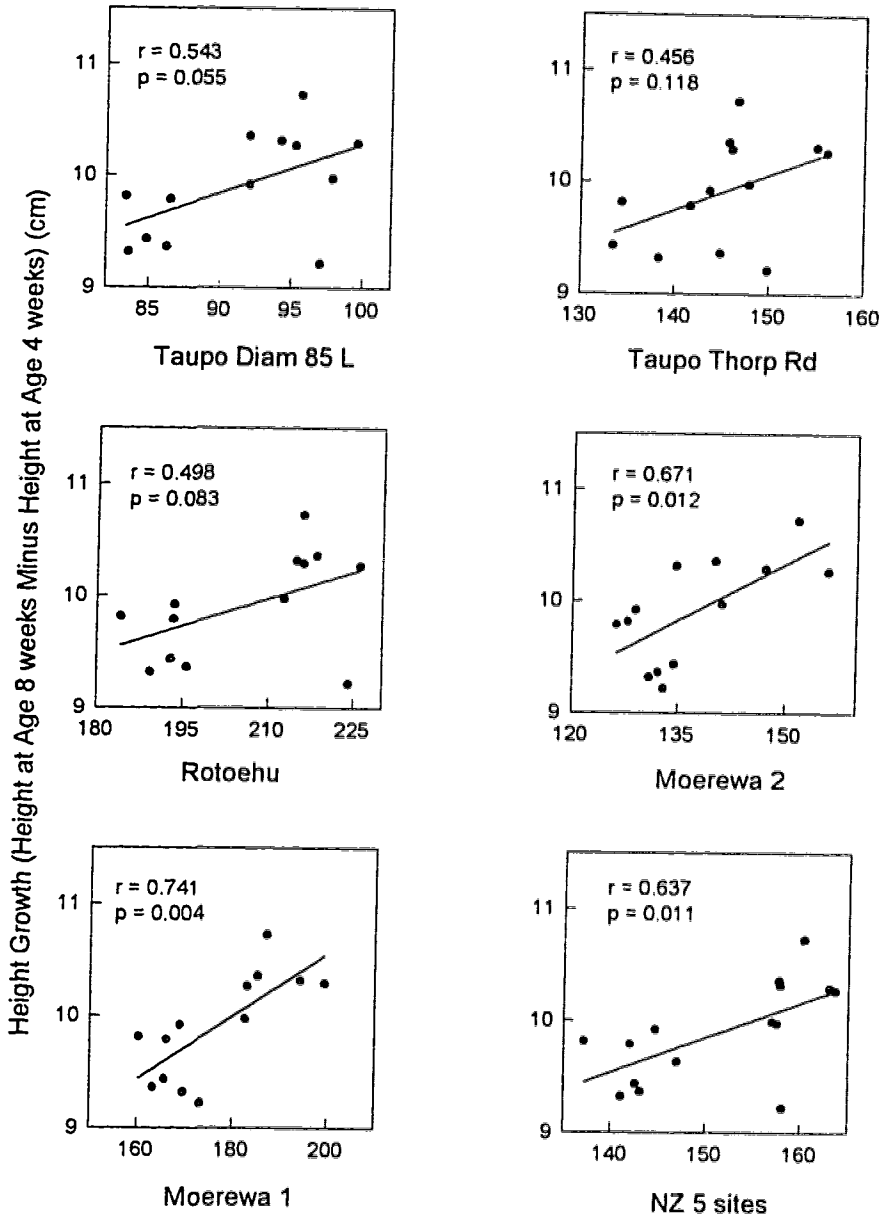


Figure 5.14 for height at age 14 weeks).

Family mean height growth between age 4 weeks and 8 weeks was then assessed in relation to field performance (dbh). All correlations were positive with p-values ranging from 0.090 (on Moerewa 1 site) to 0.531 (on Taupo Thorp Rd site) (data not shown). After retrospective use of an outlier program at 2 SE all correlations were improved, three becoming significant at $p \leq 0.05$ (Fig. 5.15). Other height growth intervals were also assessed, but none gave correlations of height growth to field dbh as good as the age 4-8 week interval.

Thus, older seedling height and height growth tended to be better indicators for later field performance than was age four month seedling biomass (Figs. 5.13 - 5.15, versus Fig. 5.12).

Diameter and Diameter Growth

Family mean diameters for age 11-week seedlings (diameter 1) were assessed first for their relationship with field performance (dbh). All correlations were positive with p-values varying from 0.073 and 0.078 to 0.551 (data not shown). Retrospective application of an outlier program at 2 SE significantly improved all correlations, five out of six becoming significant at $p \leq 0.05$ (Fig. 5.16). Similar, but NS trends were noted for family mean diameter of age 13 and 15 week seedlings (diameters 2 and 3) (data not shown).

The means of the three seedling diameter measurements (ages 11, 13 and 15 weeks) were also assessed for their relationship with field performance. All

Figure 5.16 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean seedling diameter at age 11 weeks (outlier removed in retrospect at 2 SE)

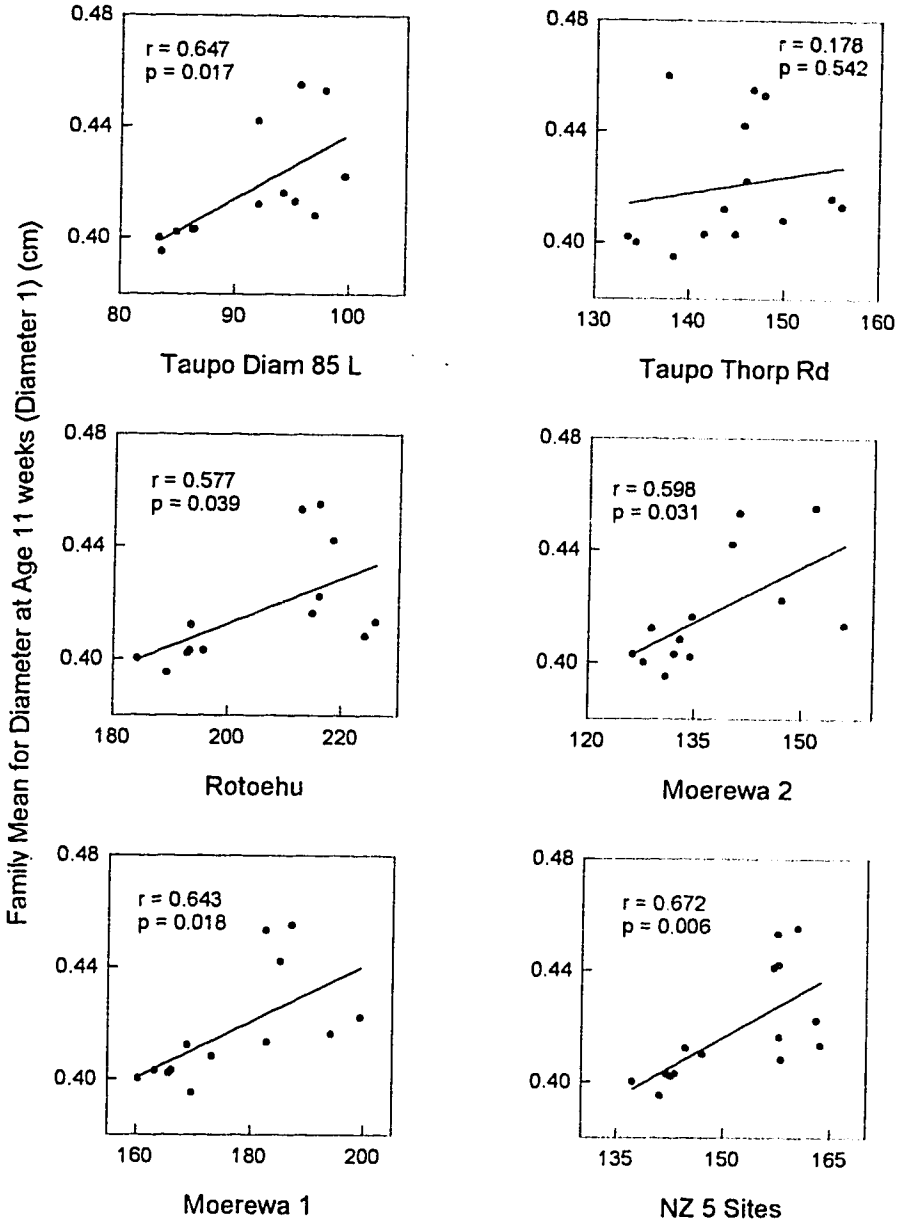
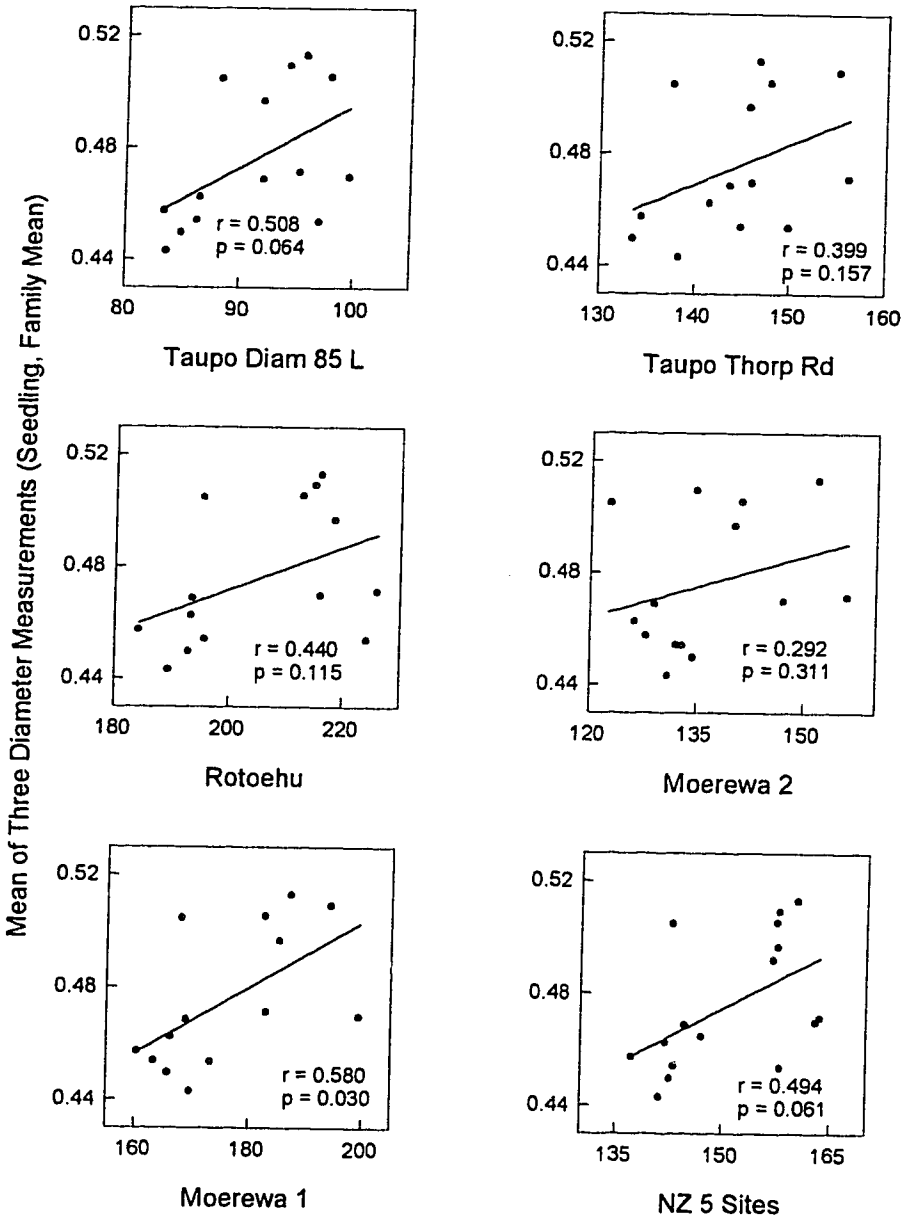


Figure 5.17 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean seedling diameter (mean of 3 diameter measurements)



correlations were positive with one comparison being significant at $p \leq 0.05$ and two reaching near significance at $p \leq 0.06$ (Fig. 5.17).

Next, family mean diameter growth was assessed for its relationship with field performance (dbh). No significant correlation was found (data not shown).

Thus, like seedling height, seedling diameter is a good indicator for later field growth, especially for the majority of the families (e.g. when one outlier family is removed).

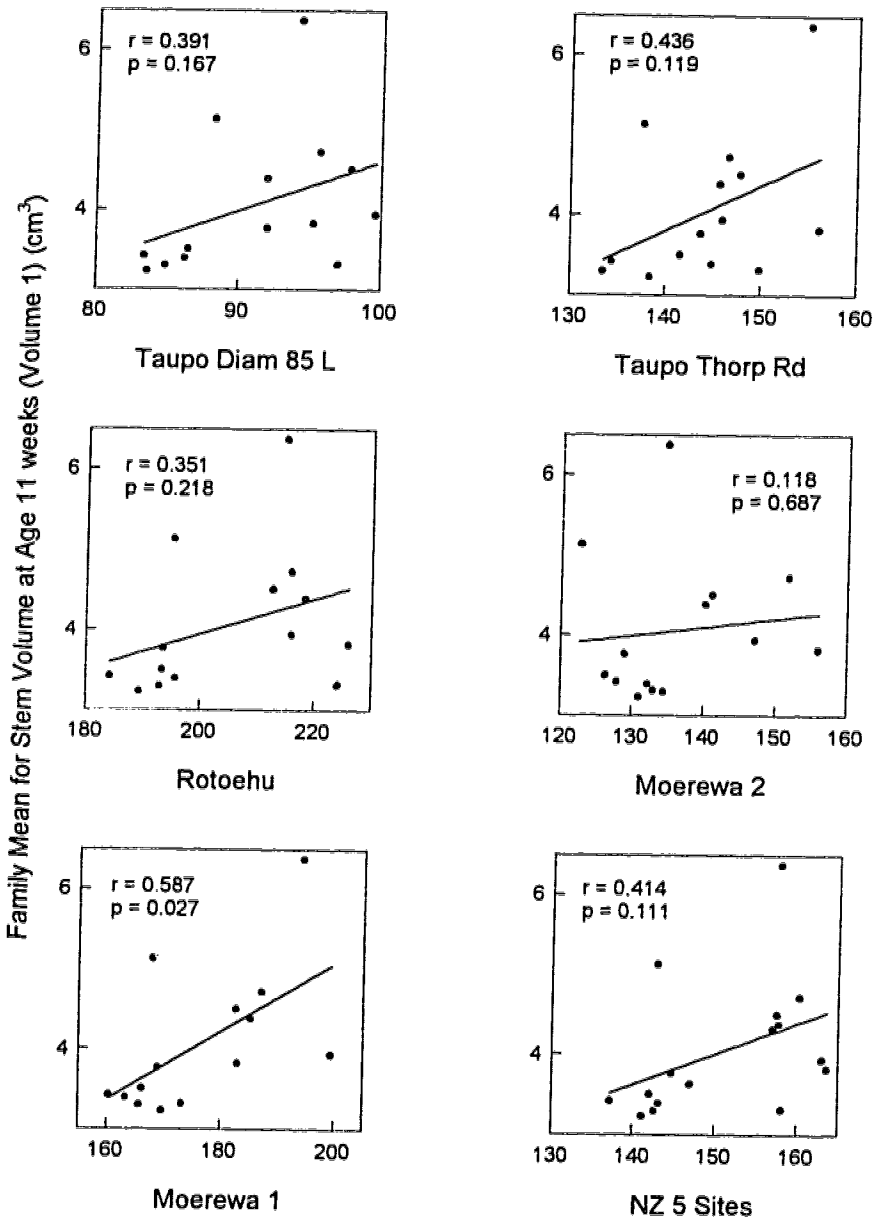
Volume

Seedling stem volumes were estimated as stem cylindrical volume using seedling diameter and height. Since the data for diameter and height were not taken at the same week, the heights used for volume one and two were the average of the two heights measured before and after that diameter, while the height used for volume three was measured the week before that diameter measurement (i.e. the very last height measurement).

Family means for stem volumes at age 11 weeks (volume 1) were assessed for their relationship with field performance (dbh). All correlations were positive with one being significant at $p \leq 0.05$ (Moerewa 1, Fig. 5.18).

Family means for stem volumes at age 14 and 16 weeks (volume 2 and 3) were then assessed in relation to the field performance (dbh). All correlations were positive, but none were significant without use of an outlier program at 2.0 SE (data not shown).

Figure 5.18 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean stem volume at age 11 weeks



Finally, the mean of the 3 stem volumes were assessed in relation to the field performance (dbh). As above, all correlations were positive, but NS unless an outlier program at 2.0 SE was utilized (data not shown).

Relative Growth Rate (RGR)

Relative growth rates were calculated for various stem height, diameter, and volume measurement intervals based on the following equation:

$$RGR = \frac{\ln m2 - \ln m1}{t1 - t2}$$

RGR = relative growth rate.

m1 = the parameter measured at time 1.

m2 = the parameter measured at time 2.

t1 = time 1.

t2 = time 2.

The RGR values were then compared with family mean seedling needle GA_9 levels (age 1 month and age 4 months), and with family mean performance in the field, significance being tested by use of the one-tail Spearman rank correlation test (Tables 5.4 and 5.6 and Appendix 5).

Neither GA_9 levels (family mean) of primary needles from seedlings at age 1 month nor GA_9 levels of fascicle needles from seedlings at age 4 months showed significant correlations (for family means) in the one-tail Spearman rank test with early or late RGRs for seedling height, diameter and volume (Table 5.4). However,

GA_9 levels of primary needles of age one month seedlings from the first subset (biggest seedlings) were significantly correlated with RGR for four out of the six height intervals and showed near significance for RGR for diameter interval 2 to 3 (Table 5.4).

The correlations of seedling RGR with field performance were negative, but many of the RGRs showed significance or near significance (Table 5.6). In fact, RGR for height yielded better correlations with family field performance than height growth per se, and similar correlations to later measurements of absolute heights (Table 5.6).

Similarly, seedling stem volume RGRs were also compared with family field performance, and the strength of the RGR correlations (stem volume measurement interval 1 to 3) was similar to that for absolute stem volume (Table 5.6).

However, when seedling stem diameter RGRs were compared with family field performance, no significant correlations were observed, unlike seedling stem diameters per se, which were highly and significantly correlated with family field performance (Table 5.6).

Hence, it appears to be the shoot elongation component of seedling relative growth rate (RGR) which is most strongly related to mean family field performance for Radiata pine.

Early Application of an Outlier Program for Correlation with Field Performance

Another approach in using an outlier program is to apply it in a non-

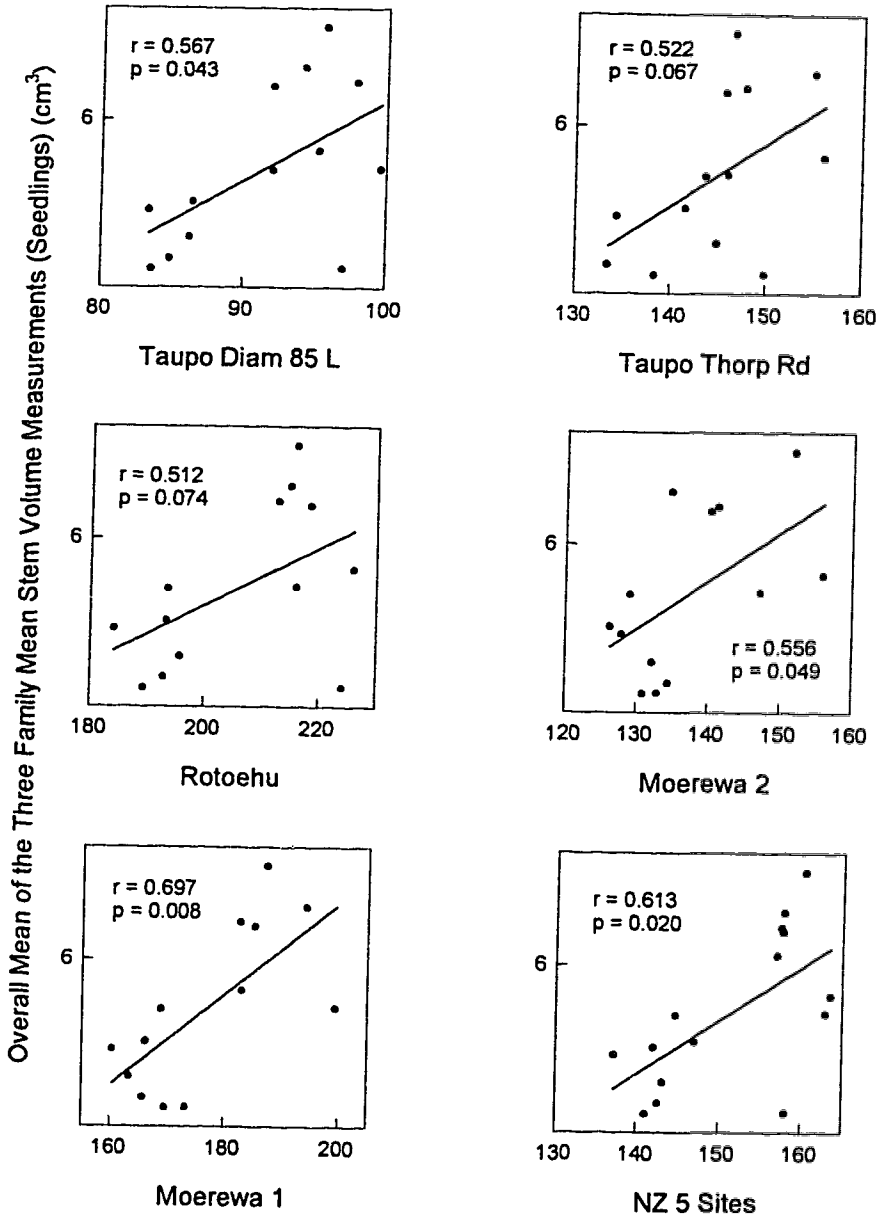
retrospective manner (i.e., apply it "early" by regression analysis of 4-month-old needle GA_9 and various seedling growth parameters, as detailed in the beginning of section 5.2).

For example, when "early" use of an outlier program at 2 SE was accomplished for primary needle (1-month-old seedling) GA_9 levels and family mean stem volume (mean of measurements at weeks 12, 14 and 16), family 25 was identified as an outlier. The remaining families were then assessed for family mean stem volume in relation to field performance (dbh). All correlations were positive with four being significant at $p \leq 0.05$ and the other two near-significant (Fig. 5.19).

The "early" use of an outlier program was also applied at 2 SE for seedling final height, needle dry weight, upper stem dry weight, needle plus upper stem dry weight and 4-month-old needle GA_9 levels. However, this exercise did not yield significant correlations between the remaining families and field performance (data not shown).

In summary, the relationship between seedling early growth parameters and field performance is consistent, with all comparisons showing positive correlations. On most field sites, and especially for NZ Average across 5 sites, the comparisons for seedling stem height, stem diameter and stem volume with field performance are significant if one uses an outlier program at 2 SE, applied "early" (Fig. 5.19) or in retrospect to remove one outlier family. Also, in general, the retrospective use of an outlier program at 2 SE gives significance for many early growth comparisons on most field sites. For older (age four months) seedlings the growth parameters

Figure 5.19 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with mean of the 3 family mean stem volume measurements (family 25 removed as an "outlier" at 2 SE "early")



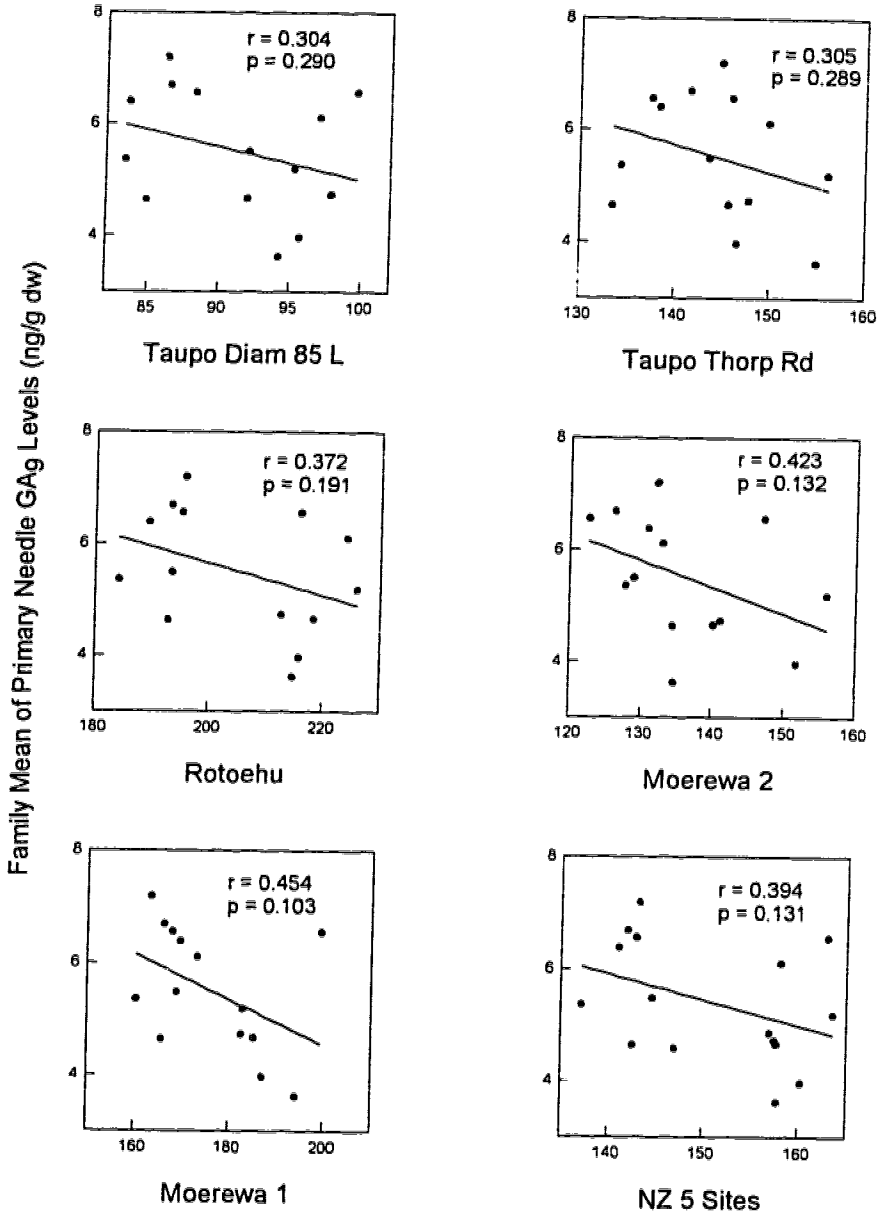
that are most closely correlated with field performance (dbh) are seedling stem height, stem diameter and stem volume, especially at the later measurement dates (ages 8 weeks to 14 weeks). These results are consistent with previous reports. For example, stem volumes of phytotron-grown Radiata pine seedlings were positively and significantly correlated with field rank at age 9 (Pharis et al., 1992). Diameters of 5-year-old Radiata pine were positively and significantly correlated with those of 17-year-old trees (King and Burdon, 1991). And, Sulzer et al. (1993) reported that seedling diameters of 3-year-old black spruce were significantly correlated with 10-year-old tree height. Finally, the heights of 4-year-old half-sib jack pine were positively correlated with 12-year field height (Magnussen and Yeatman, 1986) and the height at the end of the second growing season was positively correlated with 7-year field height for jack pine (Carter et al., 1990).

5.2.4 GA_9 in Primary Needles of One-Month-Old Seedlings in Relation to Field Progeny Trial Performance

The family means for GA_9 levels in primary needle tissue of 1-month-old seedlings were compared with mean family field performance (dbh). All comparisons were negative, with p-values ranging from 0.103 to 0.290 (Fig. 5.20). The best comparison was found on a fertile Northland site (Moerewa 1) and the poorest was found for the Taupo Diam 85L site.

Consistent with earlier results where family means for GA_9 levels at age one

Figure 5.20 Radiata pine F_2, ages 5-8 years dbh (mm) on each of 5 NZ sites and for the average of those sites (NZ 5 sites) compared with family mean of primary needle GA_9 levels



month were related to subsequent seedling early growth parameters (e.g. height, stem dry weight, stem diameter and stem volume), needle GA_9 tends to be inversely correlated with field performance (Fig. 5.20). Thus, as mentioned above, the higher needle GA_9 found in slow-growing families may result from a slower metabolism of GA_9 to downstream GAs, such as GA_4 and GA_1 or to a reduced ability of slow-growing families to translocate GA_9 from the needle to the growing stem.

5.2.5 GA_9 Levels in Fascicle Needles from Four-Month-Old Seedlings in Relation to Field Progeny Trial Performance

Family means for GA_9 levels in fascicle needles of 4-month-old seedlings were assessed in relation to field performance (dbh). No significant correlation was found even after a retrospective application of an outlier program at 2 SE (data not shown).

Then GA_9 levels in fascicle needles of 4-month-old seedlings from the 5 fastest growing genotypes for each family were assessed in relation to family mean field performance (dbh). The comparisons gave weak correlations and varied from site to site with regard to slope (positive or negative) (data not shown).

Finally, the GA_9 levels in 4-month-old seedling fascicle needle tissues, grouped from the 5 slowest growing genotypes per family were assessed for their relationship with family field performance (dbh). All comparisons showed weak negative and NS correlations (data not shown).

Thus, unlike GA_9 level in primary needles of 1-month-old seedlings, no consistent correlations were found between GA_9 level in fascicle needles (age 4-month-old seedlings) and field performance for Radiata pine. Thus, in this study using half-sib families, GA_9 level per se in fascicle needles from 4-month-old seedlings is not a good direct indicator of later field performance at the family level.

5.2.6 GA_9 Levels In Relation To Field Progeny Trial Performance -- A Second Order Comparison

Because of the interesting and significant "second order" correlations found for coastal Douglas-fir needle GA_9 levels and field progeny trial performance (see Section 5.3.4), a similar exercise was performed for Radiata pine. Thus, within each of the 16 families a slope was obtained from the regression of GA_9 level with upper stem dry weight (see slopes, shown in Fig. 5.4). These slopes (all positive) were then assessed for their relationship with field progeny trial performance (dbh) in the same manner shown for coastal Douglas-fir (Figs. 5.30 and 5.33). This is termed a "second order comparison".

A similar exercise was performed for fascicle needle GA_9 and needle dry weight along the main stem, the mean of the three seedling diameter measurements, and final seedling height, within each of the 16 families. However, unlike coastal Douglas-fir, none of the correlation coefficients were statistically significant (data not shown), even after retrospective use of an outlier program at

2 SE (data not shown).

5.2.7 Spearman Rank Correlation Test

In addition to the linear regression analysis, an one-tail Spearman rank correlation test was performed by SPSS (SPSS for Windows, Release 6.1) to test the correlations between the various early parameters, such as needle GA_9 level or stem diameter, and the field performance (dbh). All correlation coefficients and p-values are presented in Table 5.6.

For GA_9 measurements, the correlation with field performance across NZ 5 Sites varied from negative ($r = -0.333$) for family mean needle GA_9 level of 1-month-old seedlings to positive ($r = 0.097$) for family mean needle GA_9 level of 4-month-old seedlings. Among the needle GA_9 comparisons with field performance on the 5 different NZ sites the best correlation ($r = -0.546$, $p = 0.022$) was found with the Moerewa 2 site (this Northland site is subjected to high temperatures and has a relatively poor nutrient status, R. Burdon, personal communication with R.P. Pharis). The correlation on Moerewa 1 (which is also a high temperature site, but with a better nutrient status, ibid) was also relatively high ($r = -0.427$, $p = 0.064$) (Table 5.6). However, correlations with age one month needle GA_9 and family field performance were poorer for the more optimal field sites (Table 5.6).

For seedling biomass measurements, the correlation with field performance across NZ 5 Sites was better ($r = 0.384$) for family mean upper shoot dry weight of

4-month-old seedlings than for shoot dry weight of 1-month-old seedlings (r = 0.335). On four out of the five NZ sites, the biomass of 4-month-old seedlings was more strongly correlated with field performance than the biomass of 1-month-old seedlings. Among these correlations, the best (r = 0.459) was found on site Taupo Diam 85L for shoot dry weight of 1-month-old seedlings and on site Moerewa 1 for upper shoot dry weight of 4-month-old seedlings.

For the three diameter measurements, the strongest correlation (r = 0.573) with field performance across 5 NZ sites was the first diameter measurement at age 12-weeks (diameter 1). Among the correlations with field performance on the 5 different NZ sites, the strongest correlation was found on site Taupo Diam 85L for all diameter measurements. On each site, the strength of the seedling diameter correlation with field performance decreased with each successive diameter measurement (i.e. the earlest diameter measurement was best).

In contrast, for height measurements, the strength of the correlation with field performance across 5 NZ sites increased with each successive measurement. For each height measurement, the strongest correlation with field performance was found on site Taupo Diam 85L for heights at age 4 and 6 weeks and on site Moerewa 1 for heights at ages 8, 10, 12, and 14 weeks.

For height growth measurements, the best correlation (r = 0.447) with field performance across 5 NZ sites was found for height growth between ages 4 and 8 weeks. Among the 5 different NZ sites, the better positive correlations were mainly found on site Moerewa 1 and among these correlations and the best ones were

between ages 4 and 8 weeks (r = 0.543), or ages 6 and 8 weeks (r = 0.596).

In summary, seedling early growth parameters were more consistent and better indicators for later field performance of Radiata pine than were first- or second-order needle GA_9 levels. Among the seedling early growth parameters, stem diameter best reflected field performance. Interestingly, the strength of the correlation between diameter and field performance decreased with each successive measurement, while the strength of the correlation between height and field performance increased with each successive measurement. Generally, seedling early growth parameters were better correlated with field performance across NZ 5 sites, and on sites Moerewa 1 and Taupo Diam 85L.

5.3 Coastal Douglas-fir

5.3.1 GA_9 Level In Relation To Early Seedling Growth

Within each of the 16 families, GA_9 levels in needle tissues at harvest were first assessed in relation to individual seedling final heights. The correlations varied from negative (6) to positive (9), with four families exhibiting significant or near-significant trends for needle GA_9 and seedling height (Fig. 5.21).

The GA_9 levels in needle tissues were then assessed for their relationship with final seedling stem dry weight (the 10 genotypes utilized were chosen so that a representative range of stem weights was present for each of the 16 families). Comparisons for 12 out of 16 families had positive correlations, with three of these being significant at $p \leq 0.05$ (Fig. 5.22).

The GA_9 levels in needle tissues within each of the 16 families were then assessed in relation to seedling final needle dry weight. Among the 16 families, 11 correlations were positive, but only one out of the 11 positive correlations was significant at $p \leq 0.05$ (Fig. 5.23).

Finally, GA_9 levels in needle tissues within each of the 16 families were assessed for their relationship with seedling diameters (measured at ca. 3 cm above the basal swelling). Only three of the 11 positive correlations were significant at $p \leq 0.05$ (Fig. 5.24).

Thus, in contrast to the results for 4-month-old Radiata pine half-sibs, no

Figure 5.21 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling heights - within families

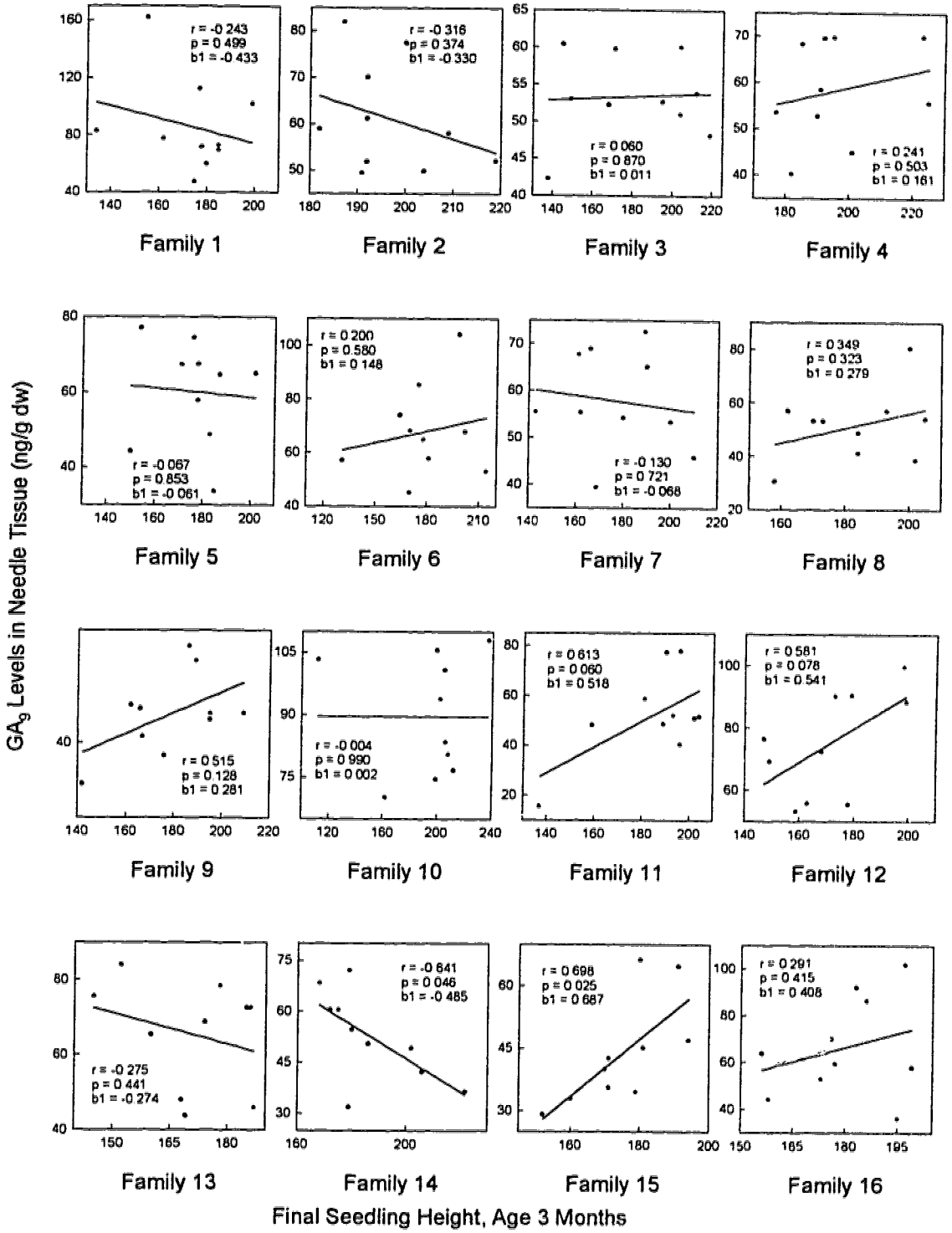


Figure 5.22 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling stem dry weights - within families

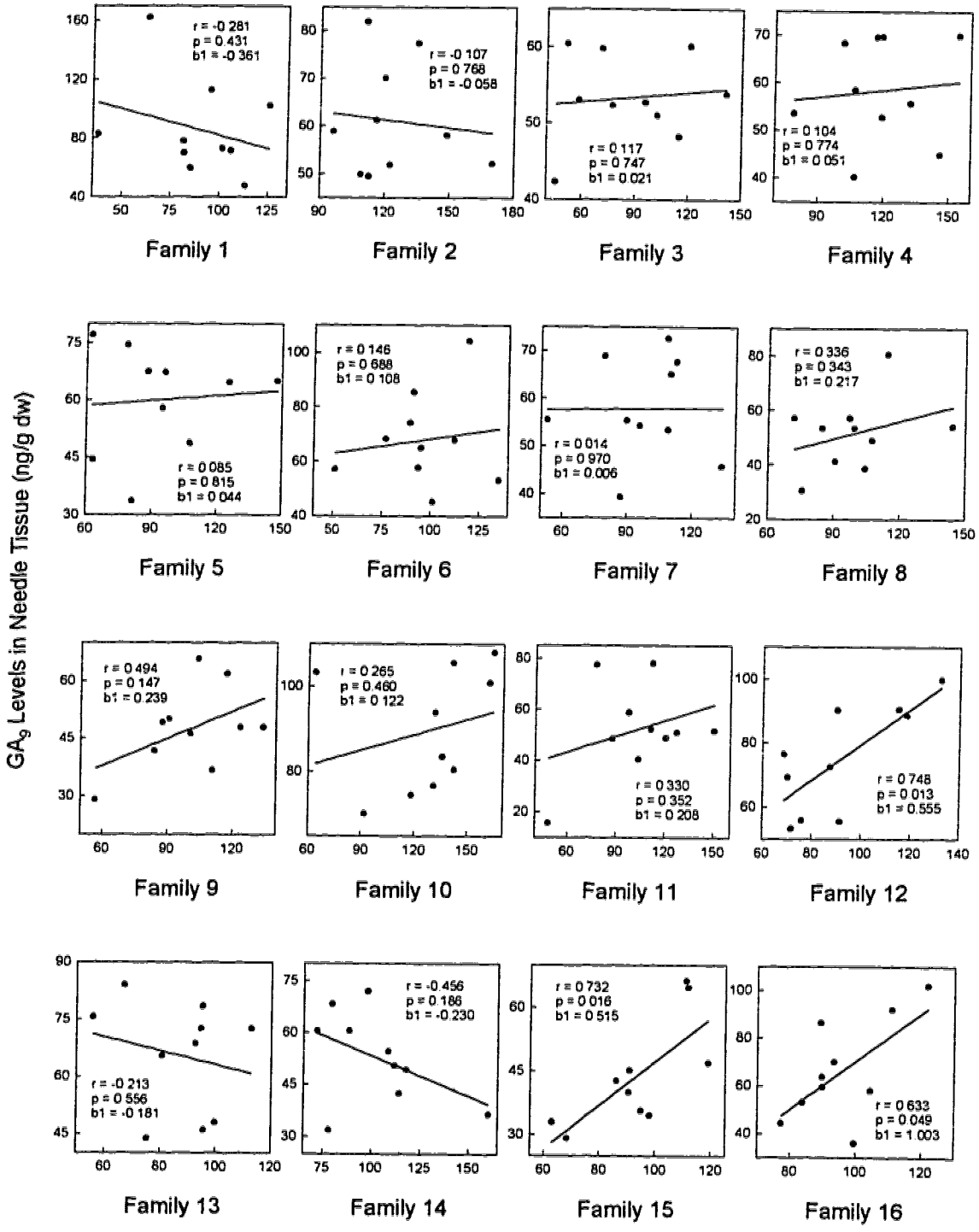


Figure 5.23 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling needle dry weights - within families

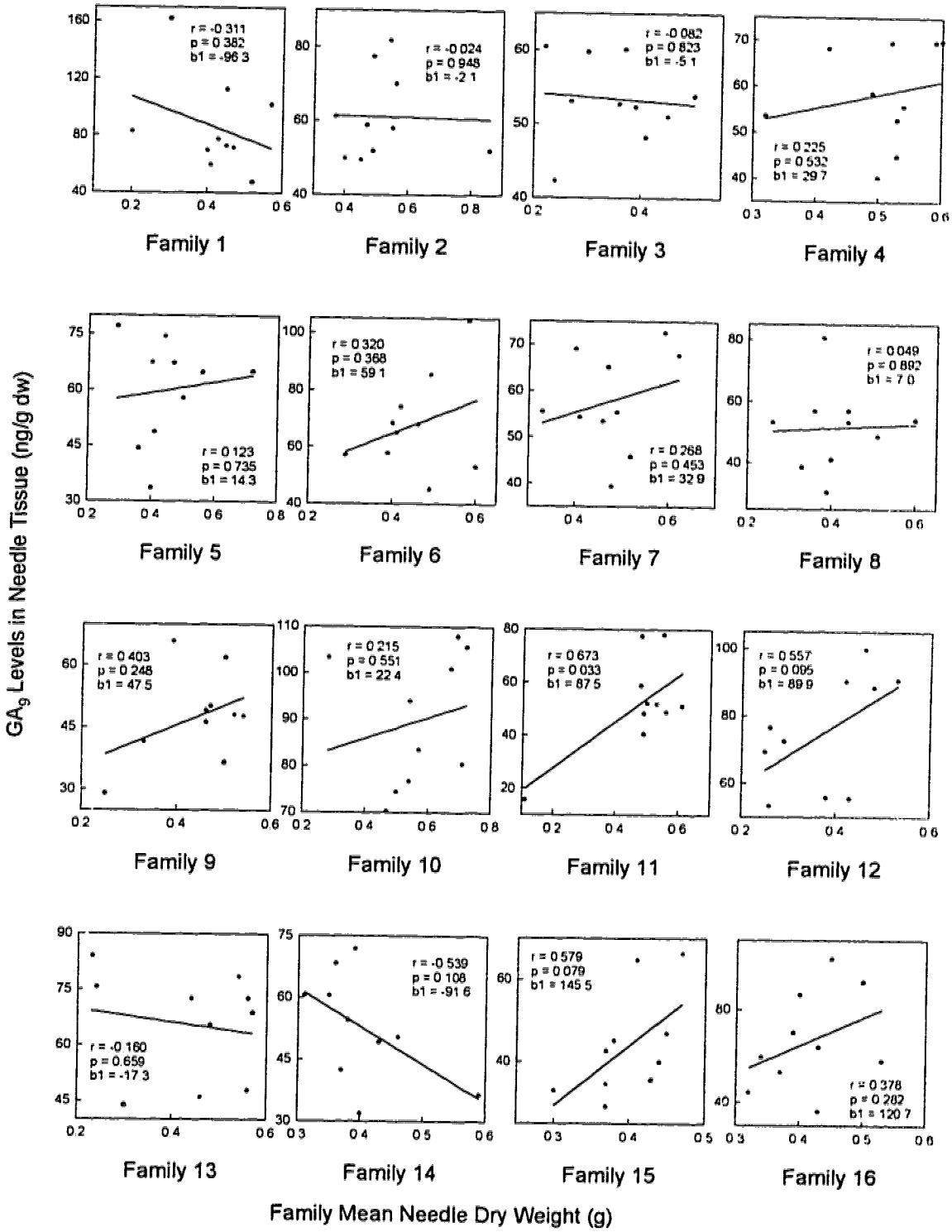
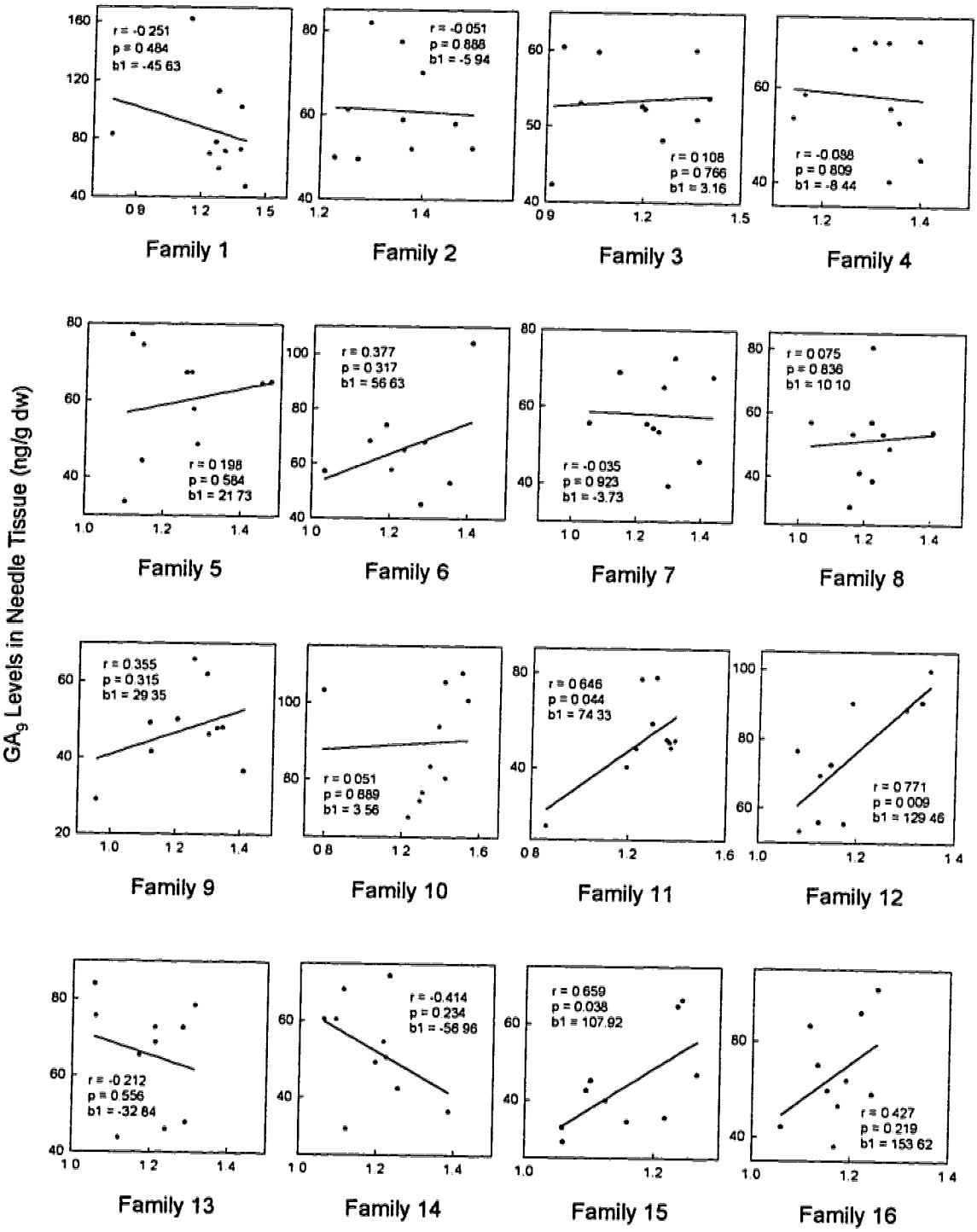


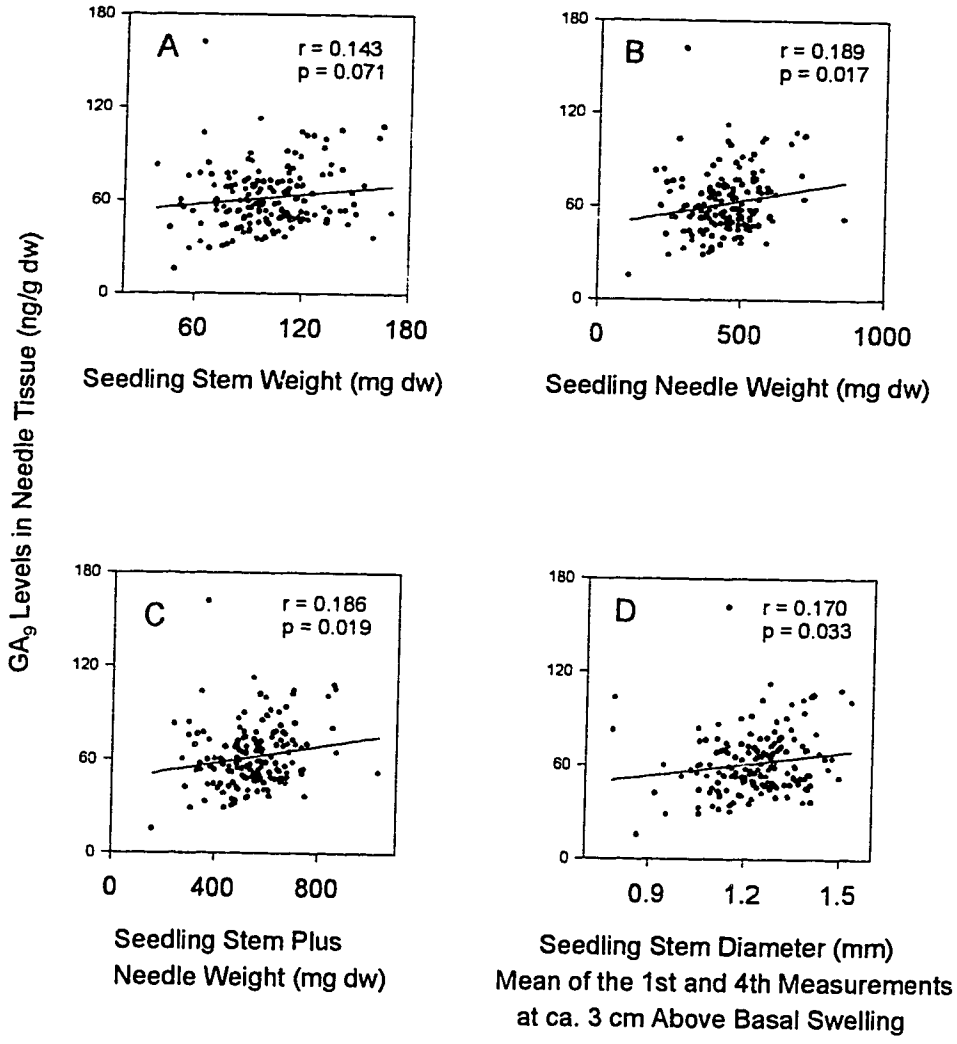
Figure 5.24 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling diameters - within families



general trend of first-order GA_9 levels to early genotype performance within a family was found for 3-month-old coastal Douglas-fir full-sibs. This suggests that the relationship between needle GA_9 and seedling growth within a family is either genus or species specific. This may result from the different genetic properties of the known parent full-sib coastal Douglas-fir versus open-pollinated half-sib Radiata pine. In full-sibs, 1/2 of additive genetic variance (GCA) is contributed to among family and the other 1/2 to within family, while 1/4 of non-additive genetic variance (SCA) is for among family and the remaining 3/4 to within family. On the other hand, in half-sibs, 1/4 of GCA is for among family and 3/4 for within family, whereas, all SCA is for within family with no SCA for among family. Thus, the within family variation in Radiata pine reflects all of SCA and 3/4 of GCA. In contrast, the within family variation in coastal Douglas-fir has 1/2 SCA and 1/2 GCA. For among family variation, 1/4 GCA and no SCA are found in Radiata pine whereas 1/2 GCA and 3/4 SCA are present in the full-sib coastal Douglas-fir. Since the GA_9 level has a significant SCA component, the different genetic properties of full-sib coastal Douglas-fir, relative to the half-sib Radiata pine could have a quite different effect on the relationship between GA_9 level and growth. Thus, the consistent positive trend found within half-sib Radiata pine families may not occur across full-sib families (this ignores possible species differences).

Then, ignoring family, GA_9 levels of needles were assessed for all 160 seedlings (across all families) for their relationship with various seedling biomass parameters. For seedling stem dry weight at final harvest, the correlation was

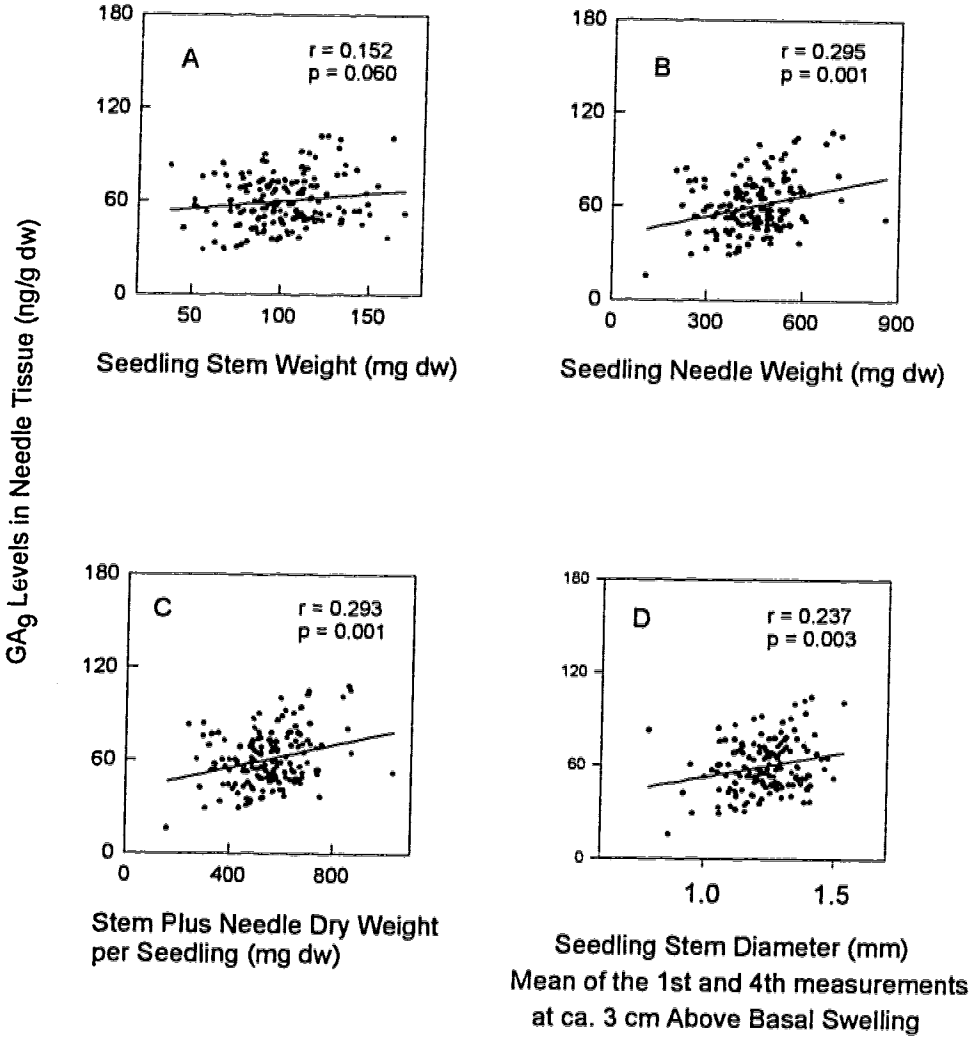
Figure 5.25 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling biomass and diameters - ignoring families



positive and near significant, with a p-value of 0.071 (Fig. 5.25.A). Similar assessments were made for GA_9 levels in relation to seedling needle dry weights at final harvest and seedling stem plus needle dry weight (lateral branch wood excluded) for the 160 seedlings across all 16 families. As above, positive correlations were found and these were significant at $p \leq 0.05$ (Figs. 5.25. B and C). The relationship of GA_9 to seedling stem diameter was also assessed. As described in the Methods section, stem diameters of the 3-month-old seedlings of coastal Douglas-fir seedling were measured four times: once at 0.6 cm, once at 1.0 cm, and twice at ca. 3 cm above the basal swelling. The correlations between the diameters measured at 0.6 and 1.0 cm and needle GA_9 levels were positive, but NS (data not shown). However, the comparison of needle GA_9 level and stem diameter at ca. 3 cm gave positive and significant correlations ($p \leq 0.05$; Fig. 5.25.D). Application of an outlier program at 2 SE improved all of the above correlations (Fig. 5.26).

Thus, in agreement with earlier results for Radiata pine, significant correlations between needle GA_9 and various seedling biomass parameters were obtained when all 160 coastal Douglas-fir seedlings were assessed across the 16 families (i.e. family ignored). These results further support the hypothesis that faster-growing conifer seedlings have higher GA levels than slower-growing seedlings. However, it must be kept in mind that there are very appreciable differences between families for coastal Douglas-fir with regard to this trend (e.g. Figs. 5.21 to 5.24), unlike Radiata pine where the positive relationship between GA_9

Figure 5.26 Coastal Douglas-fir early progeny test: needle GA_9 levels compared with seedling biomass and diameters - ignoring families (as per Fig. 5.25, only outliers removed at 2 SE)



and seedling growth is common to all families (Fig. 5.4).

5.3.2 Early Seedling Growth in Relation to Field Progeny Trial Performance

Family mean stem dry weights and family mean needle dry weights of coastal Douglas-fir seedlings were assessed in relation to the family mean diameters (dbh) of 8-year-old field-grown trees on each of three sites and for the mean across the three sites. The correlations varied from site to site, but were NS (data not shown). Thus, in contrast to Radiata pine, most parameters of seedling biomass (family mean stem or needle dw) were not significantly correlated with a family's field performance, even after retrospective use of an outlier program at 2 SE (data not shown).

Then, family mean stem diameters (age 3 months) were assessed for their relationship with the diameters of field-grown trees across the three sites. Comparisons with seedling diameters measured at 0.6 cm or 1 cm above basal swellings gave positive, but NS correlations (data not shown). Comparisons with the two seedling diameter measurements at ca. 3 cm above the basal swellings were also positive with p-values ranging from 0.028 to 0.132 (Figs. 5.27, 5.28 and 5.29).

Thus, among the various early growth parameters, for full-sib coastal Douglas-fir, seedling stem diameter at ca 3 cm showed the strongest correlation with later field performance. These results are in agreement with trends shown

Figure 5.27 Coastal Douglas-fir early progeny test: family mean stem diameter (1st measurement) compared with field performance

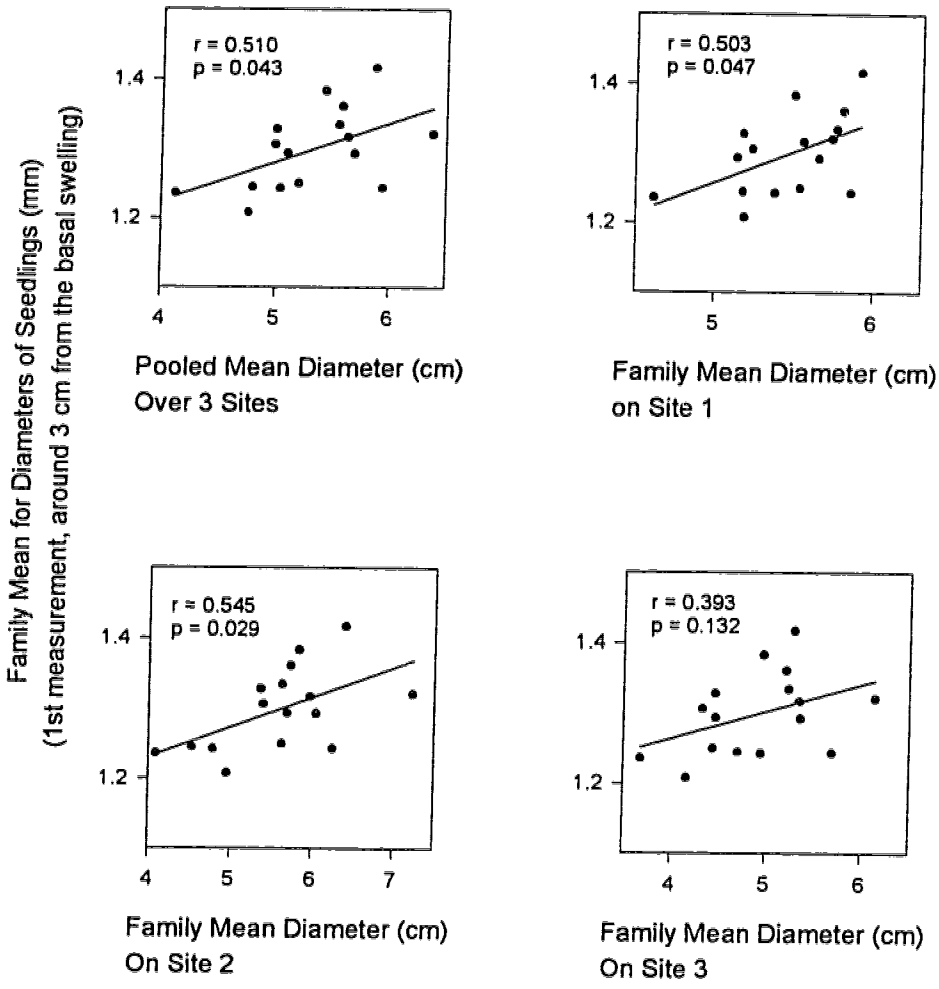


Figure 5.28 Coastal Douglas-fir early progeny test: family mean stem diameter (4th measurement) compared with field performance

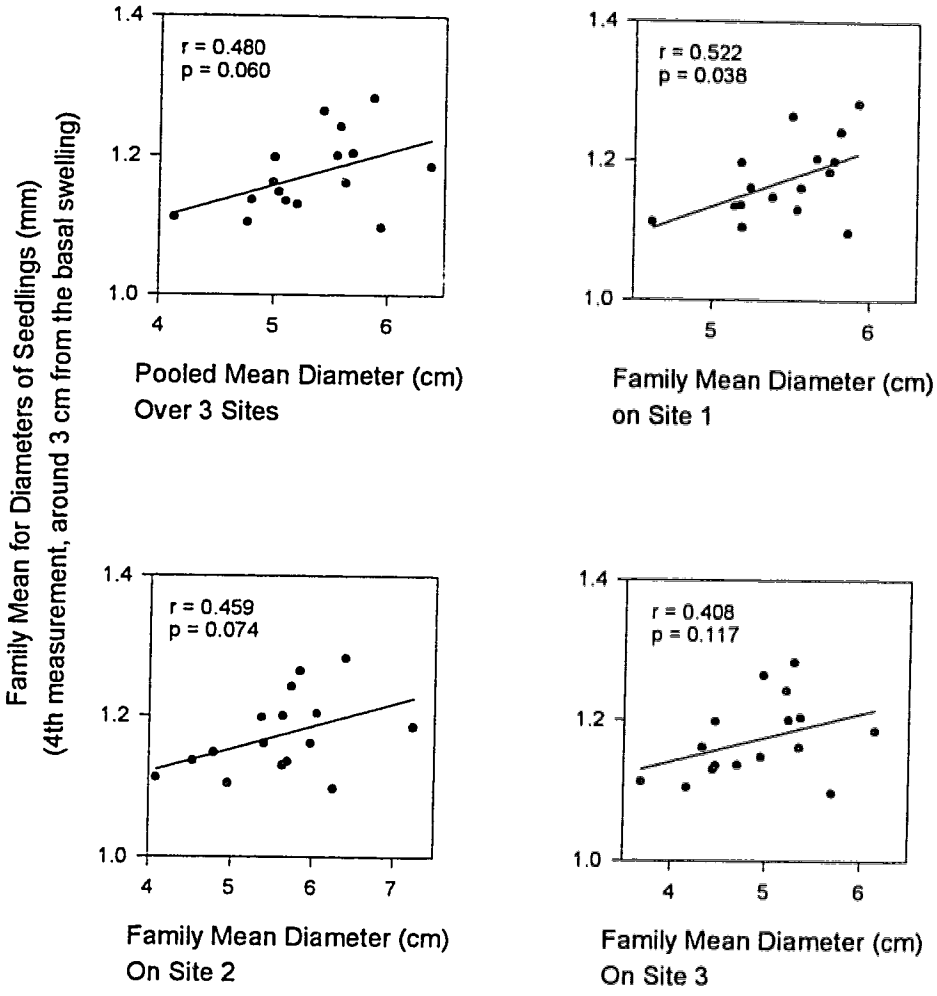
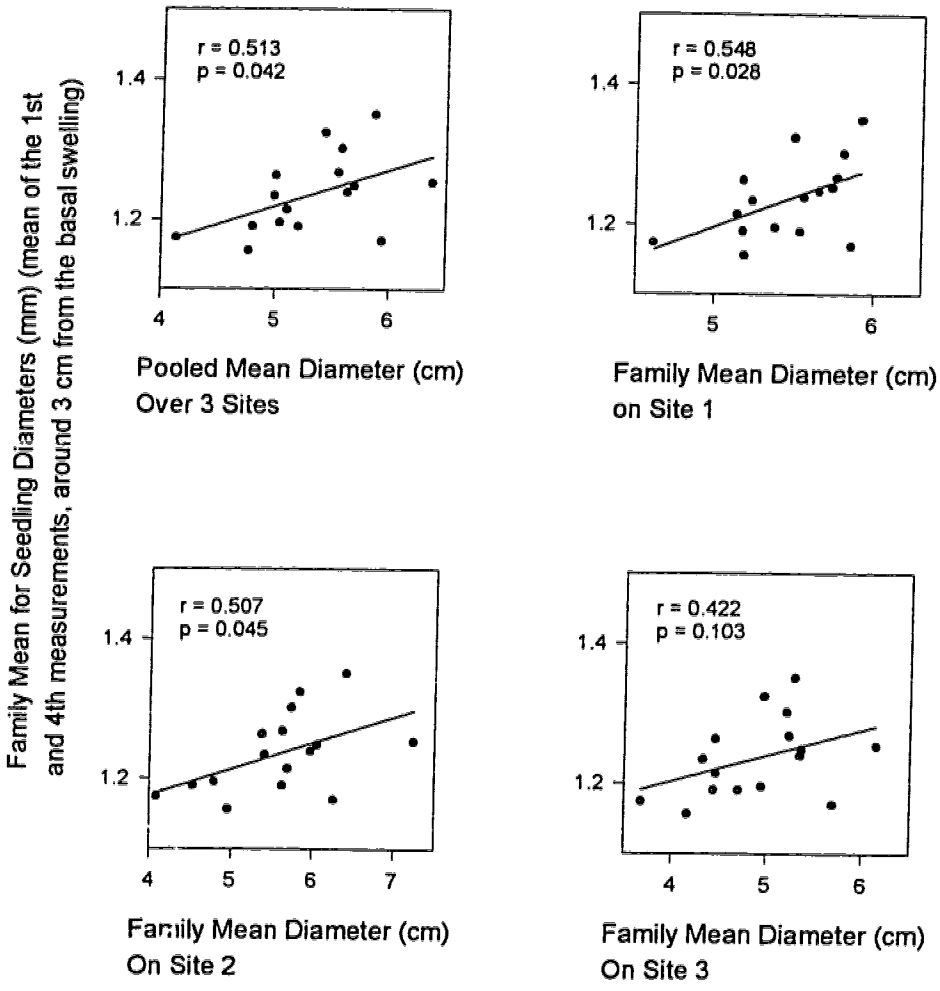


Figure 5.29 Coastal Douglas-fir early progeny test: family mean stem diameter (mean of the 1st and 4th measurements) compared with field performance



earlier for the 16 half-sib Radiata pine seedlings (Fig. 5.17) and other early reports that seedling diameters are among the best indicators for later field growth (e.g. Pharis, R.P., and R. Griffin, unpublished results with full-sib Radiata pine families) and (Sulzer et al., 1993), where diameters of 3-year-old black spruce were significantly correlated with the heights of 10-year-old field trees.

5.3.3 GA_9 Levels in Coastal Douglas-fir Needles in Relation to Field Progeny Trial Performance -- A First Order Comparison

Family mean GA_9 levels in needle tissue at harvest were assessed in relation to the family mean diameters of field-grown trees across the three sites. This is termed a "first-order comparison". The regression analysis correlations were positive, but NS, with p-values ranging from 0.418 to 0.773 (data not shown). No appreciable improvement in these correlations was found by retrospectively utilizing an outlier program (data not shown). An one-tail Spearman rank correlation test (Table 5.7), however, showed a near-significant correlation ($r = 0.374$, $p = 0.077$) for the first order needle GA_9 concentrations and family field performance [(dbh, for performance pooled across the 3 field sites (Table 5.7), pdbh].

Thus, as with the results presented earlier for fascicle needles of 4-month-old half-sib Radiata pine seedlings, the first-order comparisons of needle GA_9 from full-sib 3-month-old seedlings of coastal Douglas-fir are not a strong reflection of later field performance.

Table 5.7 One-tail Spearman Rank Correlation Coefficients for Various Seedling Needle GA_9 and Seedling Growth Parameters, When Compared to Mean Family Field Performance (dbh at age 8 years) for Coastal Douglas-fir

Field Progeny Test Site		1	2	3	pdbh***
Seedling needle GA_9	r	0.287	0.308	0.338	0.374
(1st order; ng/g dw)	p	0.141	0.123	0.100	0.077
Seedling Shoot dw	r	0.252	0.250	0.203	0.224
	p	0.174	0.175	0.225	0.203
Seedling Height	r	0.072	0.153	0.044	0.056
	p	0.395	0.286	0.436	0.419
Seedling Stem Diameter (ca. 3 cm from	r	0.483	0.508	0.447	0.495
cotyledon attachment point)	p	0.029*	0.022*	0.041*	0.026*
Seedling Needle GA_9**	r	-0.433	-0.266	-0.668	-0.438
(2nd order; Correlation coefficients)	p	0.047*	0.159	0.002*	0.045*
Seedling Needle GA_9**	r	-0.754	-0.628	-0.827	-0.765
(2nd order; Correlation coefficients; Sign ignored	p	0.001*	0.005*	0.001*	0.001*
negative values given a positive sign).					

Table 5.7 continued

Seedling Needle GA_9**	r	-0.502	-0.333	-0.712	-0.500
(2nd order; Slopes)	p	0.024*	0.104	0.001*	0.024*
Seedling Needle GA_9**	r	-0.745	-0.631	-0.747	-0.724
(2nd order; Slopes; Sign ignored, negative values given a positive sign)	p	0.001*	0.004*	0.001*	0.001*
Seedling Needle GA_9**	r	0.754	0.628	0.827	0.765
(2nd order; Probability values)	p	0.001*	0.005*	0.001*	0.001*

* Significant at $p \leq 0.05$.

** Correlation coefficients, slopes, and probabilities used above were derived from the regression of seedling needle GA_9 level:seedling stem dry weight for each of the 16 families (see Fig. 5.26. These 16 values (r, b1 or p) were then compared, using the one-tail Spearman rank test, with family field performance (dbh) on three sites, and across the three sites (pooled three sites).

*** pdbh stands for pooled dbh across the three sites.

Figure 5.30 Coastal Douglas-fir early progeny test, Slopes (sign ignored, negative values given a positive sign) were derived from the regression of seedling needle GA_9 level:seedling stem dry weight for each of the 16 families (see Fig. 5.22). These 16 slopes (b1 values) were then compared with family field performance (dbh) on three sites, and across the three sites (pooled dbh).

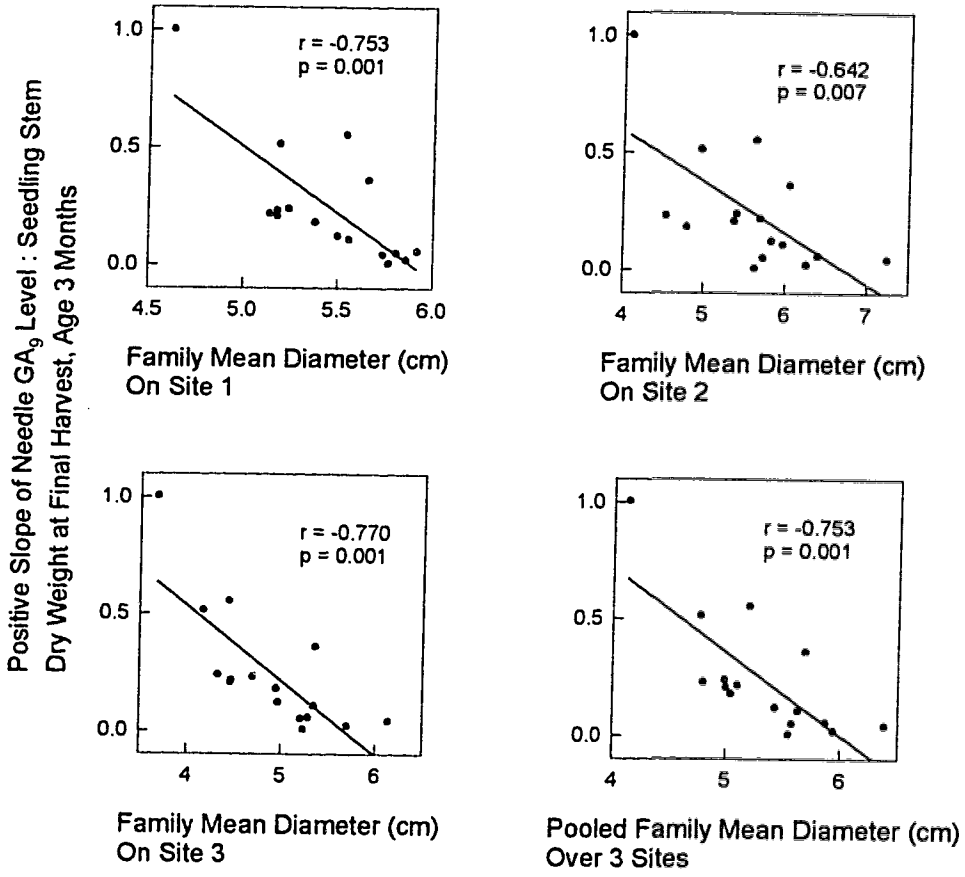
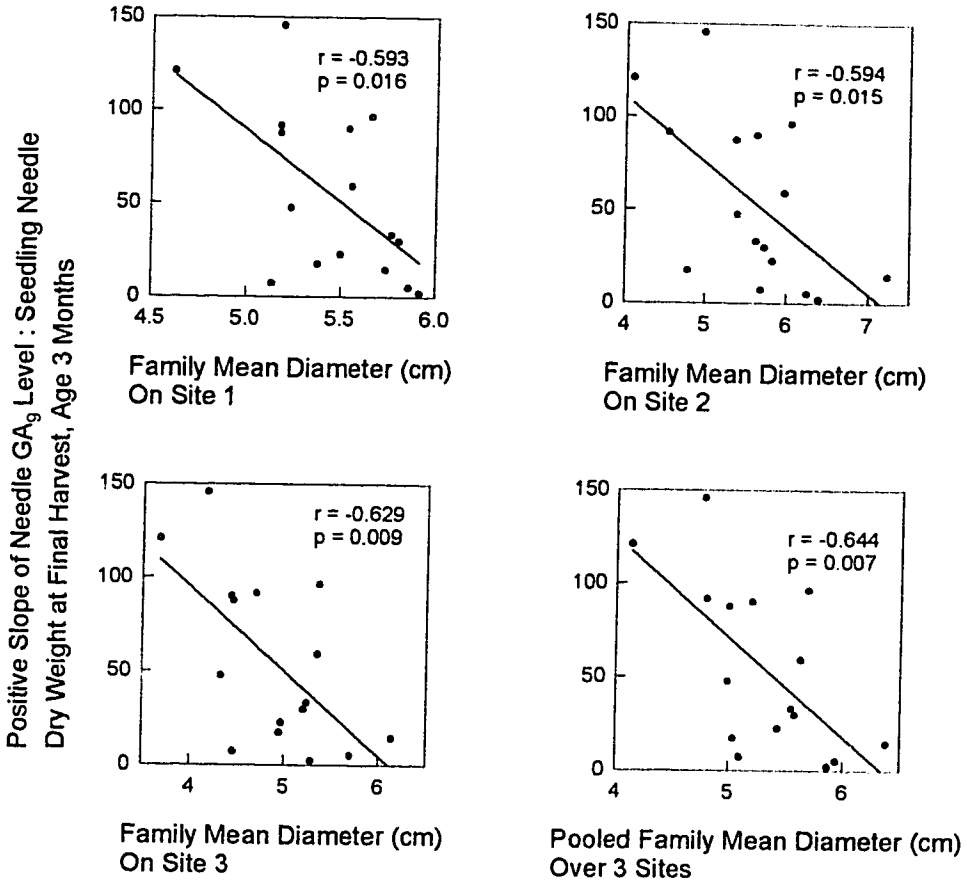


Figure 5.31 Coastal Douglas-fir early progeny test. Slopes (sign ignored, negative values given a positive sign) were derived from the regression of seedling needle GA_9:seedling needle dry weight for each of the 16 families (see Fig. 5.23). These 16 slopes (b1 values) were then compared with family field performance (dbh) on three sites, and across the three sites (pooled sites).



5.3.4 GA_9 Levels in Relation to Field Progeny Trial Performance -- A Second Order Comparison

First, 16 slopes were derived from the regression of needle GA_9 level with seedling stem dry weight at harvest within each of the 16 families (see Fig. 5.22). Although both negative and positive correlations are present, only the degree of correlation is considered. Thus, the slopes were all assigned a positive value, and then assessed for their relationship to field progeny trial performance (dbh). All comparisons gave significant correlations at $p \leq 0.01$ (Fig. 5.30).

This exercise was repeated for slopes derived from regressions of GA_9 level with needle dry weight within each of the 16 families (see Fig. 5.23), the 16 slopes then being assessed for their relationship with field progeny trial performance (dbh). All correlations were significant at $p \leq 0.05$ (Fig. 5.31).

Similarly, the 16 slopes derived from the regression of GA_9 level with seedling final height for each of the 16 families (see Fig. 5.21) were assessed in relation to field progeny trial performance (dbh). Again, all correlations were significant at $p \leq 0.05$ (Fig. 5.32).

Finally, the 16 slopes derived from the regression of GA_9 levels with seedling stem diameters for each of the 16 families (see Fig. 5.24) were related to field progeny trial performance (dbh) (Fig. 5.33). Again, all comparisons showed significance at $p \leq 0.05$.

Similar statistically significant correlations and probability values (all derived

Figure 5.32 Coastal Douglas-fir early progeny test. Slopes (sign ignored, negative values given a positive sign) were derived from the regression of seedling needle GA_9:seedling heights for each of the 16 families (see Fig. 5.21). These 16 slopes (b1 values) were then compared with family field performance (dbh) on three sites, and across the three sites (pooled dbh).

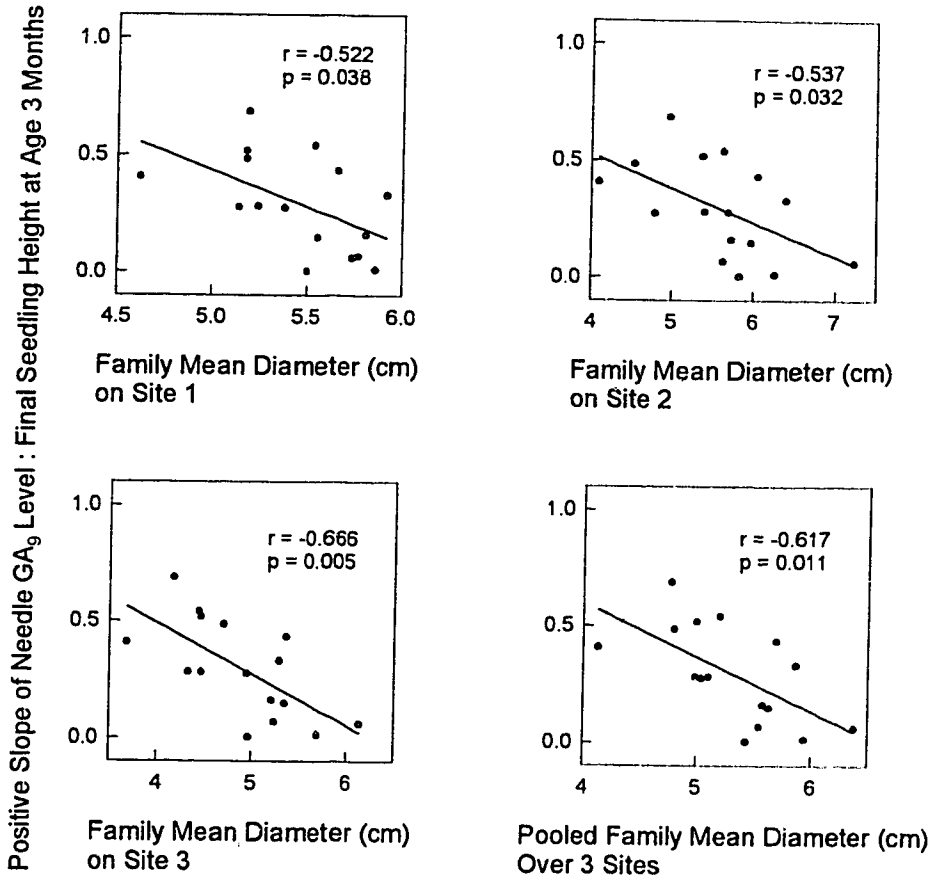
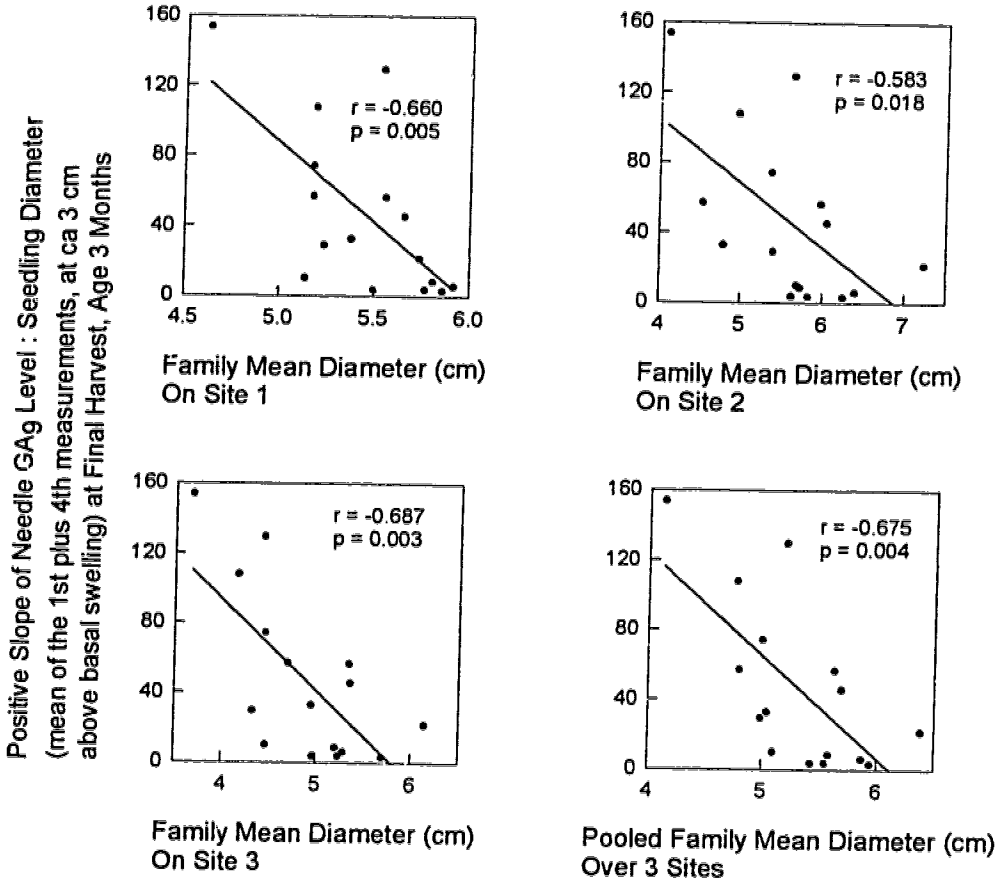


Figure 5.33 Coastal Douglas-fir early progeny test. Slopes (sign ignored, negative values given a positive sign) were derived from the regression of seedling needle GA_9 level:seedling diameters for each of the 16 families (see Fig. 5.24). These 16 slopes (b1 values) were then compared with family field performance (dbh) on three sites, and across the three sites (pooled dbh).



from Fig. 5.22) were obtained if correlation coefficients (r-values) for each of the 16 families were correlated with family field performance (data not shown, but see Table 5.7.a and 5.7.b for Spearman rank correlation test for significance for these comparisons).

Thus, in contrast to Radiata pine half-sibs, use of the slopes of GA_9 : various early growth parameters within a family yielded highly significant correlations for coastal Douglas-fir full-sibs when related to family field performance. That is, the comparisons between the family's characteristic slope of needle GA_9:stem or needle dry weights, or seedling height or stem diameter and the family field performance was highly significant. It thus appears that for coastal Douglas-fir, whenever seedling needle GA_9 level becomes an appreciable or significant variable for seedling (genotype) performance within a family, that family is likely to be slower-growing later on in the field.

5.3.5 Spearman Rank Correlation Test

The correlation coefficients and p-values of one-tail Spearman rank correlation test were summarized in the Table 5.7. Among the various early growth parameters, seedling stem diameter was the best indicator for later field performance, the first order needle GA_9 level was a moderately good indicator and seedling biomass and seedling height were the poorest.

Table 5.8 Predictive Ability at the Family Level of Various Early Parameters When Used to Rogue Out the 8 Slowest-Growing Families of Radiata Pine

	number of families rogued	
	8	
Marker used for early selection	mistakes made/max. mistakes possible	
stem dw	6/16	(37.5%)
needle dw (along main stem)	6/16	(37.5%)
upper stem+needle dw	6/16	(37.5%)
final seedling height	4/16	(25.0%)
seedling stem diameter (mean of measurements at 3 dates)	2/16	(12.5%)
seedling stem diameter (mean of measurements at 3 dates; 2 outlyers removed early)	2/16	(12.5%)
seedling stem volume (mean of measurements at 3 dates)	2/16	(12.5%)
first-order primary needle GA_9 (age 1 month)	4/16	(25.0%)
fascicle needle GA_9 (age 4 months)	8/16	(50.0%)
slope of GA_9 : needle dw	6/16	(37.5%)
slope of GA_9 : stem dw	4/16	(25.0%)

Table 5.9 Predictive Ability at the Family Level of Various Early Parameters When Used to Rogue Out the 4 or 8 Slowest-Growing Families of Coastal Douglas-fir

	number of family rogued			
	4		8	
Marker used for early selection	mistakes made/max. mistakes possible			
final seedling height	4/8	(50.0%)	8/16	(50.0%)
stem dw	6/8	(75.0%)	6/16	(37.5%)
needle dw	3/8	(37.5%)	4/16	(25.0%)
stem diameter (final)	3/8	(37.5%)	2/16	(12.5%)
needle GA_9	2/8	(25.0%)	6/16	(37.5%)
slope of GA_9 : final seedling height	2/8	(25.0%)	4/16	(25.0%)
slope of GA_9 : final stem diameter	2/8	(25.0%)	4/16	(25.0%)
slope of GA_9 : needle dw	2/8	(25.0%)	4/16	(25.0%)
slope of GA_9 : stem dw	3/8	(37.5%)	2/16	(12.5%)

5.4 Predicting Family Field Performance Based on GA_9 Levels and Other Early Measurement Parameters

This section will explore the use of various early growth parameters and other early measurements to predict family performance in the field for each of the two 16-family comparisons. Tables 5.8 and 5.9 summarize the number of mistakes that would have been made if four and eight families were rogued out based solely on various early measurement parameters. A "mistake" is defined as roguing out a faster-growing family or incorrectly (based on rank) keeping a slower-growing family after roguing. For Radiata pine, families defined as slower-growing are in the bottom 8/16, and as faster-growing are in the top 8/16 based on later field performance across 5 NZ sites. The reason for this "broad" classification in roguing is that the NZ Forest Research Institute provided us with the very slowest and very fastest families out of a ca. 220 family field progeny trial. Hence, attempts to rogue the very lowest 2 or 4 out of 16 families would not be warranted. For coastal Douglas-fir, the roguing exercise was accomplished for either the bottom 4 or 8 out of 16 families with regard to performance across three western Washington field progeny trial sites. Here, Weyerhaeuser had provided us with seedling material from families representing slow, middle, and fast-growers in their progeny trial tests.

The rational for performing this exercise is that if a significant portion of slower-growing families could be removed by early roguing, significant gains in the overall family performance should be feasible. Thus, in this context, the maximum

number of mistakes that could be made through early roguing is 8 or 16 (e.g. 4 or 8 faster-growing families rogued, and 4 or 8 slower-growing families kept.

For Radiata pine, various early growth parameters (e.g. seedling stem dry weight, seedling height, seedling diameter etc.,) could be used as the criteria in early selection of the eight slowest growing families. Thus, if any of stem dry weight, needle dry weight and stem plus needle dry weight were used as selection criteria to rogue out the bottom eight families, six mistakes (37.5%) would be made, three faster-growing families would be wrongly rogued out and three slower-growing families kept (Table 5.8). Four mistakes (25.0%) would be made if final seedling height was used (Table 5.8). However, only 2 mistakes (12.5%) would be made if the mean of three measurements of seedling stem diameter (with or without two outlier families removed early) were used as a predictor of field performance.

If the family mean for GA_9 level in primary or fascicle needles were used, the total mistakes made would be four (25.0%) or eight (50.0%) (out of 16), respectively (Table 5.8). When the second order comparisons for slope of GA_9 : needle dw were used as an early selection parameter, six mistakes (37.5%) were made (Table 5.8). Finally, if the second order slope of GA_9 : stem dw was used for selection, two faster-growing families were rogued out and two slower-growing were retained (an error rate of 25.0%) (Table 5.8).

Thus, for Radiata pine, if eight out of sixteen families were rogued out, the best early predictors are stem diameter (with or without using an outlier program early) and stem volume [2 mistakes made out of the 16 possible (12.5%)], while the

worst predictor was the first order, age 4 months fascicle needle GA_9 [8 out of 16 mistakes(50.0%); Table 5.8].

For coastal Douglas-fir, when seedling heights were used to rogue out the bottom four families, one faster-growing family was rogued out and three slower-growing families were retained [i.e. 4 mistakes (50.0%) out of 8 possible] (Table 5.9). If seedling stem dry weights were used in early selection, two faster-growing families were rogued out and four slower-growing families were retained [i.e. 6 mistakes (75.0%) out of 8 possible] (Table 5.9). If needle dry weights or stem diameters were used, one faster-growing family was rogued out and two slower-growing families were retained [i.e. 3 mistakes (37.5%) out of 8 possible] (Table 5.9). Finally, the second-order, slopes of GA_9 : various growth parameters, relationships were used (Table 5.9). All except the GA_9 slope for stem dry weight allowed for early selection with only two slower-growing families being mistakenly retained (25.0%) (Table 5.9).

Thus, for roguing the bottom four families of full-sib coastal Douglas-fir, the better predictors are first-order needle GA_9 and the several second-order slopes of GA_9 : early growth parameter.

The same exercise was performed for roguing out the bottom 8 families. The general trends are similar, with stem diameter and slope of GA_9 : stem dw being the best predictor [2 mistakes (12.5%) made out of the 16 mistakes possible]. Seedling height is the worst predictor (Table 5.9).

Thus, for coastal Douglas-fir the overall best predictors for use in early

selection are seedling stem diameter and the second-order slopes of GA_9 : various early growth parameters, especially stem diameter (bottom four families) or stem dry weight (bottom eight families).

Thus, for both Radiata pine and coastal Douglas-fir, the best predictor for early selection is seedling stem diameter, which yields 2/16 mistakes (a 12.5% rate of errors). This is somewhat higher than the rate of errors reported for lodgepole pine in a 120 family trial (< 6%) (personal communication, R. P. Pharis with F. C. Yeh) and for slash pine in a 64 family trial (5%) (personal communication, R. P. Pharis with S. E. Surles).

CHAPTER 6 GENERAL DISCUSSION

The present investigation has centered on the relationship between the gibberellin (GA) class of growth hormones and inherently rapid growth of conifers at the family level (among families) and at the genotype level within a family. Gibberellins are known to be causal for shoot growth of higher plants (Phinney, 1990; Reid, 1990), and in the context of this study they were examined in needle tissue from seedlings grown under near-optimal conditions in glasshouse environments during the long days of late spring/summer.

Conifers appear to have only a single biosynthetic pathway for GAs, the early non-hydroxylation pathway. Within that pathway (Fig. 1.2) GA_8 is the major GA (family mean) being ues thus far examined (Zhang 1990 and present study), hence its metabolism may be rate-limiting. Thus, the level of GA_8 in needles of two conifer species (Radiata pine and coastal Douglas-fir) was chosen as a biochemical trait to examine the relationship between hormone level and early seedling (genotype) growth within families, and between families. Additionally, the possible relationship of seedling GA_8 level to later family performance in the field was also assessed by a retrospective comparison. Finally, several seedling growth parameters were related to family performance in the field, again by a retrospective comparison.

Because of the large number of samples to be analyzed (> 300), the first phase of the investigation required the development of rapid, yet accurate and

precise methods of purification and analysis of GA_9 from small amounts of tissue. This was accomplished via methanolic extraction, the addition of $[^2H] + [^3H]GA_9$, purification by preparative C_{18} reversed phase columns, followed by $N(CH_3)_2$ HPLC, and where necessary C_{18} reversed phase HPLC. Having developed such a procedure, I was able to utilize tissue in amounts less than 200 mg dry weight for analysis by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM), yielding both accurate and precise measurement of endogenous GA_9 by isotope dilution.

GIBBERELLIN A_9 COMPARISONS

used as the selection criteriones

For 4-month-old seedlings, within family fascicle needle GA_9 levels were positively correlated with all early growth parameters, and especially with genotype stem dry weight or stem diameter (Fig. 5.4). For most of the 16 families assessed, this correlation was significant at $p \leq 0.05$. Therefore, within families the GA_9 level in needle tissues of 4-month-old seedlings is a strong and positive indicator of genotype (seedling) performance. That is, within any one half-sib family, seedlings having high levels of needle GA_9 are likely to be "fast growers". A similar trend occurred for 1-month-old seedlings, but because of the limited number of data points (only 2 or 3 per family; data not shown) no firm conclusion can be drawn.

When individual GA_9 levels in fascicle needles from 4-month-old seedlings were assessed for their relationship with the corresponding age 4 months stem or

needle dry weight, ignoring family, both comparisons gave highly significant correlations (Fig. 5.5). Hence, individual GA_9 levels best reflect seedling growth in terms of biomass accumulation when families were ignored.

In the between-family comparisons, the family mean GA_9 levels in primary needle tissue of age 1-month-old seedlings were, when analyzed by regression analysis, negatively, but non-significantly correlated with family mean for several early growth parameters, such as age one month shoot dry weight, and subsequent seedling height growth or seedling stem diameter or stem volume (see Fig. 5.2). However, if family mean primary needle GA_9 comparisons were analyzed by the one-tail Spearman rank correlation test (Table 5.4), then several comparisons showed significance (mean of 3 seedling diameter measurements) or near-significance (first stem volume, and mean of 3 seedling stem volume measurements). Hence, family mean primary needle GA_9 levels were negatively and significantly correlated with certain of the subsequent seedling growth measurements at the family level. This inverse relationship of GA_9 : growth of the family indicates that families having lower GA_9 in their primary needles grow faster in the subsequent growth period. The observation that faster-growing families have lower mean family GA_9 levels may indicate a faster metabolic turnover of GA_9 to other "downstream" GAs, such as GA_4 and GA_1, both of which are known to have *per se* bioactivity (Phinney, 1990; Reid, 1990).

Also at the family level, in a trend similar to that seen for primary needle GA_9, the family mean for fascicle needle GA_9 level of 4-month-old seedlings was

negatively, but non-significantly correlated with the family mean for all seedling growth parameters (Fig. 5.9 and Table 5.4). However, when GA_9 was analyzed just from needle tissues grouped from the 5 slowest-growing genotypes (seedlings) within each of 16 families, the negative correlation of this GA_9 value with family mean for seedling diameters became significant at $p \leq 0.05$, even without using an outlier program (Fig. 5.10 and Table 5.5). Similarly, when GA_9 levels of the 5 slowest-growing seedlings in each family were compared by regression analysis with seedling biomass and heights, there was also a negative, but non-significant correlation for seedling biomass (Fig. 5.3) and with most seedling heights (Table 5.5). However, GA_9 levels in needles of the 5 slowest-growing seedlings were significantly correlated with height growth (Fig. 5.8), and after removal of one outlier family at 2.0 SE, with final height (Fig. 5.7). Further, when the one-tail Spearman rank correlation test was used, several family mean seedling biomass parameters were significantly or near-significantly correlated with GA_9 levels in needles of the 5 slowest-growing seedlings (Table 5.5), as were seedling stem volumes and seedling stem diameters (Table 5.5), and even mean family seedling heights (Table 5.5).

Thus, although family mean GA_9 in fascicle needles of 4-month-old seedlings inversely reflects early family performance (fast-growers tending to have low GA_9, as was the case for primary needle GA_9), this trend for needle GA_9 from the older seedlings is statistically significant only when the 5 slowest-growing (out of 25) seedlings are used to represent the families! Thus, the best indicator for mean

family seedling performance in terms of shoot growth was the level of GA_9 in needles of the very slowest-growing genotypes within each family.

Comparisons of primary needle GA_9 at the family level were then made retrospectively with family field performance across five New Zealand sites. Consistent with the trend for early seedling growth (family rank), the family mean GA_9 levels in needle tissues of 1-month-old seedlings were negatively correlated with mean family performance (dbh) in 5- to 8-year-old field trials (Fig. 5.20 and Table 5.6). However, significance or near-significance in these comparisons was reached only when the one-tail Spearman rank correlation test was utilized, and only on the far north, Moerewa 1 and 2 sites (Table 5.6). Hence, family mean GA_9 levels in primary needles of these very young seedlings is strongly, but not always significantly correlated with family rank for shoot or stem growth at the seedling stage. Surprisingly, seedling primary needle GA_9 levels (family mean) are also strongly correlated with family performance (dbh) at ages 5 to 8 years on certain of the field progeny trial sites.

In fascicle needles of 4-month-old seedlings, although the trend of seedling GA_9 to family field performance continued to be negative; all comparisons between family mean GA_9, or even GA_9 from the 5 slowest-growing genotypes, and field performance were NS. Hence, unlike primary needle GA_9, no statistically significant "first order" relationship was found between fascicle needle GA_9 levels from these age 4-month-old half-sib seedlings and family performance in the field. This also contrasts with the positive and significant relationship found for fascicle needle GA_9

levels and field performance for F1 full-sibling Radiata pine families in a more limited (5 family) comparison (Zhang 1990 and Pharis et al., 1990).

Since the simple first-order comparisons between fascicle needle GA_9 of 4-month-old seedlings and field performance were NS, a "second order" comparison was tested (see later discussion for coastal Douglas-fir). That is, slopes for GA_9 levels : early genotype growth within a family (e.g. seedling height, diameter, tissue dry weight) were derived for each of the 16 families by regression analysis (Fig. 5.4). These 16 "GA_9 slopes" were then assessed for their relationship with family field performance. No significant correlation was found by regression analysis or by use of the one-tail Spearman rank correlation test, and no outlier families were removed by use of an outlier program at 2.0 SE. The poor correlation of this second order seedling GA_9 relationship with Radiata pine field performance contrasts markedly with the good fit found for coastal Douglas-fir (see below). That said, lowering outlier program significance to 1.5 SE did remove one outlier Radiata pine family, thereby gaining significance for the second order comparison at $p \leq 0.05$ (data not shown).

Coastal Douglas-fir Full-Sib Families

For coastal Douglas-fir full-sibs, unlike Radiata pine half-sibs, there was no consistent relationship over all 16 families for levels of needle GA_9 within a family to various seedling growth parameters, such as age three month seedling stem dry weight, stem diameter and height (Figs. 5.21 - 5.24). Nonetheless, some families

(3 to 5/16) did show statistically significant positive or negative correlations. But, when family was ignored and GA_9 levels were assessed across all 160 seedlings, relatively weak, but statistically significant correlations were found between needle GA_9 levels and a number of early growth parameters, such as seedling stem dry weight, needle dry weight, diameter and height (Fig. 5.25). Hence, the relationship of GA_9:genotype performance within a family for coastal Douglas-fir full-sibs is, for most families, much different than for Radiata pine half-sibs. However, like Radiata pine, when family is ignored, early seedling growth across all 160 genotypes for coastal Douglas-fir was positively and significantly correlated with GA_9 levels.

As for Radiata pine, GA_9 levels in coastal Douglas-fir needles were compared, in a retrospective manner, to family field performance (dbh) at year 8. The simple "first order" correlations were positive, but weak (NS). However, near significance ($r = 0.374$, $p = 0.077$) was gained for the first-order comparison of GA_9 level in seedling needles when compared with mean family field performance (dbh) for the 3 sites pooled (Table 5.7). Interestingly, all "second order" comparisons, using the slopes of needle GA_9 level : seedling growth parameters within each family (e.g. seedling diameter, height or stem or needle dry weight), were significantly ($p \leq 0.05$) related to field performance (dbh) at the family level. Thus, when seedling needle GA_9 is an appreciable to significant variable within family with regard to early genotype rank for seedling growth, that family is likely to be slower growing than are families where GA_9 has only a nominal or nil relationship. In other words, a family with a high frequency of genotypes (seedlings) exhibiting "GA_9

dependency" for genotype performance at the seedling stage, is a family which will likely perform poorly in the field (Figs. 5.30, 5.31, 5.32, 5.33)!

EARLY GROWTH COMPARISONS TO FIELD PERFORMANCE

Various early seedling growth parameters were also assessed by regression analysis for their relationship with field performance. For Radiata pine, at the family level, seedling height, height growth, stem diameter and stem dry weight were positively (but generally NS) correlated with field performance (Figs. 5.11, 5.12, 5.13, 5.17, 5.18). However, when assessed by the one-tail Spearman rank correlation test, significance or near-significance was gained on some sites, or across the NZ 5 sites for seedling stem dry weight, needle dry weight, total seedling dry weight, the later height measurements, height growth and RGR for height (Table 5.6). For seedling stem diameters and stem volumes, the Spearman correlations with mean family field rank (dbh) are very strong and usually significant (Table 5.6). Additionally, many of the positive regression analysis correlations became significant after a retrospective use of the outlier program at 2.0 SE (Figs. 5.12, 5.14, 5.15, 5.16, 5.19). Hence, family means for most seedling stem growth parameters (e.g. height, height growth, stem diameter, stem dry weight and needle dry weight along the stem) of seedlings are highly correlated with subsequent growth (dbh) of the stem in the field for most (12 or 13/14) of the Radiata pine families (see also Table 5.4 where the one-tail Spearman rank correlation test was utilized)! Although the presence of a small number of "outlier" families may limit the

use of early stem growth parameters as a predictor of field performance at the family level, it is interesting (and reassuring) to find that for most families, early stem growth, and especially early stem diameter, positively and significantly reflect family field performance (stem diameter at breast height; dbh) across a wide range of New Zealand sites.

For coastal Douglas-fir, at the family level, seedling stem and needle dry weights were only weakly correlated (NS) with field performance (dbh) (Table 5.7). However, mean family seedling diameter, taken at a point along the seedling stem where taper was minimal, showed a strong, significant and positive correlation with family field performance (dbh) (Figs. 5.27 - 5.29, Table 5.7). Thus, at the family level, early seedling diameter is the seedling shoot growth parameter most highly correlated with later family field performance (dbh) for both Radiata pine and coastal Douglas-fir.

The results of this investigation suggest that needle GA_9 level (or, likely, levels of one or more of its metabolites) is causally implicated in seedling growth at both the genotype (within family) and family levels, although the relationship may be specific to species or developmental stage. For Radiata pine half-sibs, within family or without considering family (across all 166 individual measurements), the concept that fast-growing seedlings (genotypes) biosynthesize higher levels of needle GA_9 appears correct (Figs 5.4 and 5.5). However, at the family level, the situation was more complex, with high GA_9 levels (family mean) being negatively correlated with both early glasshouse and subsequent field performance for most

families. That is, fast-growing families tended to have low GA_9 levels.

For coastal Douglas-fir full-sibs, across 160 seedlings (ignoring family) the fast-growing seedlings (rank based on needle and stem biomass) had significantly higher needle GA_9. However, at the family level, only the second order "GA_9 slope" relationship, which compared GA_9 slopes (or correlation coefficients or probability values) for each of the 16 families to family field performance (dbh) yielded highly significant correlations with field performance. Hence, for coastal Douglas-fir the direct first-order comparison of needle GA_9 level is not a good correlate with family field performance (however, see Table 5.7, where significance is gained at $p = 0.077$ if the one-tail Spearman rank correlation test is utilized). Rather, the more complex second order relationship of needle GA_9 to genotype rank within a family (GA_9 slope) best reflects family field performance for coastal Douglas-fir.

With regard to early selection (roguing of slow-growing families), although needle GA_9 in Radiata pine was closely related to early seedling growth (across all families), the highest frequency of error would be made if the first order, fascicle needle GA_9 level were used as the selection criterion for family field performance (Table 5.8). Primary needle GA_9 and the second order GA_9 slope means gave a much lower frequency of error (e.g. 4 mistakes out of a possible 16 mistakes would have been made). Among various Radiata pine seedling growth parameters, stem diameter had the lowest rate of error in predicting family performance in the field (only 2 errors out of a possible 16 would have been made). For coastal Douglas-fir, the best predictors of family field performance are seedling stem diameter and the

second order slopes of GA_9 : various seedling growth parameters (Table 5.9). The error rate of the best predictors for both species are the same (2/16 = 12.5%). This rate of error is higher than those reported for lodgepole pine in a 120 family trial (<6%) (personal communication, R. P. Pharis with F. C. Yeh) and for slash pine in a 64 family trial (ca. 5%) (personal communication, R. P. Pharis with S. E. Surles).

CHAPTER 7 CONCLUSIONS

Based on these results and on other published work, including exogenous application of gibberellins, the following conclusions can be drawn:

1. Gibberellins are causal for shoot growth of individual conifer genotypes and GA_9 level in the needles of seedlings reflects individual differences in growth capacity across a population of genotypes.

2. Individual families vary considerably in the proportion (frequency) of genotypes (seedlings) whose growth is limited by endogenous GA level (as reflected by endogenous GA_9 level in the needles). That is, for some families genotype growth is highly and positively correlated with endogenous GA_9 levels (especially for half-sib Radiata pine families), whereas for other families genotype growth and GA_9 levels show only a modest, or negligible, or nil correlation (this is especially so for the full-sib Douglas-fir families).

3. For Radiata pine half-sibs, the simple "first order relationship" of family mean GA_9 levels to family mean for seedling growth is poor, except for age 1 month primary needles, or for fascicle needles of the very slowest-growing seedlings within a family (e.g. those 5 out of 25 seedlings with the lightest stem dry weight). Gibberellin A_9 levels of these slowest-growing seedlings show strong and significant correlations with family mean rank for a wide variety of seedling growth parameters, especially stem volume and stem diameter.

4. A similar situation exists for coastal Douglas-fir. The simple first order

family mean needle GA_9 relationship to family mean for seedling stem diameters and needle dry weights, while positive, is weak (significance is gained only at p = 0.14 to 0.18).

5. The relationship of first order GA_9 levels to family field performance in Radiata pine is negative, and generally weak, the exception being primary needle (age 1 month) GA_9 levels, which show significant or near-significant correlations with field performance on 2 of the 5 field sites.

6. For coastal Douglas-fir, the first order GA_9 levels to family field performance relationship is also weak (but positive), gaining near significance (p = 0.077) only with the "3 field sites pooled" comparison.

7. However, for coastal Douglas-fir, a more complex "second order comparison", which relates the GA_9 slopes for each of the 16 families to family performance in the field, is highly and significantly correlated with field performance of the coastal Douglas-fir full-sib families.

8. For Radiata pine half-sibs, seedling height, height growth, RGR for height, stem diameter, stem volume and stem dry weight are all positively correlated with family field performance, and many of the correlations are significant, especially those involving stem diameter and stem volume.

9. For coastal Douglas-fir full-sibs, among several seedling growth parameters, only seedling stem diameter is positively and significantly correlated with family field performance (dbh at year 8).

10. The best early predictor (lowest error frequency when used to rogue out

slow growers) of field performance is seedling stem diameter for both Radiata pine half-sib and coastal Douglas-fir full-sib families. Additionally, for coastal Douglas-fir, the slope of GA_9 : various early growth parameters was equally good. These "best predictors" had a similar rate of error - 2 mistakes were made out of a possible 16 (12.5%).

LITERATURE

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ABBREVIATIONS FOR APPENDICES

Appendix 1:

R:	random number assigned to the seedling
S:	seedling hypocotyl dry weight (mg)
N:	seedling needle plus shoot dry weight (mg)
number:	family number, e.g. 400 stands for family 400

Appendix 2:

FAM:	family number
R:	random number assigned to the seedling
SW:	seed weight
H1:	height 1 (cm), age 4 weeks
H2:	height 2 (cm), age 6 weeks
H3:	height 3 (cm), age 8 weeks
H4:	height 4 (cm), age 10 weeks
H5:	height 5 (cm), age 12 weeks
H6:	height 6 (cm), age 14 weeks
D1:	diameter 1 (cm), age 11 weeks
D2:	diameter 2 (cm), age 13 weeks
D3:	diameter 3 (cm), age 15 weeks
BW:	branch weight (g)
NW:	branch needle weight (g)

BTW:	bottom stem weight (g)
UTW:	upper stem weight (g)
UNW:	upper stem needle weight (g)

Appendix 3:

number:	family number
A9:	GA_9
N:	mean weight of upper stem weight of selected seedlings
S:	mean weight of upper stem needle weight of selected seedlings

Appendix 4:

FAM:	family number
R:	random number assigned to the seedling
STDW:	stem dry weight of seedling at harvest
DIA1:	first diameter measurement
DIA2:	second diameter measurement
DIA3:	third diameter measurement
MEAND:	mean of the three diameter measurements
PVR:	rank of the average family volume mean on 3 sites
V1R:	rank of the family volume mean on site 1
V2R:	rank of the family volume mean on site 2

V3R:	rank of the family volume mean on site 3
PVOL:	average family volume mean on 3 sites
VOL1:	family volume mean on site 1
VOL2:	family volume mean on site 2
VOL3:	family volume mean on site 3
PHTR:	rank of the average family height mean on 3 sites
HT1R:	rank of the family height mean on site 1
HT2R:	rank of the family height mean on site 2
HT3R:	rank of the family height mean on site 3
PHT:	average family height mean on 3 sites
HT1:	family height mean on site 1
HT2:	family height mean on site 2
HT3:	family height mean on site 3
PDR:	rank of the average family diameter mean on 3 sites
D1R:	rank of the family diameter mean on site 1
D2R:	rank of the family diameter mean on site 2
D3R:	rank of the family diameter mean on site 3
PDBH:	average of family diameter at breast height on 3 sites
DBH1:	family diameter at breast height on site 1
DBH2:	family diameter at breast height on site 2
DBH3:	family diameter at breast height on site 3
NDW:	needle dry weight

	GA9:	needle GA_9 levei (ng/g dw)
Appendix 5:	(r):	correlation coefficient
	(p):	probability
	Taupo:	family field performance (dbh) on Taupo 85L site
	Thorp:	family field performance (dbh) on Thorp Rd site
	Rotoe:	family field performance (dbh) on Rotoehu site
	Moer2:	family field performance (dbh) on Moerewa 2 site
	Moer1:	family field performance (dbh) on Moerewa 1 site
	NZ 5 S:	family field performance (dbh) across New Zealand 5 sites
	fam1mGA9:	family mean GA_9 levels in needle tissues of 1-month-old seedlings
	fam4mGA9:	family mean GA_9 levels in needle tissues of 1-month-old seedlings
	5s4mGA9:	GA_9 levels in needle tissues taken from 5 slowest genotypes of 4-month-old seedlings
	5f4mGA9:	GA_9 levels in needle tissues taken from 5 fastest genotypes of 4-month-old seedlings
	1mtotalwt:	total biomass dry weight of 1-month-old seedlings
	4mtotalwt:	total biomass dry weight of 4-month-old seedlings (above ground part)
	diameter1:	family mean stem diameter at age 11 weeks

diameter2:	family mean stem diameter at age 13 weeks
diameter3:	family mean stem diameter at age 15 weeks
mean3dia:	mean of the 3 diameter measurements.
height1:	family mean seedling height at age 4 weeks
.....	
height6:	family mean seedling height at age 14 weeks
ht2-ht1:	height at age 6 weeks minus height at age 4 weeks
.....	
ht6-ht5:	height at age 14 weeks minus height at age 12 weeks
RGR:	relative growth rate
D2-D1:	diameter at age 13 weeks minus diameter at age 11 weeks
upstem dw:	family mean of upper stem dry weight of 4-month-old seedlings
upneedle dw:	family mean of needle dry weight along upper stem of 4-month-old seedlings
r:	correlation coefficient from Fig. 5.4
p:	probability from Fig. 5.4
b1:	slope from Fig. 5.4

Appendix 1 Radiata Pine

One Month Old Seedling Data

400R	400S	400N	500R	500S	500N	618R	618S	618N	631R	631S	631N	632R	632S	632N	645R	645S	645N	650R	650S	650N	657R	657S	657N
33	10	43	100	9	31	178	22	78	86	9	33	149	15	57	163	12	49	135	10	39	62	7	15
133	9	49	99	7	25	181	13	58	87	8	30	148	14	60	168	14	52	3	14	54	140	4	9
42	12	50	70	7	25	198	10	46	88	8	39	144	15	62	148	12	43	194	6	29	155	5	8
122	8	31	52	8	33	104	14	56	89	9	33	134	9	47	147	10	42	196	8	36	160	5	7
148	10	35	53	6	23	94	10	51	92	10	37	121	13	48	142	15	58	185	9	37	161	7	12
72	9	38	57	6	28	96	8	31	126	12	39	123	14	62	99	16	60	175	9	23	79	5	9
70	11	46	122	8	27	84	11	43	128	9	33	129	14	60	83	14	49	171	7	29	186	5	19
73	8	31	157	8	33	107	12	43	135	9	37	131	16	65	81	17	46	169	9	43	99	6	13
183	9	36	15	7	28	105	14	48	108	8	30	111	13	62	77	14	49	145	8	33			
182	7	20	129	11	38	8	10	45	111	7	22	104	15	59	76	14	51	44	6	30			
92	7	29	13	16	49	1	14	58	117	11	46	176	17	58	71	9	41	94	10	34			
96	6	29	27	9	33	39	12	40	106	10	30	182	13	64	20	12	48	95	9	46			
194	13	51	19	9	37	34	16	54	102	6	21	199	15	54	35	14	55	126	7	30			
134	10	47	64	7	18	48	11	47	107	8	31	116	16	59	18	17	61	128	7	27			
97	10	38	7	8	26	65	10	30	45	7	27	185	16	63	19	16	65	98	7	28			
66	12	38	115	7	33	60	9	34	43	9	30	198	17	64	13	14	54	81	8	20			
43	11	45				52	8	33	56	9	35	197	12	60	9	15	47	69	9	33			
105	11	49				174	12	42	54	9	41	193	11	66	8	17	62	55	8	34			
58	15	56				173	12	45	53	10	39	192	12	57	105	13	44	63	7	21			
59	13	40				153	13	56	52	9	34	189	11	56	90	13	55	42	9	25			
18	15	50				122	20	81	82	5	23	79	21	79	92	8	31	43	6	18			
29	8	30				124	7	33	78	7	31	81	12	49	93	9	43	8	11	41			
37	14	58				129	10	40	73	9	30	75	14	56	124	15	67						
177	8	28				130	12	45	70	8	26	86	14	56	101	13	54						
176	7	25				175	13	42	69	7	25	57	11	65	104	13	49						
22	11	37				193	13	66	172	12	49	103	17	66	109	20	69						
7	15	50				189	14	51	160	9	30	107	16	71	114	16	58						
166	6	25				109	12	51	159	9	35	101	17	66	117	16	53						
55	9	39				112	12	36	116	10	43	99	16	67	193	13	54						
30	10	41				115	13	43	115	10	31	97	19	72	195	10	46						
161	11	38				133	9	25	152	8	27	84	14	69	174	11	40						
167	12	36				132	9	46	148	11	37	173	13	41	157	14	54						
27	9	36							143	8	32	174	15	59	162	18	75						
178	9	37							138	8	31	169	12	56	198	11	43						
81	8	36							31	14	54	152	14	61	183	12	45						
60	11	47							28	7	27	156	15	75	5	10	31						
65	7	25							8	7	26	157	16	53	6	12	49						
39	10	37							192	6	21	158	19	75	2	12	52						
172	9	27							194	8	32	160	9	35	3	10	41						

Appendix 1 Radiata Pine

One Month Old Seedling Data

400R	400S	400N	500R	500S	500N	618R	618S	618N	631R	631S	631N	632R	632S	632N	645R	645S	645N	650R	650S	650N	657R	657S	657N
131	14	48							23	11	52	58	12	47	191	13	58						
56	6	25							121	6	21	67	16	61	192	19	73						
186	10	41							120	7	22	49	11	46	31	10	46						
32	7	21							179	6	21	43	17	77	34	11	44						
51	13	56							176	9	35	25	15	69	103	14	47						
45	9	39							15	9	28	21	12	44	127	10	45						
68	9	37							16	12	38	20	12	59	128	12	43						
50	10	38							17	10	29	36	11	49	197	10	41						
89	11	45										29	14	65	196	11	43						
87	7	24										40	8	35	175	14	53						
137	7	13										6	15	74	68	15	53						
10	12	39										161	14	52	62	23	62						
119	8	18										167	13	50	59	15	68						
198	10	44										150	17	66	52	11	46						
120	11	40													46	12	45						
188	12	46													48	15	46						
35	12	46													42	19	66						
127	11	35													45	12	45						

Appendix 1 Radiata Pine

One Month Old Seedling Data

665R	665S	665N	672R	672S	672N	692R	692S	692N	728R	728S	728N	734R	734S	734N	738R	738S	738N	754R	754S	754N	782R	782S	782N
170	5	20	151	6	18	190	8	33	43	13	44	176	6	19	49	5	14	130	10	32	66	8	31
9	8	33	124	8	23	185	8	28	45	11	38	162	8	19	48	7	14	54	11	41	32	7	21
141	8	36	163	8	26	179	6	25	34	14	42	53	8	23	45	8	26	48	6	20	65	10	37
143	8	31	145	7	21	16	6	22	52	12	38	67	5	14	10	6	25	16	9	35	97	6	21
103	11	46	144	7	23	187	6	27	33	14	50	57	7	18	188	6	21	88	10	45	17	8	29
106	7	36	167	6	20	14	10	35	20	14	47	80	5	14	12	6	20	41	9	33	95	7	27
130	8	33	97	7	19	13	7	24	54	8	31	184	7	30	178	7	17	4	11	47	11	9	31
151	9	33	93	7	23	12	5	21	18	10	36	167	9	28	58	6	15	68	10	41	108	11	34
92	9	30	85	8	30	7	6	18	90	12	39	94	6	21	63	5	14	92	8	28	104	15	50
73	7	33	78	7	24	38	6	18	160	11	46	182	8	24	31	5	15	67	12	40	102	9	28
69	10	42	63	8	23	66	9	26	159	9	34	93	5	20	166	8	21	96	9	31	31	9	34
58	7	30	35	7	23	60	7	24	170	15	54	135	7	25	141	7	22	94	10	30	20	7	15
64	8	40	91	9	23	6	9	33	30	13	45	143	7	22	152	6	16	79	5	10	34	7	29
68	10	37	74	8	26	30	11	31	145	11	46	177	7	19	104	6	18	52	11	40	29	10	32
93	8	34	92	9	24	31	6	20	15	8	30	97	4	8	79	6	19	72	8	19	112	11	45
43	8	37	11	5	16	117	8	29	179	10	39	175	7	23				32	11	45	199	13	45
19	11	47	9	12	37	111	11	33	199	9	38	23	8	20				43	8	27	180	8	31
38	8	28	6	6	20	128	8	27	46	7	23	27	5	13				42	8	24	198	11	45
12	12	44	5	8	28	130	7	26	26	10	29	42	7	28				195	8	34	177	9	31
16	7	22	2	7	27	125	6	18	12	12	34	43	7	21				2	12	50	163	10	32
4	9	39	1	5	16	124	7	24	168	11	48	75	5	17				177	10	38	121	8	23
2	7	29	103	7	20	87	7	18	167	9	33	71	6	23				173	8	27	122	9	31
11	9	33	128	5	14	139	6	22	164	6	27	172	8	25				152	6	14	179	11	39
192	9	42	108	7	27	145	9	34	151	10	44	194	6	22				156	9	25	10	12	43
178	8	36	179	8	23	148	8	24	95	6	6	19	6	20				103	10	36	11	8	23
175	9	33	166	6	20	149	7	20	134	10	41	39	7	21				151	10	40	54	9	27
29	8	34	164	6	15	164	9	38	136	10	38	4	6	24							60	7	24
7	11	42	131	8	28	173	8	24	114	10	31	5	5	19							185	12	61
173	7	25	127	4	16	174	7	22	122	6	16	14	7	27							145	19	31
50	9	39	69	7	21	69	9	36	125	9	38	1	7	21							160	10	41
			58	7	24	70	8	29	129	11	56	20	7	23							157	10	33
			102	9	21	75	5	16	142	10	42	10	5	14							150	8	31
			115	9	25	73	6	17	130	9	34	52	6	23							120	6	15
			119	8	29	72	8	20	93	7	22	166	7	30							143	10	38
			118	7	24	51	11	36	91	7	26	195	7	25							156	7	19
			105	8	22	55	5	16	174	12	55	156	8	27							118	11	43
			194	6	23	76	7	26	175	8	29	56	6	19							119	13	44
			192	5	16	53	6	21	110	9	36	186	8	27							154	11	45
			191	7	25	81	6	28	103	11	48	187	7	23							128	7	25

Appendix 1 Radiata Pine

One Month Old Seedling Data

665R	665S	665N	672R	672S	672N	692R	692S	692N	728R	728S	728N	734R	734S	734N	738R	738S	738N	754R	754S	754N	782R	782S	782N
			189	8	22	78	6	20	105	12	39	163	6	20							127	9	30
			169	6	19	79	7	34	73	11	39	192	7	23							126	11	33
			153	7	20	77	7	27	197	10	41	159	9	27							138	7	22
			173	7	25	105	7	24	74	8	24	62	6	28									
			22	6	18				61	7	31	96	8	20									
			86	6	19				85	13	41	32	7	26									
			195	7	23				98	10	29	55	6	20									
			18	8	29				118	6	19	81	4	8									
			60	8	21				133	10	37	28	8	25									
			19	7	19				86	11	39	31	9	18									
			46	9	29				181	11	44	78	7	23									
			47	6	22				186	11	49	85	7	26									
			48	5	20				89	13	41	316	8	23									
			30	8	26				80	10	30	66	6	17									
			23	6	18				77	10	31	65	5	18									
			25	7	21				96	9	33	64	5	17									
			13	9	28				36	8	25	63	5	17									
			10	7	24				11	10	36	147	8	25									
			15	8	25				57	9	23	118	7	20									
									2	10	42	123	7	18									
									1	12	44	126	8	24									
									87	13	46	120	6	21									
									37	13	41	101	8	29									
									35	7	28	117	9	23									
									196	8	35	111	8	29									
									195	8	35	110	9	27									
									32	14	47	109	7	26									
									158	13	50												
									200	10	36												
									40	7	26												
									41	8	25												
									88	7	28												
									176	14	55												
									9	9	27												

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
400	2	0.513	9.40	13.80	19.10	26.20	31.30	37.50	0.514	0.530	0.549	0.53	2.79	0.54	1.27	3.95
400	4	0.343	11.20	15.50	20.60	27.20	33.10	35.20	0.420	0.502	0.516	0.36	1.96	0.38	1.23	3.30
400	9	0.430	9.20	14.60	22.00	25.50	29.80	31.10	0.458	0.492	0.513	0.39	2.11	0.39	1.33	3.64
400	11	0.348	9.60	14.40	20.10	27.50	33.50	37.50	0.468	0.542	0.577	0.52	3.55	0.51	1.76	4.15
400	15	0.365	9.50	14.10	18.50	24.50	30.20	33.00	0.449	0.487	0.516	0.34	1.43	0.33	0.95	3.20
400	19	0.410	11.50	17.00	22.10	27.90	34.00	35.60	0.426	0.477	0.515	0.33	1.81	0.41	1.32	3.69
400	23	0.456	11.30	16.50	21.50	28.50	33.60	36.10	0.444	0.500	0.539	0.30	1.95	0.38	1.57	4.95
400	26	0.317	9.50	14.90	21.40	26.10	29.50	31.20	0.483	0.548	0.573	0.55	3.11	0.47	1.54	4.81
400	36	0.290	9.60	14.90	20.60	26.10	30.50	33.00	0.467	0.540	0.564	0.42	2.63	0.42	1.43	4.07
400	48	0.399	9.40	14.50	20.20	25.00	30.80	32.50	0.450	0.522	0.551	0.63	3.12	0.53	1.22	3.37
400	52	0.378	11.20	17.50	24.50	32.00	39.00	43.00	0.412	0.489	0.514	0.44	1.91	0.41	2.21	4.32
400	63	0.351	9.20	14.60	19.60	26.50	33.50	34.60	0.458	0.506	0.536	0.51	2.69	0.40	1.51	5.28
400	75	0.292	8.00	13.00	18.10	24.50	31.00	35.50	0.438	0.496	0.516	0.35	1.94	0.39	1.32	3.98
400	79	0.447	9.80	15.20	20.50	28.50	35.20	38.50	0.495	0.571	0.581	0.82	3.83	0.53	1.80	4.61
400	91	0.448	8.50	14.10	18.60	25.10	30.10	32.50	0.394	0.445	0.490	0.20	1.20	0.24	0.99	3.22
400	104	0.464	8.90	14.50	18.60	23.80	28.00	29.50	0.461	0.503	0.520	0.42	2.49	0.53	1.17	4.10
400	106	0.360	8.90	13.20	16.60	22.10	27.50	30.20	0.411	0.455	0.502	0.25	1.68	0.36	0.99	2.83
400	111	0.359	8.50	13.40	18.10	24.40	31.80	34.50	0.428	0.487	0.523	0.43	2.26	0.36	1.28	3.42
400	116	0.384	10.00	12.40	16.90	22.00	27.60	30.00	0.350	0.490	0.506	0.35	2.18	0.36	0.93	3.36
400	129	0.498	9.90	13.50	18.50	23.50	27.50	28.50	0.433	0.479	0.533	0.31	2.28	0.42	1.03	3.09
400	130	0.399	8.60	12.70	17.00	20.50	26.00	27.30	0.390	0.447	0.480	0.36	2.14	0.35	0.80	2.74
400	154	0.306	8.60	13.60	18.00	22.30	27.30	30.50	0.464	0.515	0.547	0.65	3.33	0.34	1.04	2.71
400	162	0.380	9.60	14.50	19.50	25.50	32.50	35.60	0.444	0.531	0.548	0.26	1.31	0.41	1.41	4.51
400	181	0.302	9.00	13.60	18.60	23.50	31.60	35.20	0.420	0.479	0.508	0.19	1.22	0.33	1.31	3.95
400	191	0.341	11.00	16.00	20.50	27.10	33.20	35.80	0.460	0.532	0.581	0.40	2.81	0.39	1.53	3.75
500	9	0.169	7.6	11.6	16.7	21.7	27.0	29.5	0.382	0.455	0.480	0.21	1.50	0.30	0.93	3.26
500	12	0.311	9.2	12.8	17.1	22.5	27.0	29.3	0.386	0.450	0.478	0.32	2.02	0.29	1.07	3.29
500	16	0.352	8.6	13.9	18.6	25.5	30.1	33.5	0.454	0.520	0.544	0 31	1.84	0.45	1.43	4.13
500	23	0.359	7.9	12.0	18.0	24.8	29.5	32.6	0.367	0.402	0.444	0.14	0.70	0.28	1.09	3.66
500	25	0.211	10.0	14.0	19.5	27.5	32.6	35.5	0.425	0.478	0.542	0.35	1.96	0.37	1.57	3.59
500	35	0.227	8.4	12.5	17.5	24.1	29.6	32.3	0.200	0.415	0.449	0.20	1.28	0.29	1.10	3.18
500	38	0.265	8.0	12.8	18.1	25.1	19.1	31.2	0.387	0.442	0.488	0.23	1.00	0.37	1.02	3.17
500	39	0.291	10.5	15.6	21.0	27.5	35.4	38.5	0.404	0.469	0.534	0.12	0.86	0.24	1.73	3.71
500	44	0.334	8.6	13.0	18.2	24.6	32.3	33.4	0.463	0.512	0.556	0.30	1.60	0.34	1.36	3.63
500	60	0.281	9.6	13.0	15.5	23.5	29.3	31.4	0.435	0.483	0.506	0.43	2.53	0.33	1.21	3.29
500	67	0.437	8.1	13.5	17.9	24.5	30.4	33.5	0.394	0.434	0.468	0.33	1.50	0.40	1.03	3.02
500	73	0.316	9.5	14.5	19.6	27.1	34.0	36.0	0.475	0.531	0.576	0.22	1.05	0.47	1.85	4.79
500	77	0.252	9.7	14.3	19.6	25.6	30.6	34.0	0 399	0.432	0.473	0.33	1.20	0.32	1.23	3.29

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
500	79	0.334	8.5	13.0	18.0	24.6	30.0	32.5	0.368	0.413	0.438	0.25	1.33	0.21	1.10	2.81
500	112	0.219	6.5	10.5	14.5	20.0	25.3	28.1	0.393	0.452	0.488	0.36	1.52	0.33	0.87	2.78
500	114	0.228	10.5	14.5	20.0	25.7	31.5	34.5	0.450	0.536	0.562	0.50	3.05	0.40	1.25	3.54
500	146	0.326	9.2	14.2	18.6	23.5	29.0	31.2	0.436	0.489	0.567	0.39	2.94	0.30	0.94	2.53
500	150	0.227	10.1	15.1	21.1	27.5	34.5	37.1	0.400	0.502	0.513	0.27	1.53	0.27	1.55	4.14
500	153	0.290	8.4	13.0	18.1	24.5	30.2	33.1	0.385	0.427	0.473	0.20	1.41	0.23	1.11	2.94
500	181	0.267	8.6	14.2	20.5	27.0	32.8	34.2	0.445	0.509	0.536	0.34	1.40	0.28	1.24	4.07
500	187	0.317	8.6	13.2	16.6	21.5	25.1	26.7	0.422	0.446	0.480	0.29	1.84	0.31	1.09	4.15
500	189	0.178	9.1	14.6	20.0	26.5	30.4	32.1	0.408	0.487	0.522	0.34	1.76	0.25	1.25	4.07
500	191	0.257	9.9	14.5	20.5	27.4	34.1	36.2	0.498	0.526	0.551	0.55	2.60	0.27	1.44	3.98
500	193	0.190	9.5	14.6	20.1	26.8	33.5	36.7	0.418	0.479	0.535	0.38	1.79	0.36	1.21	3.53
500	195	0.194	8.5	13.1	18.5	23.5	29.0	31.4	0.452	0.541	0.562	0.52	2.81	0.34	1.31	3.32
618	14	0.399	9.1	15.1	21.6	28.9	36.4	41.0	0.475	0.522	0.570	0.26	1.54	0.47	1.82	4.56
618	15	0.499	7.6	13.1	18.3	25.5	32.5	36.1	0.459	0.524	0.559	0.25	1.94	0.46	1.25	3.85
618	28	0.471	9.8	15.1	21.0	28.5	36.5	39.4	0.476	0.534	0.570	0.38	2.08	0.40	1.89	5.10
618	47	0.323	8.2	13.5	19.3	26.0	33.6	36.7	0.438	0.511	0.531	0.47	2.35	0.44	1.70	4.06
618	80	0.387	8.4	13.1	18.2	23.5	29.3	32.5	0.436	0.500	0.536	0.41	1.73	0.37	1.45	4.15
618	81	0.383	8.2	12.6	18.0	24.6	30.6	32.6	0.418	0.512	0.533	0.42	2.25	0.43	1.07	3.69
618	88	0.478	9.0	14.7	21.4	28.8	34.5	37.1	0.490	0.594	0.634	0.55	2.42	0.45	1.74	5.11
618	93	0.322	8.2	12.9	19.7	25.7	32.6	35.3	0.437	0.521	0.560	0.49	2.58	0.39	1.64	4.16
618	95	0.431	6.5	10.6	14.6	18.2	23.5	26.5	0.418	0.460	0.476	0.22	1.21	0.30	0.88	2.27
618	99	0.549	9.0	14.6	19.8	25.5	30.0	31.5	0.543	0.632	0.678	0.17	1.17	0.71	2.96	6.21
618	108	0.350	6.2	9.8	14.5	20.0	23.6	25.5	0.439	0.474	0.490	0.22	1.38	0.44	0.87	3.24
618	125	0.490	10.1	16.2	22.5	30.1	36.7	39.5	0.471	0.507	0.531	0.34	1.42	0.34	1.81	4.61
618	142	0.434	9.8	14.5	21.0	28.3	34.6	37.5	0.501	0.556	0.586	0.36	1.88	0.46	1.94	5.75
618	143	0.407	8.9	13.5	18.6	25.6	32.5	35.7	0.435	0.494	0.552	0.22	0.97	0.30	1.54	4.25
618	152	0.418	9.1	13.6	19.1	25.2	32.1	33.8	0.489	0.551	0.576	0.58	1.54	0.53	1.73	4.46
618	154	0.476	9.4	14.1	17.7	23.5	26.2	27.6	0.430	0.514	0.527	0.27	1.97	0.37	1.01	3.11
618	166	0.468	9.5	14.5	21.8	30.1	36.6	39.5	0.445	0.511	0.533	0.28	2.18	0.37	1.59	3.78
618	182	0.439	10.8	16.5	24.4	32.1	38.5	40.5	0.484	0.564	0.609	0.28	1.32	0.60	1.94	5.58
618	183	0.384	7.6	11.0	15.7	22.0	27.5	30.2	0.300	0.534	0.564	0.30	1.37	0.35	1.49	4.70
618	187	0.231	6.6	10.4	14.5	20.1	25.6	29.5	0.432	0.451	0.485	0.18	1.35	0.32	0.89	3.63
618	188	0.422	10.3	17.1	23.5	30.7	36.8	40.0	0.490	0.542	0.603	0.49	2.14	0.43	1.77	4.58
618	191	0.278	9.2	15.5	20.5	27.1	32.6	34.5	0.503	0.586	0.598	0.78	2.56	0.44	1.37	3.87
618	194	0.360	8.8	14.6	20.4	27.2	35.0	37.6	0.476	0.537	0.583	0.53	2.26	0.52	1.50	3.51
618	196	0.174	8.5	14.0	19.0	25.1	30.4	33.5	0.441	0.490	0.511	0.27	1.48	0.36	1.30	4.06
618	197	0.477	9.6	15.5	21.4	27.5	33.7	35.6	0.448	0.525	0.560	0.36	2.18	0.41	1.59	3.70
631	3	0.266	8.1	13.2	18.9	25.5	31.7	35.1	0.412	0.474	0.523	0.25	1.28	0.42	1.68	4.00

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
631	5	0.287	6.9	10.8	14.8	20.2	25.5	28.1	0.382	0.449	0.470	0.29	1.45	0.27	0.95	2.73
631	10	0.275	7.2	11.0	16.1	22.3	28.6	31.0	0.390	0.459	0.478	0.23	1.66	0.38	1.17	3.75
631	58	0.244	8.5	14.5	21.1	28.5	35.0	37.6	0.453	0.507	0.538	0.53	2.13	0.42	1.91	4.30
631	64	0.290	8.2	12.4	17.0	24.5	30.5	32.5	0.448	0.507	0.551	0.37	1.87	0.34	1.33	4.05
631	77	0.269	8.3	13.1	18.1	25.5	30.2	34.1	0.397	0.486	0.509	0.42	2.77	0.35	1.32	4.20
631	85	0.341	7.5	11.4	17.0	22.6	29.3	33.1	0.408	0.449	0.476	0.23	1.21	0.29	1.21	3.32
631	101	0.347	9.5	14.3	19.0	24.5	29.6	31.5	0.471	0.556	0.584	0.67	3.05	0.43	1.36	2.70
631	105	0.322	7.2	11.4	16.8	24.0	29.1	32.5	0.389	0.424	0.448	0.11	0.93	0.28	0.98	3.37
631	118	0.357	9.3	15.0	20.6	25.1	28.1	30.3	0.462	0.507	0.600	0.47	2.09	0.46	1.03	3.65
631	123	0.297	10.6	16.0	21.1	27.1	33.5	35.5	0.424	0.472	0.503	0.36	2.10	0.39	1.58	4.24
631	134	0.299	7.3	10.4	13.1	17.6	23.0	24.6	0.388	0.446	0.494	0.32	1.59	0.38	0.85	2.91
631	136	0.323	11.5	17.0	22.5	28.5	33.5	35.5	0.453	0.512	0.555	0.29	1.29	0.48	1.76	4.00
631	137	0.301	10.6	16.4	22.2	28.5	35.5	39.5	0.427	0.487	0.516	0.39	1.40	0.42	1.59	2.92
631	168	0.293	8.6	13.5	19.5	25.2	29.2	31.5	0.413	0.480	0.518	0.27	1.60	0.44	1.12	3.89
631	169	0.396	7.9	13.5	20.0	27.5	33.6	37.5	0.395	0.446	0.480	0.11	0.70	0.27	1.22	3.55
631	173	0.288	7.0	10.9	14.6	21.1	26.3	29.5	0.351	0.431	0.456	0.28	1.30	0.30	0.81	2.33
631	174	0.192	9.5	14.6	19.9	25.8	31.2	33.5	0.414	0.455	0.564	0.22	1.10	0.35	1.15	3.43
631	179	0.283	8.7	12.6	17.2	23.7	29.1	31.5	0.394	0.447	0.480	0.23	1.43	0.34	1.19	3.97
631	183	0.255	10.2	15.5	19.6	26.5	33.1	36.6	0.408	0.465	0.509	0.52	2.56	0.39	1.30	3.70
631	187	0.233	8.1	12.6	18.1	24.5	31.5	34.2	0.160	0.467	0.513	0.16	0.79	0.38	1.29	4.18
631	189	0.286	8.1	12.5	17.5	22.8	27.1	28.5	0.427	0.470	0.518	0.36	2.61	0.46	0.99	3.94
631	190	0.255	9.2	13.9	18.0	26.5	32.3	36.2	0.411	0.468	0.510	0.18	1.62	0.31	1.51	3.99
631	191	0.304	9.6	14.5	20.6	27.6	33.5	34.6	0.398	0.480	0.518	0.46	1.60	0.31	1.49	3.61
631	195	0.256	8.0	12.7	17.0	21.5	26.1	27.5	0.396	0.457	0.483	0.26	0.88	0.25	1.28	3.47
632	13	0.308	9.9	15.0	21.0	28.5	34.5	36.2	0.441	0.481	0.488	0.49	2.20	0.36	1.24	3.83
632	16	0.463	10.6	17.1	23.5	29.5	35.0	36.5	0.466	0.545	0.557	0.50	2.55	0.42	1.97	4.93
632	23	0.381	10.5	15.4	21.0	27.5	32.5	33.6	0.468	0.538	0.545	0.43	2.33	0.25	1.20	3.36
632	48	0.305	10.5	16.0	22.5	27.5	33.5	36.2	0.461	0.516	0.525	0.29	1.65	0.39	1.77	4.34
632	62	0.407	9.0	12.6	19.0	24.2	30.0	32.8	0.446	0.523	0.531	0.34	1.76	0.35	1.34	4.30
632	64	0.377	12.2	18.0	25.5	31.5	35.5	38.5	0.489	0.562	0.604	0.55	2.68	0.55	2.37	3.83
632	89	0.406	8.6	14.2	19.4	25.5	32.5	34.2	0.515	0.561	0.584	0.50	3.12	0.44	1.35	3.01
632	96	0.458	9.2	14.0	18.1	25.9	33.5	36.1	0.431	0.469	0.504	0.32	1.74	0.40	1.54	4.32
632	102	0.379	11.5	17.6	24.5	31.5	35.6	36.7	0.412	0.459	0.485	0.28	1.39	0.28	1.45	2.95
632	110	0.418	9.2	14.7	20.6	18.1	33.2	36.3	0.477	0.531	0.562	0.41	3.14	0.52	1.55	4.64
632	113	0.357	8.5	12.2	16.5	23.0	28.5	31.5	0.351	0.398	0.431	0.32	1.52	0.28	1.03	2.64
632	122	0.106	11.5	14.0	22.3	29.1	35.1	37.5	0.496	0.534	0.536	0.42	1.88	0.41	1.40	4.00
632	127	0.516	11.0	15.9	21.1	28.1	35.1	37.0	0.492	0.513	0.531	0.50	2.90	0.49	1.55	4.66
632	135	0.278	10.3	15.5	22.0	28.5	33.6	35.1	0.481	0.542	0.550	0.65	2.99	0.59	1.53	4.60

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
632	137	0.336	10.2	16.5	22.5	29.2	36.7	37.6	0.477	0.517	0.543	0.39	2.66	0.36	1.83	4.87
632	143	0.270	10.2	14.5	21.2	27.0	32.1	35.5	0.444	0.501	0.537	0.21	0.89	0.35	1.58	4.14
632	170	0.366	10.2	16.0	21.2	30.6	35.1	37.5	0.466	0.539	0.568	0.32	1.72	0.47	1.89	4.47
632	172	0.350	9.5	13.7	20.1	28.2	35.1	37.5	0.446	0.536	0.587	0.63	2.40	0.44	1.34	3.68
632	181	0.093	12.0	17.6	24.0	32.2	36.4	38.5	0.492	0.535	0.550	0.42	2.18	0.48	1.58	4.23
632	183	0.405	8.5	12.5	17.5	24.5	30.8	34.5	0.404	0.476	0.504	0.25	1.94	0.37	1.29	3.84
632	185	0.413	9.1	13.6	20.0	25.0	31.5	33.5	0.426	0.499	0.515	0.43	1.98	0.44	1.45	4.11
632	188	0.383	9.9	16.0	23.1	31.5	39.6	42.5	0.488	0.531	0.568	0.75	3.53	0.53	1.90	4.62
632	190	0.327	10.2	16.0	22.0	29.6	36.1	37.6	0.472	0.528	0.566	0.50	2.09	0.58	1.54	3.72
632	191	0.408	8.5	13.9	18.9	25.2	32.2	34.3	0.467	0.498	0.526	0.41	2.13	0.42	1.31	3.85
632	196	0.449	10.5	17.0	22.8	30.8	38.6	42.5	0.487	0.539	0.574	0.31	1.66	0.42	1.98	3 82
645	1	0.373	8.2	13.0	16.7	22.0	27.0	29.1	0.475	0.505	0.527	0.67	3.53	0.38	1.07	2.68
645	16	0.362	9.0	13.5	17.1	23.5	27.1	29.5	0.435	0.458	0.496	0.24	1.31	0.31	1.00	2.75
645	33	0.377	10.4	16.6	21.6	29.9	35.2	36.5	0.445	0.496	0.499	0.33	2.08	0.35	1.42	4.76
645	47	0.318	9.8	15.5	20.6	27.5	32.5	35.5	0.518	0.554	0.610	0.76	3.32	0.46	1.42	4.17
645	65	0.267	9.9	15.0	19.2	24.6	30.6	32.3	0.501	0.545	0.574	0.79	4.63	0.39	1.35	3.41
645	72	0.316	11.2	15.0	19.5	24.3	30.5	31.8	0.452	0.529	0.571	0.45	2.46	0.41	1.31	3.58
645	74	0.285	9.5	13.5	18.5	24.3	30.5	32.1	0.493	0.557	0.588	0.73	4.36	0.31	1.36	3.37
645	79	0.335	10.0	16.2	22.0	28.0	33.6	30.1	0.460	0.514	0.558	0.54	3.04	0.39	1.27	2.85
645	85	0.404	8.1	12.5	18.1	24.5	31.0	32.5	0.416	0.523	0.581	0.36	2.01	0.34	1.33	3.82
645	86	0.258	7.6	11.9	17.2	24.5	31.0	33.5	0.441	0.499	0.574	0.44	2.21	0.30	1.03	3.19
645	87	0.325	7.0	11.0	14.0	18.5	23.4	25.0	0.435	0.481	0.533	0.79	2.55	0.28	0.73	2.43
645	91	0.335	7.1	10.9	14.5	19.5	23.5	25.2	0.441	0.474	0.515	0.44	1.20	0.39	0.83	3.26
645	95	0.508	10.2	15.6	21.1	27.5	32.5	34.5	0.458	0.515	0.546	0.28	2.07	0.37	1.57	3.59
645	97	0.339	6.5	12.0	18.7	24.5	30.2	32.5	0.430	0.504	0.546	0.50	2.36	0.49	0.98	3.84
645	98	0.398	8.6	12.0	17.2	22.1	26.5	33.7	0.500	0.543	0.593	0.50	2.29	0.37	1.24	4.00
645	110	0.362	8.5	12.6	17.1	23.5	29.4	32.5	0.381	0.452	0.531	0.18	0.99	0.35	1.19	3.54
645	113	0.339	8.4	13.1	18.5	23.6	30.1	32.4	0.440	0.505	0.537	0.23	1.31	0.43	1.15	4.09
645	126	0.233	9.5	14.3	19.6	27.4	34.6	38.2	0.441	0.527	0.556	0.29	1.35	0.40	1.36	3.92
645	133	0.323	8.1	12.2	18.1	23.2	28.3	29.5	0.428	0.478	0.505	0.38	1.78	0.34	1.08	3.06
645	140	0.404	11.6	17.3	23.0	30.0	36.8	40.0	0.440	0.504	0.542	0.27	1.33	0.41	1.56	4.15
645	159	0.391	11.0	17.5	23.9	30.5	36.2	38.5	0.475	0.550	0.588	0.46	1.85	0.47	1.97	4.47
645	169	0.397	8.9	14.0	18.4	23.5	28.5	31.5	0.444	0.453	0.496	0.20	1.21	0.34	1.06	3.63
645	180	0.313	10.5	16.6	24.4	31.5	37.9	41.1	0.515	0.603	0.611	1.00	3.54	0.58	1.79	3.80
645	186	0.263	10.0	14.1	20.1	26.5	33.2	36.0	0.475	0.561	0.591	0.59	2.32	0.38	1.43	3.65
645	199	0.377	7.9	12.0	17.7	23.6	29.6	36.2	0.390	0.475	0.514	0.39	1.98	0.40	1.20	3.55
650	1	0.425	8.1	13.9	19.5	25.2	31.5	34.2	0.416	0.472	0.496	0.20	1.23	0.28	1.30	4.71
650	4	0.381	7.6	11.1	15.5	23.5	29.6	32.6	0.433	0.485	0.514	0.46	2.53	0.49	1.29	3.89

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
650	6	0.269	6.5	10.2	14.1	17.5	23.3	25.7	0.347	0.407	0.436	0.21	1.01	0.26	0.78	2.53
650	9	0.395	8.3	13.1	18.5	24.5	30.5	34.5	0.414	0.493	0.514	0.47	2.76	0.44	1.21	3.53
650	10	0.386	9.1	13.5	17.0	21.2	26.5	29.6	0.418	0.453	0.518	0.52	2.60	0.44	1.23	3.16
650	47	0.339	8.0	11.5	15.5	20.0	25.0	27.4	0.430	0.524	0.539	0.23	1.40	0.41	0.91	3.27
650	51	0.404	9.4	14.2	20.5	27.8	33.6	36.5	0.452	0.514	0.536	0.49	2.59	0.33	1.46	3.93
650	64	0.488	8.0	12.8	18.0	24.7	31.6	35.1	0.397	0.467	0.492	0.27	1.53	0.42	1.26	3.47
650	86	0.410	7.5	12.0	16.1	22.2	27.2	29.5	0.421	0.487	0.524	0.31	2.31	0.37	1.57	4.24
650	88	0.392	8.2	13.1	18.6	20.5	30.5	32.5	0.402	0.488	0.510	0.33	1.97	0.30	1.15	3.63
650	92	0.348	7.2	11.8	16.5	22.5	28.5	31.1	0.368	0.421	0.445	0.32	2.09	0.34	0.96	2.87
650	97	0.356	8.5	12.9	18.5	25.0	30.8	33.1	0.423	0.479	0.503	0.34	2.06	0.44	1.08	3.69
650	100	0.318	8.0	12.5	18.2	24.5	30.4	33.4	0.405	0.445	0.484	0.27	1.25	0.37	1.13	3.38
650	108	0.398	7.4	11.6	15.8	21.2	24.5	26.5	0.469	0.494	0.517	0.42	1.99	0.32	0.99	2.67
650	113	0.396	11.4	16.5	21.1	27.6	33.1	35.6	0.423	0.479	0.506	0.30	2.18	0.33	1.17	2.74
650	119	0.244	9.2	14.0	19.5	24.5	30.3	32.3	0.399	0.473	0.503	0.28	1.37	0.32	1.26	3.47
650	127	0.337	8.1	13.5	18.0	25.1	31.2	35.5	0.394	0.415	0.451	0.23	0.80	0.27	1.27	2.90
650	151	0.401	7.5	11.1	16.7	22.5	27.5	29.5	0.391	0.436	0.469	0.26	1.78	0.30	0.99	3.16
650	160	0.482	8.2	13.6	18.7	26.8	33.2	36.5	0.425	0.495	0.534	0.28	1.59	0.49	1.59	4.36
650	176	0.407	10.0	14.5	20.0	26.4	32.3	35.9	0.350	0.508	0.555	0.35	1.87	0.49	0.81	2.33
650	181	0.379	9.7	13.9	19.1	26.1	32.0	34.5	0.415	0.468	0.484	0.17	0.98	0.42	1.25	3.56
650	187	0.343	8.8	14.9	20.4	26.2	34.0	37.6	0.401	0.465	0.474	0.23	1.26	0.34	1.45	4.00
650	188	0.298	8.2	12.7	17.5	23.5	28.4	31.2	0.356	0.418	0.460	0.16	1.45	0.28	0.88	2.29
650	189	0.263	9.5	10.9	15.2	21.0	28.0	31.1	0.376	0.443	0.477	0.19	0.99	0.38	0.90	2.75
650	198	0.304	8.2	12.0	16.2	21.2	24.5	27.2	0.344	0.389	0.401	0.23	1.33	0.20	0.64	2.44
657	9	0.262	7.2	11.6	15.6	21.5	27.6	29.7	0.364	0.419	0.443	0.17	1.26	0.28	0.88	3.24
657	16	0.312	8.9	13.0	18.1	24.2	30.7	23.5	0.423	0.452	0.474	0.14	0.71	0.33	1.06	2.95
657	20	0.241	6.2	10.7	15.2	22.1	27.8	31.5	0.409	0.462	0.511	0.19	1.03	0.23	0.92	2.60
657	27	0.232	4.1	8.1	13.4	20.2	26.1	28.5	0.323	0.368	0.398	0.09	0.30	0.18	0.78	2.34
657	31	0.321	9.3	14.9	19.6	27.0	34.1	37.2	0.444	0.507	0.540	0.21	1.05	0.39	1.36	3.28
657	41	0.303	5.4	8.8	12.5	17.5	21.5	24.6	0.317	0.380	0.417	0.11	0.85	0.23	0.63	2.06
657	42	0.323	6.5	10.1	14.8	21.6	28.0	31.2	0.414	0.490	0.515	0.22	1.46	0.26	1.19	3.04
657	47	0.270	8.2	12.4	18.6	24.5	29.2	33.2	0.440	0.482	0.507	0.25	1.19	0.37	1.37	3.28
657	53	0.271	6.2	12.2	17.6	23.2	28.5	32.3	0.364	0.425	0.444	0.12	0.88	0.25	0.99	2.15
657	88	0.212	6.2	11.1	16.5	21.6	27.6	29.5	0.373	0.443	0.461	0.13	0.85	0.24	1.07	3.14
657	94	0.271	7.5	10.7	17.0	21.5	27.0	29.1	0.447	0.466	0.512	0.33	1.41	0.25	1.08	3.13
657	95	0.229	4.4	9.3	14.4	20.2	27.6	30.4	0.328	0.385	0.424	0.09	0.34	0.17	0.81	2.20
657	97	0.275	9.0	13.7	20.3	26.8	34.1	36.1	0.456	0.528	0.563	0.22	1.06	0.34	1.60	3.97
657	100	0.188	7.9	12.5	19.0	26.2	33.5	36.7	0.440	0.477	0.515	0.25	1.47	0.28	1.44	4.23
657	106	0.227	6.5	10.1	13.6	19.5	25.2	28.5	0.418	0.445	0.483	0.16	1.15	0.22	0.82	2.17

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
657	107	0.269	8.2	12.2	16.6	22.5	27.5	29.5	0.431	0.488	0.569	0.32	1.57	0.28	1.04	3.13
657	109	0.260	6.5	10.0	14.2	20.0	24.7	25.6	0.417	0.464	0.488	0.27	1.39	0.28	0.63	2.30
657	113	0.287	7.6	11.8	15.5	20.5	26.5	29.2	0.419	0.506	0.525	0.30	1.69	0.29	1.26	3.54
657	136	0.215	7.1	10.5	14.5	21.1	25.7	28.1	0.417	0.427	0.478	0.11	0.48	0.22	0.98	3.07
657	177	0.184	7.5	12.0	16.5	22.5	28.2	31.3	0.397	0.445	0.493	0.39	1.70	0.28	1.09	3.06
657	187	0.274	10.5	15.6	21.5	26.7	33.5	36.5	0.412	0.434	0.464	0.13	0.90	0.26	1.52	4.22
657	188	0.299	8.4	12.0	16.6	21.6	25.5	27.5	0.489	0.514	0.575	0.31	1.71	0.30	1.09	3.20
657	189	0.296	9.0	13.9	19.5	25.5	30.7	33.2	0.429	0.509	0.518	0.32	1.55	0.44	1.11	3.82
657	194	0.245	7.5	11.6	17.6	23.3	29.0	31.5	0.401	0.491	0.505	0.40	1.71	0.27	1.20	3.69
657	195	0.199	7.6	11.5	15.1	20.6	25.5	28.5	0.426	0.487	0.512	0.29	1.60	0.22	1.11	3.41
665	17	0.278	8.40	12.60	18.60	24.5	31.3	33.5	0.407	0.480	0.495	0.44	2.46	0.37	1.29	4.42
665	25	0.255	10.50	15.60	22.10	27.2	30.6	31.3	0.443	0.533	0.562	0.67	2.67	0.36	1.26	4.00
665	47	0.275	9.00	14.70	20.20	27.0	32.0	34.5	0.461	0.513	0.555	0.62	2.67	0.45	1.73	3.51
665	85	0.277	8.30	12.80	18.90	22.5	27.1	28.2	0.428	0.488	0.512	0.40	2.20	0.30	0.98	3.82
665	91	0.258	8.50	13.60	19.20	26.8	35.4	37.2	0.456	0.550	0.581	0.46	2.25	0.40	1.89	4.39
665	97	0.306	7.10	12.20	17.50	24.1	31.5	34.5	0.378	0.449	0.499	0.29	1.42	0.26	1.15	3.29
665	98	0.237	8.50	14.10	20.10	27.6	35.3	38.2	0.445	0.537	0.568	0.23	1.51	0.36	1.65	4.71
665	102	0.250	9.00	12.40	16.50	21.5	28.2	30.3	0.400	0.444	0.499	0.23	1.55	0.32	1.08	3.13
665	108	0.273	8.90	12.40	17.50	23.5	27.5	28.5	0.382	0.429	0.471	0.25	1.54	0.32	0.86	3.21
665	112	0.316	10.50	15.60	22.00	28.5	34.5	38.1	0.468	0.534	0.559	0.42	2.51	0.44	1.51	4.00
665	117	0.254	9.10	13.30	19.20	25.6	31.4	34.1	0.410	0.453	0.473	0.32	1.84	0.27	1.17	4.06
665	134	0.314	10.50	15.40	21.50	27.2	34.5	37.1	0.446	0.502	0.518	0.53	2.38	0.34	1.41	4.64
665	135	0.312	8.20	12.60	17.60	24.3	29.0	30.6	0.499	0.525	0.569	0.43	1.67	0.45	1.41	4.55
665	139	0.300	9.80	15.10	20.40	29.0	35.2	39.8	0.364	0.404	0.441	0.04	0.29	0.24	1.11	3.09
665	160	0.257	10.00	12.90	18.00	23.8	30.4	32.5	0.440	0.510	0.522	0.23	1.47	0.31	1.26	3.93
665	162	0.276	8.60	12.50	17.00	23.4	30.2	33.2	0.381	0.443	0.492	0.23	1.51	0.37	1.11	3.60
665	164	0.283	10.00	14.00	19.50	25.2	30.5	32.5	0.420	0.489	0.518	0.26	1.12	0.31	1.28	3.83
665	182	0.343	9.80	14.00	18.50	25.5	32.2	35.2	0.386	0.439	0.453	0.20	1.18	0.30	1.21	3.60
665	187	0.229	8.40	12.90	17.40	23.0	27.1	29.1	0.381	0.440	0.469	0.25	1.34	0.26	1.06	3.11
665	190	0.240	9.70	15.00	20.10	26.7	32.5	36.4	0.449	0.498	0.527	0.41	2.02	0.31	1.60	4.44
665	196	0.236	8.90	14.20	20.00	27.5	34.1	37.1	0.444	0.522	0.594	0.50	2.64	0.43	1.46	4.84
665	198	0.212	9.30	12.60	16.50	21.0	27.6	30.0	0.408	0.484	0.533	0.31	1.87	0.30	1.01	2.77
665	199	0.232	10.10	15.40	21.50	27.5	34.5	38.5	0.220	0.458	0.497	0.22	1.27	0.31	1.45	4.02
665	200	0.234	9.50	14.10	19.20	24.1	30.5	33.5	0.391	0.458	0.496	0.25	1.69	0.26	0.93	3.59
665	201	0.207	8.50	13.50	18.10	23.5	28.5	29.8	0.392	0.414	0.458	0.18	1.21	0.31	1.15	2.93
672	4	0.332	9.8	13.3	19.1	26.6	31.8	32.5	0.415	0.496	0.505	0.21	1.10	0.42	1.72	3.61
672	7	0.266	8.0	10.5	15.5	20.5	26.8	29.2	0.367	0.437	0.462	0.39	2.49	0.35	0.90	2.96
672	16	0.314	7.6	14.3	17.5	25.5	31.6	33.7	0.368	0.408	0.438	0.20	1.36	0.29	1.22	3.15

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
672	44	0.326	7.5	12.4	19.1	24.3	31.1	34.1	0.372	0.431	0.444	0.16	1.11	0.28	1.21	2.99
672	52	0.278	8.5	13.5	18.0	25.5	32.5	36.1	0.362	0.407	0.432	0.15	0.86	0.28	1.11	2.61
672	57	0.278	8.1	12.5	17.6	23.6	29.5	32.1	0.438	0.495	0.527	0.46	2.19	0.42	1.14	3.60
672	76	0.253	6.4	9.7	13.5	19.3	24.0	26.0	0.384	0.444	0.481	0.26	1.28	0.36	0.73	2.71
672	81	0.200	6.2	10.9	15.5	22.2	27.6	30.3	0.367	0.424	0.455	0.12	0.72	0.26	1.13	4.10
672	82	0.276	7.6	12.9	19.0	26.5	34.3	38.1	0.406	0.468	0.493	0.14	1.07	0.39	1.45	4.44
672	84	0.304	8.5	13.2	16.4	22.5	28.1	30.5	0.377	0.441	0.457	0.22	0.96	0.34	1.05	2.38
672	89	0.282	7.5	12.2	18.5	24.7	30.6	32.6	0.481	0.560	0.601	0.79	3.61	0.44	1.54	3.41
672	94	0.325	8.2	12.9	18.5	26.7	33.0	35.6	0.416	0.456	0.495	0.21	1.16	0.30	1.41	4.53
672	96	0.282	6.9	10.3	16.2	21.5	27.1	30.0	0.391	0.453	0.477	0.24	1.56	0.29	0.96	2.66
672	100	0.258	7.6	11.3	16.2	22.1	28.2	31.0	0.355	0.418	0.450	0.15	0.97	0.30	0.93	3.11
672	107	0.346	6.6	11.2	17.2	24.1	28.3	32.2	0.360	0.409	0.433	0.07	0.33	0.31	1.04	2.74
672	110	0.237	7.4	11.1	16.5	22.1	28.5	31.6	0.411	0.463	0.486	0.26	1.40	0.32	1.16	3.11
672	112	0.299	8.2	12.6	19.5	26.5	35.7	38.2	0.389	0.475	0.505	0.30	1.00	0.37	1.77	4.43
672	113	0.270	7.4	11.0	16.1	22.5	29.0	33.0	0.412	0.461	0.502	0.28	1.57	0.29	1.16	3.11
672	122	0.250	5.6	9.9	12.7	18.5	22.5	24.3	0.330	0.387	0.422	0.04	0.45	0.29	0.66	1.91
672	136	0.307	6.7	8.9	12.2	15.1	18.5	20.6	0.323	0.375	0.407	0.17	1.35	0.26	0.53	2.21
672	157	0.290	8.2	12.1	17.4	22.8	28.3	31.0	0.407	0.455	0.499	0.38	2.32	0.36	1.10	3.75
672	165	0.301	6.5	10.5	14.3	20.4	27.0	29.2	0.419	0.452	0.496	0.17	2.42	0.38	0.95	3.12
672	190	0.301	8.3	13.9	19.8	27.5	36.0	39.8	0.450	0.515	0.543	0.34	1.86	0.49	1.73	4.42
672	196	0.301	9.1	13.5	19.1	25.6	32.1	34.1	0.449	0.496	0.537	0.40	2.11	0.43	1.19	3.99
672	198	0.295	9.5	14.1	19.5	25.8	32.0	35.2	0.423	0.462	0.494	0.23	1.17	0.26	1.43	3.87
692	1	0.303	8.0	13.6	19.1	27.5	35.4	39.0	0.382	0.420	0.468	0.18	0.82	0.30	1.38	3.77
692	15	0.312	8.5	13.0	19.1	28.2	35.5	38.2	0.427	0.478	0.516	0.19	0.98	0.28	1.57	4.25
692	34	0.271	8.6	14.1	20.1	28.0	35.1	38.4	0.460	0.500	0.523	0.44	2.28	0.37	1.33	3.70
692	43	0.339	10.0	15.1	21.2	28.5	36.0	39.7	0.400	0.448	0.485	0.51	2.13	0.38	1.48	4.09
692	44	0.310	7.3	10.6	26.6	22.0	28.3	31.5	0.345	0.418	0.430	0.28	1.56	0.22	0.95	2.63
692	47	0.285	8.6	14.0	18.6	26.8	34.5	38.5	0.398	0.465	0.484	0.09	0.64	0.39	1.52	4.48
692	56	0.305	7.4	11.3	15.6	21.1	25.7	27.5	0.432	0.503	0.519	0.30	1.88	0.32	1.03	2.96
692	71	0.258	8.6	12.3	17.9	24.1	29.5	32.6	0.428	0.472	0.500	0.26	2.04	0.26	1.15	4.11
692	90	0.280	9.4	13.9	19.5	27.1	33.6	37.2	0.364	0.400	0.436	0.28	1.55	0.25	1.01	2.42
692	94	0.345	8.0	12.0	17.7	25.0	31.0	33.6	0.394	0.469	0.504	0.28	1.62	0.27	1.27	3.74
692	96	0.277	8.1	11.0	15.6	22.6	28.5	31.5	0.449	0.478	0.488	0.32	1.69	0.31	1.33	3.81
692	98	0.267	8.6	12.8	18.5	25.1	32.5	35.4	0.439	0.505	0.520	0.36	1.61	0.38	1.52	3.95
692	100	0.351	6.6	11.1	16.4	21.6	28.5	31.7	0.414	0.470	0.508	0.34	1.62	0.27	1.10	3.19
692	103	0.292	8.3	13.2	17.4	22.6	28.5	31.7	0.454	0.520	0.548	0.33	2.12	0.37	1.25	3.90
692	106	0.348	9.2	13.2	18.1	25.0	31.6	34.5	0.290	0.469	0.518	0.29	2.33	0.31	1.32	3 42
692	108	0.313	9.8	15.3	21.0	29.2	36.5	39.7	0.400	0.471	0.513	0.14	1.00	0.26	1.70	4 14

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
692	110	0.210	8.6	13.8	19.9	27.0	34.0	37.5	0.414	0.487	0.508	0.34	1.45	0.34	1.31	3.98
692	112	0.296	8.7	14.2	19.6	26.5	32.1	34.7	0.443	0.497	0.509	0.41	0.04	0.37	1.31	3.79
692	135	0.317	8.1	12.5	18.6	25.8	32.6	36.2	0.462	0.530	0.557	0.40	2.35	0.43	1.59	4.95
692	175	0.328	9.3	14.5	19.6	26.0	31.1	34.1	0.438	0.522	0.541	0.59	2.26	0.32	1.29	3.97
692	177	0.360	9.4	14.2	20.5	26.4	31.8	33.5	0.472	0.545	0.586	0.56	2.46	0.42	1.29	3.59
692	186	0.335	8.8	12.3	17.0	23.4	29.0	31.5	0.416	0.464	0.540	0.35	1.68	0.25	1.17	2.82
692	191	0.220	7.5	12.2	16.7	23.6	27.7	30.6	0.407	0.439	0.468	0.17	1.07	0.34	1.03	3.60
692	194	0.283	8.3	12.4	17.6	20.8	24.5	26.0	0.430	0.530	0.580	0.41	2.15	0.27	0.78	1.87
692	196	0.233	8.1	12.5	17.8	24.1	31.3	35.0	0.436	0.499	0.540	0.24	1.23	0.26	1.28	3.62
728	3	0.280	10.2	16.4	23.4	31.8	38.6	42.3	0.403	0.494	0.523	0.22	1.48	0.41	1.93	4.74
728	8	0.291	8.7	13.2	18.0	23.0	28.2	30.5	0.394	0.417	0.441	0.18	1.21	0.23	1.06	3.11
728	14	0.271	7.2	11.5	13.9	16.5	20.5	22.6	0.396	0.465	0.473	0.23	1.32	0.29	0.72	2.26
728	16	0.309	8.5	12.5	17.2	23.6	30.5	33.5	0.400	0.467	0.510	0.07	0.36	0.35	1.05	3.27
728	29	0.250	9.5	14.1	18.5	24.7	29.6	31.2	0.408	0.470	0.507	0.31	2.13	0.31	1.38	2.96
728	67	0.345	9.1	13.2	19.5	26.5	31.4	34.2	0.471	0.553	0.592	0.20	1.07	0.48	2.45	7.37
728	69	0.228	8.7	12.9	19.1	26.7	32.4	36.5	0.405	0.472	0.519	0.10	1.15	0.33	1.34	4.34
728	71	0.251	8.5	12.6	19.6	26.5	35.5	39.5	0.454	0.489	0.544	0.12	0.75	0.35	1.93	5.79
728	79	0.256	9.5	13.0	18.5	26.5	31.4	35.5	0.473	0.516	0.555	0.27	1.59	0.29	1.47	3.39
728	81	0.259	9.0	13.5	17.8	23.6	28.0	30.5	0.455	0.495	0.516	0.43	2.46	0.36	1.25	4.36
728	82	0.250	8.5	14.1	20.5	23.5	27.5	30.1	0.381	0.429	0.447	0.13	0.87	0.31	1.11	2.63
728	94	0.164	8.1	11.8	16.5	20.2	25.1	27.2	0.408	0.456	0.470	0.22	1.85	0.32	0.99	4.65
728	113	0.302	10.3	16.6	21.6	26.8	34.2	37.5	0.385	0.466	0.511	0.37	1.65	0.32	1.82	4.65
728	115	0.260	10.1	14.9	21.3	27.5	35.1	39.2	0.394	0.497	0.524	0.26	1.68	0.35	1.62	4.12
728	126	0.221	9.0	14.5	18.2	26.0	32.5	33.5	0.446	0.529	0.547	0.30	1.63	0.38	1.24	3.10
728	128	0.201	9.6	15.0	22.5	30.1	36.7	41.2	0.417	0.529	0.569	0.33	1.49	0.42	1.84	4.33
728	135	0.211	8.5	13.1	19.2	26.8	34.2	36.5	0.425	0.476	0.523	0.24	1.84	0.34	1.48	4.41
728	140	0.312	10.2	15.1	21.1	28.5	35.5	38.6	0.413	0.452	0.492			0.31	1.54	4.77
728	143	0.243	8.8	13.5	18.5	22.5	30.7	34.3	0.260	0.485	0.515	0.26	1.55	0.34	1.33	3.41
728	144	0.301	9.8	15.5	20.6	28.1	33.4	36.5	0.399	0.467	0.504	0.15	0.97	0.34	1.37	3.63
728	169	0.285	7.8	12.6	17.1	23.5	30.6	34.5	0.426	0.492	0.543	0.15	1.68	0.39	1.47	4.58
728	188	0.274	9.5	14.9	22.1	28.5	35.3	38.5	0.449	0.521	0.563	0.17	2.24	0.38	1.94	4.82
728	190	0.310	9.5	15.5	21.4	28.2	33.6	36.5	0.430	0.502	0.525	0.36	1.85	0.34	1.60	4.32
728	192	0.232	9.0	11.9	16.6	22.5	28.2	31.3	0.357	0.416	0.452	0.22	1.20	0.32	0.95	3.01
728	193	0.206	8.9	14.9	20.5	25.4	30.6	33.4	0.479	0.522	0.581	0.56	2.97	0.40	1.32	3.62
734	2	0.260	6.6	10.6	15.0	22.5	28.6	33.1	0.386	0.427	0.454	0.28	1.22	0.29	0.92	2.41
734	8	0.323	7.1	11.5	16.6	23.1	29.5	32.7	0.400	0.456	0.484	0.40	1.42	0.29	0.96	2.19
734	12	0.290	7.1	11.6	16.6	25.1	30.5	34.2	0.347	0.422	0.439	0.09	0.51	0.27	1.00	3.05
734	18	0.273	6.0	9.4	13.1	19.0	24.5	27.5	0.373	0.409	0.440	0.18	1.01	0.27	0.76	2.22

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
734	25	0.236	6.2	11.5	18.1	25.1	33.2	36.8	0.415	0.496	0.547	0.50	2.17	0.33	1.30	2.50
734	33	0.358	7.5	12.1	17.5	20.5	22.5	24.5	0.387	0.447	0.483	0.57	2.62	0.32	0.89	2.68
734	36	0.283	6.8	12.0	17.4	25.5	31.8	34.7	0.423	0.464	0.516	0.36	1.34	0.41	1.29	3.58
734	48	0.348	6.8	11.6	16.8	23.6	29.2	32.7	0.369	0.392	0.428	0.21	1.41	0.29	0.94	2.59
734	59	0.382	8.1	12.9	17.6	23.4	30.3	33.5	0.448	0.538	0.550	0.49	2.06	0.41	1.18	2.91
734	60	0.249	7.5	12.6	17.7	23.8	30.5	33.5	0.411	0.471	0.498	0.49	2.20	0.40	1.11	2.46
734	70	0.233	5.6	11.2	15.4	21.2	26.8	31.2	0.349	0.400	0.404	0.03	0.28	0.22	0.81	2.54
734	82	0.238	8.6	13.9	19.1	27.5	32.6	34.0	0.437	0.491	0.502	0.60	3.12	0.31	1.37	2.82
734	98	0.339	7.2	11.5	17.2	24.3	32.6	36.1	0.487	0.529	0.532	0.32	1.75	0.43	1.83	4.66
734	102	0.274	7.5	11.6	17.0	23.9	30.5	33.0	0.430	0.478	0.511	0.26	1.25	0.32	1.32	3.71
734	105	0.383	7.5	11.2	17.0	21.8	27.5	29.5	0.357	0.450	0.514	0.26	1.37	0.27	1.00	2.51
734	108	0.356	7.6	12.0	17.1	24.5	30.4	34.5	0.404	0.458	0.490	0.08	0.47	0.33	1.25	3.77
734	122	0.275	6.6	11.6	15.5	21.0	26.4	28.5	0.428	0.479	0.512	0.28	1.56	0.30	1.02	3.14
734	154	0.285	7.5	11.1	15.5	21.6	28.2	30.5	0.392	0.450	0.479	0.45	1.88	0.29	0.90	2.67
734	164	0.309	7.4	11.4	15.5	21.0	25.7	28.2	0.443	0.496	0.529	0.46	2.24	0.37	1.14	3.11
734	179	0.240	6.5	9.6	13.1	18.5	22.3	24.5	0.367	0.400	0.452	0.22	1.08	0.28	0.75	2.58
734	188	0.239	7.7	11.5	16.0	21.5	28.8	31.5	0.412	0.477	0.508	0.38	1.83	0.32	1.12	2.63
734	191	0.254	7.5	11.4	16.5	23.2	28.0	31.0	0.405	0.452	0.500	0.34	1.69	0.28	1.14	3.20
734	198	0.268	7.2	12.3	18.5	24.0	29.3	31.8	0.388	0.444	0.453	0.42	1.78	0.31	1.01	2.69
734	199	0.255	8.5	13.0	19.2	26.7	34.5	37.4	0.419	0.477	0.511	0.38	2.34	0.44	1.49	5.04
734	200	0.214	7.0	11.1	16.4	22.8	29.0	31.4	0.381	0.449	0.488	0.53	2.05	0.33	0.96	2.68
738	8	0.322	4.3	11.6	17.1	24.6	30.6	34.3	0.383	0.445	0.476	0.18	0.95	0.26	1.17	3.63
738	18	0.324	8.0	12.6	18.6	25.5	30.5	33.5	0.362	0.461	0.473	0.05	0.42	0.25	1.37	3.16
738	41	0.363	10.0	15.1	20.5	26.8	33.1	36.5	0.420	0.515	0.527	0.47	1.69	0.37	1.26	4.11
738	47	0.331	8.9	14.5	19.4	29.2	36.0	39.3	0.418	0.493	0.541	0.36	1.57	0.31	1.57	3.45
738	52	0.300	8.1	12.7	18.0	24.5	31.4	33.8	0.404	0.485	0.541	0.18	0.94	0.39	1.80	4.47
738	56	0.368	8.5	12.6	17.0	24.2	30.7	33.5	0.436	0.479	0.517	0.42	2.28	0.37	1.43	3.68
738	60	0.306	7.5	12.1	18.9	25.2	33.0	36.0	0.411	0.508	0.513	0.42	2.05	0.30	1.22	3.16
738	61	0.342	7.1	11.2	16.6	21.5	27.5	30.5	0.433	0.488	0.530	0.42	2.02	0.32	1.08	3.55
738	67	0.407	8.4	11.6	17.1	23.5	30.4	33.2	0.461	0.519	0.526	0.38	2.15	0.34	1.29	4.30
738	68	0.324	7.5	12.7	18.6	26.5	34.1	38.0	0.458	0.510	0.539	0.43	1.95	0.38	1.49	3.92
738	69	0.321	6.4	11.2	16.5	22.1	28.6	31.5	0.381	0.440	0.482	0.52	2.32	0.30	0.83	2.44
738	74	0.246	8.2	11.3	15.5	21.1	28.0	30.7	0.401	0.468	0.588	0.29	1.55	0.28	1.12	3.03
738	76	0.317	7.8	12.1	17.0	23.5	28.5	31.5	0.367	0.410	0.468	0.09	0.85	0.28	1.02	2.89
738	81	0.290	8.2	16.6	17.6	24.2	30.6	34.5	0.415	0.457	0.486	0.12	1.04	0.32	1.27	3.86
738	87	0.330	7.8	11.1	16.2	22.5	29.5	33.0	0.403	0.456	0.497	0.17	1.19	0.38	1.21	3.33
738	90	0.302	7.2	11.6	17.0	24.5	30.5	33.5	0.412	0.466	0.502	0.45	1.86	0.35	1.36	2.88
738	92	0.301	7.5	12.0	16.6	22.5	28.4	32.1	0.433	0.465	0.515	0.42	2.61	0.31	1.11	2.47

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
738	97	0.278	5.7	8.5	11.6	16.5	20.6	23.0	0.350	0.392	0.437	0.22	1.33	0.21	0.54	2.08
738	103	0.350	8.0	12.6	19.0	25.0	30.5	33.5	0.394	0.433	0.489	0.15	1.02	0.34	1.14	3.02
738	119	0.311	7.1	12.5	18.6	26.0	32.4	36.0	0.200	0.418	0.463	0.20	1.18	0.43	1.16	3.12
738	137	0.313	7.5	11.5	17.5	24.5	31.6	35.2	0.380	0.477	0.515	0.16	0.88	0.36	1.31	3.88
738	171	0.274	8.6	12.7	19.5	25.6	33.1	35.5	0.393	0.476	0.508	0.40	1.78	0.31	1.31	3.63
738	173	0.313	7.4	13.1	18.2	24.4	31.2	33.5	0.464	0.475	0.494	0.42	2.39	0.31	1.18	2.90
738	176	0.387	8.0	12.1	16.4	23.4	28.6	32.1	0.419	0.463	0.528	0.16	0.74	0.35	1.19	3.61
738	192	0.317	6.5	10.5	16.5	22.5	29.5	32.6	0.397	0.467	0.481	0.38	1.59	0.34	1.33	3.62
754	7	0.275	7.0	11.6	17.5	24.5	31.5	35.2	0.413	0.476	0.508	0.44	2.01	0.36	1.30	3.99
754	9	0.280	8.2	12.1	17.2	23.6	30.5	34.0	0.407	0.449	0.478	0.31	1.41	0.31	1.11	3.12
754	12	0.302	11.3	16.3	23.6	30.7	36.7	40.1	0.435	0.509	0.530	0.32	1.21	0.42	1.87	4.33
754	13	0.298	7.6	11.6	16.7	23.2	29.6	32.6	0.455	0.498	0.544	0.51	2.87	0.42	1.37	3.97
754	14	0.115	9.2	14.2	19.5	24.2	28.5	31.2	0.393	0.424	0.466	0.21	1.27	0.24	1.10	3.28
754	15	0.360	7.0	10.1	18.9	24.5	32.3	35.0	0.424	0.485	0.507	0.44	1.79	0.36	1.35	2.92
754	35	0.324	9.2	13.5	18.5	25.2	31.5	34.4	0.426	0.474	0.502	0.26	1.48	0.28	1.44	3.72
754	57	0.271	8.5	12.9	19.5	25.2	30.2	33.5	0.404	0.481	0.502	0.37	1.77	0.27	1.28	3.85
754	59	0.262	9.0	13.1	16.6	22.3	27.5	30.6	0.386	0.449	0.474	0.29	1.71	0.25	0.94	2.67
754	71	0.265	7.4	12.1	16.6	22.5	28.0	30.2	0.350	0.390	0.414	0.17	1.25	0.20	0.90	3.09
754	73	0.310	7.5	12.2	17.7	24.1	29.0	32.2	0.424	0.489	0.502	0.38	2.10	0.46	1.41	3.23
754	97	0.294	8.5	13.6	19.5	25.8	32.5	36.2	0.497	0.571	0.590	1.13	4.37	0.41	1.62	3.50
754	98	0.202	8.9	14.4	20.4	27.7	34.4	38.0	0.436	0.519	0.520	0.28	1.31	0.39	1.56	4.27
754	106	0.261	9.1	15.1	21.7	30.5	37.6	40.5	0.419	0.484	0.523	0.30	1.54	0.45	1.75	4.58
754	107	0.345	9.8	15.0	18.5	24.0	28.5	30.0	0.399	0.450	0.462	0.21	1.43	0.31	1.15	2.68
754	112	0.353	8.5	13.4	18.1	25.1	31.5	35.5	0.415	0.560	0.586	0.45	1.93	0.32	1.19	3.08
754	117	0.339	7.6	12.1	16.4	22.6	28.5	31.2	0.378	0.446	0.463	0.21	2.23	0.28	1.01	2.79
754	119	0.314	7.6	12.5	17.4	24.5	31.1	33.4	0.398	0.448	0.477	0.20	1.45	0.32	1.23	3.52
754	123	0.234	8.8	13.6	19.5	25.3	31.2	33.5	0.457	0.526	0.559	0.60	3.29	0.38	1.39	3.50
754	174	0.356	9.1	13.4	19.5	25.4	30.5	32.0	0.455	0.492	0.498	0.32	1.84	0.32	1.57	3.61
754	179	0.299	8.2	12.6	18.6	23.5	29.5	32.5	0.445	0.479	0.525	0.47	1.94	0.44	1.15	3.59
754	191	0.243	11.5	15.9	23.6	26.5	29.4	30.2	0.486	0.543	0.567	0.43	2.03	0.34	1.57	3.74
754	193	0.334	9.6	14.0	21.0	28.5	36.5	38.5	0.418	0.476	0.495	0.40	1.89	0.32	1.78	3.78
754	197	0.263	8.7	13.0	18.5	24.6	30.4	33.4	0.417	0.461	0.490	0.28	1.68	0.29	1.17	2.92
754	198	0.121	8.0	13.2	18.1	24.8	32.5	36.5	0.407	0.450	0.475	0.22	1.40	0.31	1.34	2.86
782	9	0.249	10.5	15.8	21.1	28.4	35.5	39.2	0.416	0.506	0.546	0.31	1.61	0.29	1.52	4.01
782	12	0.282	10.6	15.7	21.6	28.7	35.1	37.5	0.456	0.506	0.524	0.55	3.09	0.45	1.75	4.51
782	18	0.295	7.9	11.1	15.6	22.5	27.0	30.5	0.415	0.431	0.468	0.22	1.23	0.30	0.97	3.42
782	24	0.280	9.3	13.6	19.1	25.5	32.3	35.0	0.420	0.465	0.539	0.42	2.10	0.39	1.18	3.81
782	30	0.280	9.8	14.8	22.0	29.5	36.5	39.5	0.535	0.587	0.613	0.73	4.43	0.53	3.32	6.09

Appendix 2 Radiata Pine

Seedling Growth Data

FAM	R	SW	H1	H2	H3	H4	H5	H6	D1	D2	D3	BW	NW	BTW	UTW	UNW
782	37	0.337	10.0	5.1	19.5	27.5	31.2	33.5	0.421	0.461	0.506	0.24	1.76	0.29	1.22	2.56
782	52	0.296	9.5	15.0	22.3	29.6	38.5	41.5	0.513	0.568	0.606	0.34	1.96	0.49	1.97	5.37
782	56	0.322	10.1	16.3	22.5	27.5	34.1	36.5	0.469	0.550	0.564	0.75	3.11	0.43	1.88	3.66
782	75	0.292	8.2	12.5	16.7	22.5	28.4	31.1	0.374	0.466	0.515	0.10	0.83	0.39	1.02	3.65
782	78	0.288	7.9	11.3	17.5	24.2	32.0	34.5	0.454	0.536	0.564	0.62	2.87	0.42	1.57	4.41
782	79	0.323	7.5	11.5	15.2	20.5	25.6	27.9	0.478	0.529	0.561	0.66	4.03	0.41	1.18	2.78
782	86	0.290	7.1	10.6	15.1	20.1	24.7	28.5	0.426	0.474	0.516	0.37	2.26	0.29	1.09	4.18
782	90	0.326	9.1	13.9	20.5	27.5	33.7	36.4	0.405	0.519	0.553	0.35	2.01	0.43	1.37	4.80
782	92	0.276	9.5	14.5	20.4	26.8	33.1	35.0	0.468	0.585	0.593	0.37	1.69	0.49	1.52	5.02
782	93	0.273	9.6	14.1	20.6	28.4	36.2	38.0	0.497	0.550	0.577	0.55	2.31	0.42	1.77	4.50
782	107	0.283	8.6	11.4	17.4	21.5	27.5	28.4	0.466	0.537	0.572	0.90	5.08	0.44	1.17	4.28
782	116	0.213	7.6	11.9	17.5	24.5	30.5	33.1	0.392	0.438	0.449	0.17	0.95	0.29	1.26	2.99
782	117	0.299	6.2	11.1	15.3	20.2	25.5	27.5	0.360	0.423	0.468	0.34	1.44	0.32	0.80	2.50
782	130	0.258	9.1	14.2	20.5	26.5	33.7	36.5	0.441	0.505	0.540	0.15	1.13	0.37	1.67	4.56
782	161	0.283	9.2	13.9	20.0	26.5	32.5	34.5	0.478	0.546	0.580	0.73	3.45	0.47	1.77	4.71
782	169	0.246	8.4	12.3	18.0	23.5	28.5	31.5	0.409	0.479	0.499	0.35	1.81	0.64	1.21	3.54
782	175	0.275	10.0	14.0	21.1	23.6	29.5	32.5	0.436	0.539	0.543	0.48	2.70	0.39	1.62	4.15
782	187	0.270	9.9	14.6	20.6	25.1	30.0	31.5	0.417	0.483	0.517	0.68	2.79	0.45	1.22	3.01
782	194	0.293	10.0	16.3	22.5	30.1	36.5	40.6	0.432	0.481	0.501	0.22	1.19	0.37	1.62	4.75
782	195	0.295	8.6	13.6	20.6	27.2	33.0	34.5	0.476	0.549	0.572	0.45	2.96	0.38	1.65	4.39

Appendix 3 Radiata Pine

Four Month Old Seedling Data

400A9	400N	400S	500A9	500N	500S	618A9	618N	618S	631A9	631N	631S	632A9	632N	632S	645A9	645N	645S	650A9	650N	650S	657A9	657N	657S
15.49	4.32	2.21	11.28	4.13	1.43	46.29	6.21	2.96	22.19	4.30	1.91	15.08	3.83	2.37	9.30	3.59	1.57	23.59	3.89	1.29	14.14	3.97	1.60
15.49	4.61	1.80	11.28	3.63	1.36	46.29	5.75	1.94	22.19	4.00	1.76	15.08	4.62	1.90	9.30	4.15	1.56	23.59	3.93	1.46	14.14	4.22	1.52
15.49	4.15	1.76	11.28	3.32	1.31	46.29	5.58	1.94	22.19	4.00	1.68	15.08	3.82	1.98	9.30	4.47	1.97	23.59	4.24	1.57	14.14	3.28	1.36
15.49	4.95	1.57	11.28	4.07	1.25	46.29	5.10	1.89	22.19	2.92	1.59	15.08	4.93	1.97	9.30	3.80	1.79	23.59	4.36	1.59	14.14	3.28	1.37
15.49	4.81	1.54	11.28	3.54	1.25	46.29	4.56	1.82	22.19	4.24	1.58	15.08	4.47	1.89	9.30	3.65	1.43	23.59	4.00	1.45	14.14	4.23	1.44
14.55	3.75	1.53	11.71	4.07	1.24	28.99	4.61	1.81	17.42	3.99	1.51	14.02	4.34	1.77	10.77	4.76	1.42	17.79	4.71	1.30	17.13	3.04	1.19
14.55	5.28	1.51	11.71	3.29	1.23	28.99	4.58	1.77	17.42	3.61	1.49	14.02	4.64	1.55	10.77	4.17	1.42	17.79	3.47	1.26	17.13	3.54	1.26
14.55	4.07	1.43	11.71	3.53	1.21	28.99	5.11	1.74	17.42	2.70	1.36	14.02	4.87	1.83	10.77	3.41	1.35	17.79	3.47	1.26	17.13	3.82	1.11
14.55	4.51	1.41	11.71	3.29	1.21	28.99	4.46	1.73	17.42	4.05	1.33	14.02	4.14	1.58	10.77	3.37	1.36	17.79	2.90	1.27	17.13	3.69	1.20
14.55	3.64	1.33	11.71	3.18	1.10	28.99	4.06	1.70	17.42	4.20	1.32	14.02	4.23	1.58	10.77	3.92	1.36	17.79	3.56	1.25	17.13	3.41	1.11
11.36	3.69	1.32	13.01	2.94	1.11	18.26	4.16	1.64	15.74	3.70	1.30	12.03	4.32	1.54	10.48	3.58	1.31	21.61	3.16	1.23	13.23	2.95	1.06
11.36	3.98	1.32	13.01	2.81	1.10	18.26	3.70	1.59	15.74	4.18	1.29	12.03	4.66	1.55	10.48	2.85	1.27	21.61	3.53	1.21	13.23	3.14	1.07
11.36	3.95	1.31	13.01	3.66	1.09	18.26	3.78	1.59	15.74	3.47	1.28	12.03	4.60	1.53	10.48	3.82	1.33	21.61	2.74	1.17	13.23	3.13	1.08
11.36	3.42	1.28	13.01	4.15	1.09	18.26	4.25	1.54	15.74	3.55	1.22	12.03	4.11	1.45	10.48	4.00	1.24	21.61	3.63	1.15	13.23	3.06	1.09
11.36	3.95	1.27	13.01	3.29	1.07	18.26	3.51	1.50	15.74	3.32	1.21	12.03	3.72	1.54	10.48	3.55	1.20	21.61	3.38	1.13	13.23	3.20	1.09
12.29	3.30	1.23	11.17	3.02	1.03	26.03	4.70	1.49	19.92	3.97	1.19	12.57	4.30	1.34	13.89	2.68	1.07	22.69	3.69	1.08	12.90	3.24	0.88
12.29	3.37	1.22	11.17	3.17	1.02	26.03	4.15	1.45	19.92	3.75	1.17	12.57	3.01	1.35	13.89	3.54	1.19	22.69	2.67	0.99	12.90	2.60	0.92
12.29	4.10	1.17	11.17	2.53	0.94	26.03	3.87	1.37	19.92	3.43	1.15	12.57	2.95	1.45	13.89	4.09	1.15	22.69	3.16	0.99	12.90	2.15	0.99
12.29	2.71	1.04	11.17	3.26	0.93	26.03	4.06	1.30	19.92	3.89	1.12	12.57	4.00	1.40	13.89	3.06	1.08	22.69	2.87	0.96	12.90	3.13	1.04
12.29	3.09	1.03	11.17	2.78	0.87	26.03	3.85	1.25	19.92	3.65	1.03	12.57	3.68	1.34	13.89	3.63	1.06	22.69	3.27	0.91	12.90	3.07	0.98
11.74	3.22	0.99	17.88	4.79	1.85	22.15	3.69	1.07	18.10	3.94	0.99	11.86	3.83	2.37	8.13	2.75	1.00	14.13	2.75	0.90	16.27	2.34	0.78
11.74	2.83	0.99	17.88	3.71	1.73	22.15	3.11	1.01	18.10	3.37	0.98	11.86	3.36	1.20	8.13	2.43	0.73	14.13	2.29	0.88	16.27	2.06	0.63
11.74	3.20	0.95	17.88	3.59	1.57	22.15	3.63	0.89	18.10	2.73	0.95	11.86	2.64	1.03	8.13	3.19	1.03	14.13	2.33	0.81	16.27	2.20	0.81
11.74	3.36	0.93	17.88	4.14	1.55	22.15	2.27	0.88	18.10	2.91	0.85	11.86	3.84	1.29	8.13	3.26	0.83	14.13	2.53	0.78	16.27	2.17	0.82
11.74	2.74	0.80	17.88	3.98	1.44	22.15	3.24	0.87	18.10	2.33	0.81	11.86	3.85	1.31	8.13	3.84	0.98	14.13	2.44	0.64	16.27	2.30	0.83
21.07	4.32	2.21	10.13	3.26	0.93	15.33	6.21	2.96	19.10	4.30	1.91	17.07	3.83	2.37	12.41	3.59	1.57	18.65	4.36	1.59	18.87	3.97	1.60
21.07	4.61	1.80	10.13	3.17	1.02	15.33	5.75	1.94	19.10	4.00	1.76	17.07	4.62	1.90	12.41	4.15	1.56	18.65	4.24	1.57	18.87	4.22	1.52
21.07	4.15	1.76	10.13	3.02	1.03	15.33	5.58	1.94	19.10	4.00	1.68	17.07	3.82	1.98	12.41	4.47	1.97	18.65	3.93	1.46	18.87	3.28	1.36
21.07	4.95	1.57	10.13	2.78	0.87	15.33	5.10	1.89	19.10	2.92	1.59	17.07	4.93	1.97	12.41	3.80	1.79	18.65	4.00	1.45	18.87	3.28	1.37
21.07	4.81	1.54	10.13	2.53	0.94	15.33	4.56	1.82	19.10	4.24	1.58	17.07	4.47	1.89	12.41	3.65	1.43	18.65	4.71	1.30	18.87	4.23	1.44
11.70	3.22	0.99	17.42	3.18	1.10	8.39	3.69	1.07	13.99	3.94	0.99	10.04	3.85	1.31	6.20	3.84	0.98	10.22	2.75	0.90	13.73	2.30	0.83
11.70	2.83	0.99	17.42	3.17	1.02	8.39	3.11	1.01	13.99	3.37	0.98	10.04	3.84	1.29	6.20	3.19	1.03	10.22	2.29	0.88	13.73	2.17	0.82
11.70	3.20	0.95	17.42	3.29	1.23	8.39	3.63	0.89	13.99	2.73	0.95	10.04	3.83	2.37	6.20	2.75	1.00	10.22	2.33	0.81	13.73	2.20	0.81
11.70	3.36	0.93	17.42	2.78	0.87	8.39	2.27	0.88	13.99	2.91	0.85	10.04	3.36	1.20	6.20	3.26	0.83	10.22	2.53	0.78	13.73	2.34	0.78
11.70	2.74	0.80	17.42	4.07	1.25	8.39	3.24	0.87	13.99	2.33	0.81	10.40	2.64	1.03	6.20	2.43	0.73	10.22	2.44	0.64	13.73	2.06	0.63
22.75	3.30	1.23	12.72	3.18	1.10	12.53	3.85	1.25	14.15	4.00	1.76	8.72	3.01	1.35	9.05	3.26	0.83	18.42	3.27	0.91	12.69	3.24	0.88
22.75	3.20	0.95	12.72	3.17	1.02	12.53	3.24	0.87	14.15	3.65	1.03	8.72	4.64	1.55	9.05	3.84	0.98	18.42	3.38	1.13	12.69	2.95	1.06
22.75	3.95	1.31	12.72	3.29	1.23	12.53	5.58	1.94	14.15	2.33	0.81	8.72	3.68	1.34	9.05	4.15	1.56	18.42	3.56	1.25	12.69	2.60	0.92

Appendix 3 Radiata Pine

Four Month Old Seedling Data

400A9	400N	400S	500A9	500N	500S	618A9	618N	618S	631A9	631N	631S	632A9	632N	632S	645A9	645N	645S	650A9	650N	650S	657A9	657N	657S
22.75	2.83	0.99	12.72	2.78	0.87	12.53	3.63	0.89	14.15	3.43	1.15	8.72	3.72	1.54	9.05	4.47	1.97	18.42	4.00	1.45	12.69	2.15	0.99
22.75	3.09	1.03	12.72	4.07	1.25	12.53	3.51	1.50	14.15	4.18	1.29	8.72	3.85	1.31	9.05	3.80	1.79	18.42	2.75	0.90	12.69	3.13	1.04
17.53	3.30	1.23				9.54	3.85	1.25	11.97	4.00	1.76	7.14	3.01	1.35	7.87	3.26	0.83	15.38	3.27	0.91	10.46	3.24	0.88
17.53	3.20	0.95				9.54	3.24	0.87	11.97	3.65	1.03	7.14	4.64	1.55	7.87	3.84	0.98	15.38	3.38	1.13	10.46	2.95	1.06
17.53	3.95	1.31				9.54	5.58	1.94	11.97	2.33	0.81	7.14	3.68	1.34	7.87	4.15	1.56	15.38	3.56	1.25	10.46	2.60	0.92
17.53	2.83	0.99				9.54	3.63	0.89	11.97	3.43	1.15	7.14	3.72	1.54	7.87	4.47	1.97	15.38	4.00	1.45	10.46	2.15	0.99
17.53	3.09	1.03				9.54	3.51	1.50	11.97	4.18	1.29	7.14	3.85	1.31	7.87	3.80	1.79	15.38	2.75	0.90	10.46	3.13	1.04
						12.10	2.27	0.88															
						12.10	4.61	1.81															
						12.10	5.75	1.94															
						12.10	3.63	0.89															
						12.10	3.51	1.50															
						15.00	2.27	0.88															
						15.00	4.61	1.81															
						15.00	5.75	1.94															
						15.00	3.63	0.89															
						15.00	3.51	1.50															
						12.00	5.10	1.89															
						12.00	4.56	1.82															
						12.00	3.69	1.07															
						12.00	3.11	1.01															
						12.00	4.58	1.77															
						14.70	5.10	1.89															
						14.70	4.56	1.82															
						14.70	3.69	1.07															
						14.70	3.11	1.01															
						14.70	4.58	1.77															

Appendix 3 Radiata Pine

Four Month Old Seedling Data

665A9	665N	665S	672A9	672N	672S	692A9	692N	692S	728A9	728N	728S	734A9	734N	734S	738A9	738N	738S	754A9	754N	754S	782A9	782N	782S
19.46	4.39	1.89	21.34	4.43	1.77	20.29	4.14	1.70	15.53	7.37	2.45	15.81	4.66	1.83	17.19	4.47	1.80	22.43	4.33	1.87	14.56	6.09	3.32
19.46	3.51	1.73	21.34	4.42	1.73	20.29	4.95	1.59	15.53	4.82	1.94	15.81	5.04	1.49	17.19	3.45	1.57	22.43	3.78	1.78	14.56	5.37	1.97
19.46	4.71	1.65	21.34	3.61	1.72	20.29	4.25	1.57	15.53	5.79	1.93	15.81	2.82	1.37	17.19	3.92	1.49	22.43	4.58	1.75	14.56	3.66	1.88
19.46	4.44	1.60	21.34	3.41	1.54	20.29	4.48	1.52	15.53	4.74	1.93	15.81	3.71	1.32	17.19	3.68	1.43	22.43	3.50	1.62	14.56	4.50	1.77
19.46	4.00	1.51	21.34	4.44	1.45	20.29	3.95	1.52	15.53	4.33	1.84	15.81	2.50	1.30	17.19	2.88	1.36	22.43	3.74	1.57	14.56	4.71	1.77
16.87	4.84	1.46	20.17	3.87	1.43	17.89	4.09	1.48	13.60	4.65	1.82	16.16	3.58	1.29	14.25	3.16	1.37	19.95	3.97	1.37	12.13	4.51	1.75
16.87	4.02	1.45	20.17	4.53	1.41	17.89	3.77	1.38	13.60	4.12	1.62	16.16	3.77	1.25	14.25	4.30	1.29	19.95	3.72	1.44	12.13	4.56	1.67
16.87	4.64	1.41	20.17	3.15	1.22	17.89	3.70	1.33	13.60	4.32	1.60	16.16	2.91	1.18	14.25	3.88	1.31	19.95	3.23	1.41	12.13	4.39	1.65
16.87	4.55	1.41	20.17	2.99	1.21	17.89	3.81	1.33	13.60	4.77	1.54	16.16	3.20	1.14	14.25	3.63	1.31	19.95	3.61	1.57	12.13	4.15	1.62
16.87	4.42	1.29	20.17	3.99	1.19	17.89	3.42	1.32	13.60	4.41	1.48	16.16	3.11	1.14	14.25	3.62	1.33	19.95	4.27	1.56	12.13	4.75	1.62
14.14	3.83	1.28	18.08	3.11	1.16	25.95	3.98	1.31	12.27	3.39	1.47	12.74	2.63	1.12	12.73	3.16	1.37	13.72	3.99	1.30	9.60	4.41	1.57
14.14	3.93	1.26	18.08	3.11	1.16	25.95	3.79	1.31	12.27	4.58	1.47	12.74	2.46	1.11	12.73	3.86	1.27	13.72	2.92	1.35	9.60	4.01	1.52
14.14	4.00	1.26	18.08	3.60	1.14	25.95	3.97	1.29	12.27	2.96	1.38	12.74	3.14	1.02	12.73	3.33	1.21	13.72	3.85	1.28	9.60	5.02	1.52
14.14	3.60	1.21	18.08	4.10	1.13	25.95	3.59	1.29	12.27	3.63	1.37	12.74	2.69	1.01	12.73	4.11	1.26	13.72	3.50	1.39	9.60	4.80	1.37
14.14	4.06	1.17	18.08	2.61	1.11	25.95	3.62	1.28	12.27	4.34	1.34	12.74	2.51	1.00	12.73	3.12	1.16	13.72	2.86	1.34	9.60	2.99	1.26
16.49	2.93	1.15	19.22	3.75	1.10	19.59	3.74	1.27	13.34	3.41	1.33	12.83	3.05	1.00	11.85	3.63	1.31	13.48	3.52	1.23	9.99	3.81	1.18
16.49	3.29	1.15	19.22	2.38	1.05	19.59	3.90	1.25	13.34	3.62	1.32	12.83	2.19	0.96	11.85	3.03	1.12	13.48	3.08	1.19	9.99	2.56	1.22
16.49	3.09	1.11	19.22	2.74	1.04	19.59	2.82	1.17	13.34	4.36	1.25	12.83	2.68	0.96	11.85	3.02	1.14	13.48	2.92	1.35	9.99	4.28	1.17
16.49	3.60	1.11	19.22	2.66	0.96	19.59	4.11	1.15	13.34	3.10	1.24	12.83	2.59	0.94	11.85	2.90	1.18	13.48	2.68	1.15	9.99	3.54	1.21
16.49	3.13	1.08	19.22	3.12	0.95	19.59	3.19	1.10	13.34	2.63	1.11	12.83	2.41	0.92	11.85	3.61	1.19	13.48	3.59	1.15	9.99	3.01	1.22
14.40	3.11	1.06	17.20	3.11	0.93	18.61	2.96	1.03	9.43	3.11	1.06	14.23	2.67	0.90	8.35	3.55	1.08	16.51	3.12	1.11	11.70	3.42	0.97
14.40	2.77	1.01	17.20	2.96	0.90	18.61	3.60	1.03	9.43	3.27	1.05	14.23	2.68	0.89	8.35	2.44	0.83	16.51	3.28	1.10	11.70	3.65	1.02
14.40	3.82	0.98	17.20	2.71	0.73	18.61	2.42	1.01	9.43	4.65	0.99	14.23	2.54	0.81	8.35	2.89	1.02	16.51	2.79	1.01	11.70	2.78	1.18
14.40	3.59	0.93	17.20	1.91	0.66	18.61	2.63	0.95	9.43	3.01	0.95	14.23	2.22	0.76	8.35	2.47	1.11	16.51	2.67	0.94	11.70	4.18	1.09
14.40	3.21	0.86	17.20	2.21	0.53	18.61	1.87	0.78	9.43	2.26	0.72	14.23	2.58	0.75	8.35	2.08	0.54	16.51	3.09	0.90	11.70	2.50	0.80
24.73	4.39	1.89	24.05	4.43	1.77	22.68	4.14	1.70	19.60	7.37	2.45	14.70	4.66	1.83	18.42	3.16	1.37	24.27	4.33	1.87	19.53	6.09	3.32
24.73	3.51	1.73	24.05	4.42	1.73	22.68	4.95	1.59	19.60	4.82	1.94	14.70	5.04	1.49	18.42	3.45	1.57	24.27	3.78	1.78	19.53	5.37	1.97
24.73	4.71	1.65	24.05	3.61	1.72	22.68	4.25	1.57	19.60	5.79	1.93	14.70	2.82	1.37	18.42	4.47	1.80	24.27	4.58	1.75	19.53	3.66	1.88
24.73	4.44	1.60	24.05	3.41	1.54	22.68	4.48	1.52	19.60	4.74	1.93	14.70	3.71	1.32	18.42	3.68	1.43	24.27	3.50	1.62	19.53	4.50	1.77
24.73	4.00	1.51	24.05	4.44	1.45	22.68	3.95	1.52	19.60	4.33	1.84	14.70	2.50	1.30	18.42	3.92	1.49	24.27	3.74	1.57	19.53	4.71	1.77
12.96	3.11	1.06	12.16	3.11	0.93	11.92	2.96	1.03	10.31	3.11	1.06	11.76	2.67	0.90	9.18	2.47	1.11	10.94	3.12	1.11	9.74	4.28	1.17
12.96	2.77	1.01	12.16	2.96	0.90	11.92	3.60	1.03	10.31	3.27	1.05	11.76	2.68	0.89	9.18	3.55	1.08	10.94	3.28	1.10	9.74	4.18	1.09
12.96	3.82	0.98	12.16	2.71	0.73	11.92	2.42	1.01	10.31	4.65	0.99	11.76	2.54	0.81	9.18	2.89	1.02	10.94	2.79	1.01	9.74	3.65	1.02
12.96	3.59	0.93	12.16	1.91	0.66	11.92	2.63	0.95	10.31	3.01	0.95	11.76	2.22	0.76	9.18	2.44	0.83	10.94	2.67	0.94	9.74	3.42	0.97
12.96	3.21	0.86	12.16	2.21	0.53	11.92	1.87	0.78	10.31	2.26	0.72	11.76	2.58	0.75	9.18	2.08	0.54	10.94	3.09	0.90	9.74	2.50	0.80
23.84	4.71	1.65	11.27	2.96	0.90	18.61	4.09	1.48	12.65	3.39	1.47	11.37	2.41	0.92	13.22	3.92	1.49	16.58	4.33	1.87	11.29	3.66	1.88
23.84	4.00	1.51	11.27	3.60	1.14	18.61	3.19	1.10	12.65	2.63	1.11	11.37	2.19	0.96	13.22	2.44	0.83	16.58	4.58	1.75	11.29	3.65	1.02
23.84	4.64	1.41	11.27	3.11	0.93	18.61	3.98	1.31	12.65	4.65	1.82	11.37	2.22	0.76	13.22	3.33	1.21	16.58	3.08	1.19	11.29	5.02	1.52

Appendix 3 Radiata Pine

Four Month Old Seedling Data

665A9	665N	665S	672A9	672N	672S	692A9	692N	692S	728A9	728N	728S	734A9	734N	734S	738A9	738N	738S	754A9	754N	754S	782A9	782N	782S
23.84	3.83	1.28	11.27	3.75	1.10	18.61	3.59	1.29	12.65	3.10	1.24	11.37	2.50	1.30	13.22	3.12	1.16	16.58	3.52	1.23	11.29	3.54	1.21
23.84	4.02	1.45	11.27	3.12	0.95	18.61	3.60	1.03	12.65	3.63	1.37	11.37	2.68	0.96	13.22	3.88	1.31	16.58	3.59	1.15	11.29	3.01	1.22
18.23	4.71	1.65	11.17	2.96	0.90	17.01	4.09	1.48	11.77	3.39	1.47	8.40	2.41	0.92	10.36	3.92	1.49	16.45	4.33	1.87	9.74	3.66	1.88
18.23	4.00	1.51	11.17	3.60	1.14	17.01	3.19	1.10	11.77	2.63	1.11	8.40	2.19	0.96	10.36	2.44	0.83	16.45	4.58	1.75	9.74	3.65	1.02
18.23	4.64	1.41	11.17	3.11	0.93	17.01	3.98	1.31	11.77	4.65	1.82	8.40	2.22	0.76	10.36	3.33	1.21	16.45	3.08	1.19	9.74	5.02	1.52
18.23	3.83	1.28	11.17	3.75	1.10	17.01	3.59	1.29	11.77	3.10	1.24	8.40	2.50	1.30	10.36	3.12	1.16	16.45	3.52	1.23	9.74	3.54	1.21
18.23	4.02	1.45	11.17	3.12	0.95	17.01	3.60	1.03	11.77	3.63	1.37	8.40	2.68	0.96	10.36	3.88	1.31	16.45	3.59	1.15	9.74	3.01	1.22
			18.90	2.96	0.90																		
			18.90	4.53	1.41																		
			18.90	2.66	0.96																		
			18.90	3.11	1.16																		
			18.90	3.12	0.95																		
			18.30	2.99	1.21																		
			18.30	4.10	1.13																		
			18.30	2.21	0.53																		
			18.30	3.99	1.19																		
			18.30	3.87	1.43																		
			19.50	2.99	1.21																		
			19.50	4.10	1.13																		
			19.50	2.21	0.53																		
			19.50	3.99	1.19																		
			19.50	3.87	1.43																		

Appendix 4

Coastal Douglas-fir Data

FAM	R	STDW	STHT	DIA1	DIA2	DIA3	MEAND	PVR	V1R	V2R	V3R	PVOL	VOL1	VOL2	VOL3	PHTR	HT1R
1	241	37.88	134	1.34	1.42	1.31	1.36	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	40	101.61	185	1.46	1.35	1.32	1.38	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	19	81.87	185	1.15	1.22	1.14	1.17	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	231	85.15	180	1.27	1.33	1.23	1.28	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	148	125.70	199	1.26	1.26	1.22	1.25	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	406	113.41	175	1.30	1.31	1.33	1.31	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	144	81.58	162	1.55	1.57	1.53	1.55	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	63	63.13	155	1.01	1.07	1.04	1.04	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	427	95.47	177	1.26	1.27	1.24	1.25	17	21	13	16	8.64	8.38	9.95	7.61	30	38
1	472	105.84	178	1.22	1.24	1.21	1.23	17	21	13	16	8.64	8.38	9.95	7.61	30	38
2	309	119.88	192	1.28	1.31	1.30	1.30	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	208	148.87	209	1.22	1.18	1.11	1.17	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	143	135.09	200	1.32	1.35	1.31	1.33	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	56	111.26	187	1.44	1.37	1.27	1.36	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	182	96.25	182	1.34	1.28	1.22	1.28	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	123	122.50	192	1.31	1.32	1.40	1.34	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	163	170.03	219	1.36	1.36	1.34	1.35	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	359	109.24	204	1.18	1.21	1.12	1.17	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	145	116.07	192	1.47	1.50	1.37	1.45	6	8	4	10	10.01	9.70	12.12	8.21	11	19
2	64	112.78	191	1.10	1.10	1.02	1.07	6	8	4	10	10.01	9.70	12.12	8.21	11	19
3	86	120.96	204	1.39	1.41	1.42	1.41	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	211	77.39	168	1.35	1.34	1.29	1.33	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	349	102.52	204	1.24	1.28	1.16	1.23	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	79	141.29	212	1.15	1.15	1.06	1.12	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	465	51.36	145	1.24	1.15	1.15	1.18	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	418	58.40	149	1.17	1.20	1.13	1.17	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	25	45.40	138	1.27	1.29	1.29	1.28	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	47	95.74	195	1.26	1.26	1.21	1.24	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	120	114.97	219	1.24	1.27	1.07	1.19	4	5	7	3	10.32	9.82	11.57	9.58	3	3
3	119	71.26	171	1.31	1.32	1.24	1.29	4	5	7	3	10.32	9.82	11.57	9.58	3	3
4	297	116.69	192	1.48	1.44	1.42	1.44	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	426	79.03	177	1.11	1.12	1.08	1.10	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	395	132.39	225	1.30	1.28	1.35	1.31	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	194	154.51	223	1.13	1.12	1.09	1.12	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	113	119.38	190	1.39	1.41	1.39	1.40	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	433	119.24	195	1.08	1.10	1.11	1.10	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	137	106.83	191	1.17	1.18	1.16	1.17	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	181	146.02	201	1.29	1.27	1.22	1.26	13	9	15	14	9.08	9.60	9.79	7.86	8	5

Appendix 4

Coastal Douglas-fir Data

FAM	R	STDW	STHT	DIA1	DIA2	DIA3	MEAND	PVR	V1R	V2R	V3R	PVOL	VOL1	VOL2	VOL3	PHTR	HT1R
4	445	106.85	182	1.25	1.30	1.30	1.28	13	9	15	14	9.08	9.60	9.79	7.86	8	5
4	129	101.62	185	1.18	1.18	1.17	1.18	13	9	15	14	9.08	9.60	9.79	7.86	8	5
5	350	125.50	187	1.20	1.14	1.17	1.17	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	80	88.01	178	1.43	1.43	1.42	1.43	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	195	63.19	150	1.40	1.28	1.21	1.30	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	2	107.44	183	0.85	0.86	0.76	0.82	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	212	78.43	176	1.21	1.27	1.22	1.23	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	101	62.13	154	1.44	1.49	1.39	1.44	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	312	94.94	178	1.42	1.41	1.37	1.40	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	96	81.17	185	1.35	1.34	1.28	1.32	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	199	96.10	171	1.45	1.46	1.46	1.45	1	15	1	1	11.68	9.11	15.06	10.88	2	13
5	205	147.89	202	1.43	1.46	1.38	1.42	1	15	1	1	11.68	9.11	15.06	10.88	2	13
6	471	119.17	198	1.30	1.33	1.21	1.28	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	92	95.09	178	1.40	1.41	1.34	1.38	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	279	89.43	164	0.83	0.82	0.77	0.81	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	335	77.04	170	1.18	1.16	1.20	1.18	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	385	93.64	181	1.28	1.33	1.24	1.28	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	29	112.33	202	1.35	1.44	1.31	1.37	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	215	101.36	170	1.53	1.69	1.43	1.55	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	438	91.16	175	1.26	1.27	1.24	1.25	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	59	51.04	131	1.21	1.19	1.19	1.20	10	16	10	8	9.32	9.00	10.53	8.42	5	2
6	430	135.25	214	1.24	1.20	1.22	1.22	10	16	10	8	9.32	9.00	10.53	8.42	5	2
7	31	95.79	180	1.53	1.51	1.37	1.47	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	306	89.83	162	1.37	1.39	1.28	1.35	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	311	109.84	190	0.95	0.88	0.86	0.90	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	5	52.93	143	1.27	1.25	1.16	1.23	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	229	87.25	169	1.25	1.28	1.27	1.27	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	330	112.52	161	1.19	1.22	1.24	1.22	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	267	79.27	166	1.52	1.59	1.51	1.54	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	192	133.67	210	1.36	1.34	1.35	1.35	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	298	108.36	189	1.23	1.41	1.26	1.30	16	14	25	11	8.72	9.20	8.89	8.07	16	16
7	411	108.84	200	1.12	1.14	1.06	1.11	16	14	25	11	8.72	9.20	8.89	8.07	16	16
8	473	84.57	170	1.20	1.32	1.21	1.24	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	310	99.27	173	1.35	1.26	1.26	1.29	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	287	75.74	158	1.43	1.48	1.46	1.46	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	118	144.23	205	1.54	1.51	1.51	1.52	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	117	107.45	184	1.33	1.27	1.22	1.27	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	43	90.79	184	1.40	1.42	1.30	1.37	24	25	16	35	8.03	8.20	9.78	6.11	22	24

Appendix 4

Coastal Douglas-fir Data

FAM	R	STDW	STHT	DIA1	DIA2	DIA3	MEAND	PVR	V1R	V2R	V3R	PVOL	VOL1	VOL2	VOL3	PHTR	HT1R
8	78	104.34	202	1.26	1.27	1.22	1.25	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	15	114.16	200	1.49	1.51	1.38	1.46	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	159	97.18	193	1.29	1.27	1.23	1.27	24	25	16	35	8.03	8.20	9.78	6.11	22	24
8	97	71.72	162	1.26	1.31	1.24	1.27	24	25	16	35	8.03	8.20	9.78	6.11	22	24
9	321	110.63	176	1.37	1.37	1.33	1.36	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	436	83.81	167	1.21	1.32	1.27	1.26	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	246	87.63	166	1.35	1.41	1.37	1.38	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	250	100.22	195	1.37	1.35	1.30	1.34	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	53	56.62	142	1.26	1.31	1.18	1.25	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	139	103.72	186	1.32	1.41	1.32	1.35	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	415	117.08	189	1.34	1.32	1.32	1.33	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	355	90.67	162	1.21	1.28	1.13	1.21	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	236	123.46	195	1.19	1.21	1.24	1.21	33	31	26	42	7.34	7.87	8.82	5.31	14	6
9	46	134.12	209	1.58	1.70	1.48	1.59	33	31	26	42	7.34	7.87	8.82	5.31	14	6
10	166	118.28	199	1.38	1.23	1.19	1.27	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	459	141.43	199	1.29	1.26	1.25	1.27	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	455	131.69	202	1.31	1.37	1.32	1.33	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	466	162.26	205	1.22	1.22	1.26	1.24	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	280	142.33	208	1.55	1.55	1.51	1.54	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	60	135.71	206	1.44	1.37	1.30	1.37	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	300	91.95	162	1.41	1.51	1.42	1.45	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	327	63.76	112	1.23	1.13	1.23	1.20	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	470	164.59	237	1.08	1.11	1.11	1.10	20	20	21	22	8.27	8.43	9.31	7.07	26	25
10	265	130.98	212	1.60	1.59	1.56	1.58	20	20	21	22	8.27	8.43	9.31	7.07	26	25
11	141	120.48	189	1.26	1.17	1.18	1.20	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	332	112.10	196	1.15	1.17	1.14	1.15	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	352	150.15	204	1.32	1.37	1.33	1.34	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	360	103.66	196	1.29	1.26	1.19	1.25	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	326	127.18	202	1.41	1.37	1.38	1.39	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	149	97.85	181	1.28	1.25	1.24	1.26	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	14	87.49	159	1.49	1.48	1.39	1.45	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	278	77.58	190	1.31	1.34	1.27	1.31	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	244	48.47	137	1.30	1.30	1.27	1.29	36	40	30	31	7.20	7.41	7.97	6.20	33	33
11	232	111.35	193	1.17	1.18	1.12	1.16	36	40	30	31	7.20	7.41	7.97	6.20	33	33
12	338	132.44	198	1.37	1.39	1.35	1.37	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	62	118.57	199	1.51	1.48	1.36	1.45	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	240	70.25	149	1.21	1.21	1.24	1.22	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	372	91.66	178	1.44	1.49	1.44	1.46	26	18	24	40	7.82	8.50	9.24	5.73	25	15

Appendix 4

Coastal Douglas-fir Data

FAM	R	STDW	STHT	DIA1	DIA2	DIA3	MEAND	PVR	V1R	V2R	V3R	PVOL	VOL1	VOL2	VOL3	PHTR	HT1R
12	394	71.88	159	1.30	1.33	1.24	1.29	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	416	87.41	168	1.33	1.34	1.29	1.32	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	283	115.23	179	1.38	1.43	1.37	1.39	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	202	68.62	147	1.23	1.25	1.18	1.22	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	458	90.32	173	1.24	1.27	1.19	1.23	26	18	24	40	7.82	8.50	9.24	5.73	25	15
12	234	76.00	163	1.48	1.44	1.43	1.45	26	18	24	40	7.82	8.50	9.24	5.73	25	15
13	367	99.87	168	1.37	1.43	1.33	1.37	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	366	92.52	174	1.12	1.17	1.13	1.14	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	429	95.74	187	1.09	1.16	1.13	1.13	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	263	80.58	160	1.40	1.41	1.38	1.40	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	288	66.85	152	1.34	1.36	1.32	1.34	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	6	75.44	169	1.35	1.36	1.19	1.30	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	257	94.62	185	1.53	1.54	1.44	1.50	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	37	112.72	186	1.44	1.49	1.38	1.44	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	247	55.73	145	1.27	1.28	1.26	1.27	37	26	41	26	6.91	8.07	6.11	6.56	35	22
13	35	95.15	178	1.48	1.52	1.38	1.46	37	26	41	26	6.91	8.07	6.11	6.56	35	22
14	87	78.14	179	1.44	1.44	1.39	1.42	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	255	160.17	222	1.47	1.48	1.45	1.47	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	169	88.31	175	1.17	1.10	1.03	1.10	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	17	117.66	202	1.30	1.30	1.28	1.29	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	275	103.54	180	1.39	1.40	1.36	1.38	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	39	97.85	179	1.39	1.32	1.27	1.33	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	361	111.78	186	1.29	1.25	1.34	1.29	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	353	79.48	168	1.26	1.27	1.31	1.28	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	228	114.27	206	1.30	1.33	1.24	1.29	41	39	43	33	6.48	7.43	5.87	6.15	36	31
14	377	71.84	172	1.27	1.24	1.25	1.25	41	39	43	33	6.48	7.43	5.87	6.15	36	31
15	150	110.99	180	1.23	1.29	1.19	1.24	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	74	119.03	194	1.25	1.25	1.30	1.27	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	206	90.88	181	1.38	1.34	1.33	1.35	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	177	68.27	152	1.41	1.40	1.37	1.39	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	83	95.00	171	1.18	1.17	1.10	1.15	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	207	86.06	171	1.33	1.35	1.34	1.34	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	256	98.03	179	1.32	1.37	1.37	1.35	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	67	63.01	160	1.05	0.97	0.97	1.00	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	368	90.56	170	1.15	1.20	1.14	1.16	39	36	38	43	6.55	7.58	7.09	4.97	28	21
15	223	111.68	191	1.32	1.34	1.33	1.33	39	36	38	43	6.55	7.58	7.09	4.97	28	21
16	3	89.66	156	1.41	1.47	1.37	1.42	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	243	104.55	199	1.08	1.09	0.99	1.06	45	45	45	45	4.67	5.86	4.54	3.63	45	44

Appendix 4

Coastal Douglas-fir Data

FAM	R	STDW	STHT	DIA1	DIA2	DIA3	MEAND	PVR	V1R	V2R	V3R	PVOL	VOL1	VOL2	VOL3	PHTR	HT1R
16	224	83.73	173	1.30	1.21	1.17	1.23	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	376	121.89	197	1.13	1.15	1.13	1.14	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	397	89.73	177	1.15	1.18	1.13	1.15	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	54	99.57	195	1.36	1.30	1.24	1.30	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	356	77.26	158	1.09	1.09	1.06	1.08	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	167	110.94	183					45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	347	89.22	186	1.14	1.14	1.08	1.12	45	45	45	45	4.67	5.86	4.54	3.63	45	44
16	354	93.38	176	1.31	1.37	1.28	1.32	45	45	45	45	4.67	5.86	4.54	3.63	45	44

Appendix 4

Coastal Douglas-fir Data

FAM	R	HT2R	HT3R	PHT	HT1	HT2	HT3	PDR	D1R	D2R	D3R	PDBH	DBH1	DBH2	DBH3	NDW	GA9
1	241	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.20	82.8
1	40	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.45	73.1
1	19	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.40	69.8
1	231	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.41	59.7
1	148	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.57	102.1
1	406	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.52	47.6
1	144	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.43	77.8
1	63	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.30	162.0
1	427	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.45	112.5
1	472	29	18	5.01	5.09	5.04	4.90	12	17	12	13	5.69	5.66	6.05	5.36	0.47	71.6
2	309	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.56	70.1
2	208	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.55	58.0
2	143	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.49	77.4
2	56	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.54	81.9
2	182	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.47	58.9
2	123	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.49	51.9
2	163	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.86	52.1
2	359	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.40	49.9
2	145	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.37	61.2
2	64	12	16	5.26	5.43	5.40	4.96	6	6	4	15	5.87	5.92	6.40	5.29	0.45	49.5
3	86	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.37	60.1
3	211	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.39	52.3
3	349	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.45	51.0
3	79	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.50	53.8
3	465	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.22	60.4
3	418	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.27	53.0
3	25	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.24	42.3
3	47	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.36	52.7
3	120	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.41	48.2
3	119	9	1	5.59	5.71	5.45	5.63	4	8	7	4	5.94	5.86	6.26	5.70	0.30	59.8
4	297	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.52	69.6
4	426	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.32	53.5
4	395	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.54	55.7
4	194	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.60	69.9
4	113	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.53	52.8
4	433	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.59	69.7
4	137	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.49	58.5
4	181	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.53	44.9

Appendix 4

Coastal Douglas-fir Data

FAM	R	HT2R	HT3R	PHT	HT1	HT2	HT3	PDR	D1R	D2R	D3R	PDBH	DBH1	DBH2	DBH3	NDW	GA9
4	445	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.50	40.3
4	129	10	9	5.45	5.68	5.43	5.26	16	11	22	17	5.58	5.81	5.73	5.21	0.42	68.3
5	350	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.56	64.7
5	80	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.40	67.4
5	195	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.36	44.3
5	2	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.41	48.7
5	212	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.44	74.5
5	101	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.29	77.1
5	312	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.50	57.8
5	96	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.40	33.6
5	199	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.47	67.3
5	205	1	2	5.61	5.50	5.81	5.51	1	14	1	1	6.38	5.74	7.24	6.14	0.72	65.0
6	471	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.58	104.2
6	92	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.41	65.0
6	279	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.42	74.0
6	335	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.40	68.3
6	385	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.39	57.8
6	29	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.46	67.9
6	215	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.49	45.2
6	438	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.49	85.4
6	59	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.29	57.1
6	430	4	7	5.55	5.78	5.60	5.28	14	20	15	14	5.63	5.56	5.97	5.35	0.60	53.1
7	31	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.41	54.3
7	306	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.49	55.4
7	311	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.47	65.1
7	5	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.33	55.5
7	229	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.48	39.4
7	330	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.62	67.7
7	267	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.40	68.9
7	192	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.52	45.8
7	298	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.59	72.6
7	411	19	19	5.19	5.46	5.19	4.90	17	12	25	16	5.55	5.77	5.63	5.24	0.46	53.4
8	473	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.26	53.3
8	310	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.44	53.3
8	287	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.39	30.5
8	118	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.60	54.0
8	117	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.51	48.8
8	43	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.40	41.1

Appendix 4

Coastal Douglas-fir Data

FAM	R	HT2R	HT3R	PHT	HT1	HT2	HT3	PDR	D1R	D2R	D3R	PDBH	DBH1	DBH2	DBH3	NDW	GA9
8	78	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.33	38.6
8	15	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.38	80.6
8	159	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.44	57.0
8	97	11	36	5.14	5.33	5.41	4.69	31	40	23	37	5.10	5.14	5.69	4.47	0.36	56.9
9	321	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.50	36.6
9	436	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.33	41.6
9	246	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.46	49.1
9	250	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.46	46.2
9	53	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.25	29.0
9	139	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.39	65.9
9	415	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.50	62.0
9	355	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.47	50.1
9	236	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.54	47.8
9	46	18	31	5.20	5.66	5.20	4.75	36	34	29	42	4.99	5.24	5.40	4.33	0.52	48.0
10	166	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.50	74.5
10	459	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.72	105.6
10	455	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.54	93.9
10	466	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.67	100.9
10	280	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.71	80.5
10	60	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.57	83.5
10	300	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.47	70.2
10	327	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.28	103.3
10	470	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.69	108.0
10	265	22	21	5.10	5.31	5.12	4.88	22	23	18	23	5.43	5.50	5.83	4.97	0.54	76.7
11	141	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.56	48.7
11	332	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.55	78.0
11	352	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.53	51.6
11	360	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.49	40.5
11	326	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.61	51.0
11	149	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.48	58.8
11	14	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.49	48.3
11	278	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.48	77.4
11	244	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.11	15.6
11	232	26	39	4.95	5.21	5.08	4.56	35	36	30	38	5.00	5.18	5.37	4.46	0.50	52.1
12	338	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.46	99.7
12	62	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.48	88.4
12	240	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.25	69.3
12	372	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.43	55.5

Appendix 4

Coastal Douglas-fir Data

FAM	R	HT2R	HT3R	PHT	HT1	HT2	HT3	PDR	D1R	D2R	D3R	PDBH	DBH1	DBH2	DBH3	NDW	GA9
12	394	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.26	53.3
12	416	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.29	72.4
12	283	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.53	90.5
12	202	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.26	76.4
12	458	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.42	90.2
12	234	15	40	5.10	5.47	5.29	4.55	28	21	24	40	5.20	5.54	5.63	4.44	0.38	55.8
13	367	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.56	47.9
13	366	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.57	68.7
13	429	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.46	46.0
13	263	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.48	65.4
13	288	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.23	84.0
13	6	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.30	43.7
13	257	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.56	72.6
13	37	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.44	72.6
13	247	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.24	75.6
13	35	40	30	4.89	5.34	4.57	4.76	33	28	41	24	5.04	5.38	4.79	4.95	0.54	78.5
14	87	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.40	31.8
14	255	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.59	36.5
14	169	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.31	60.5
14	17	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.43	49.2
14	275	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.38	54.6
14	39	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.39	71.9
14	361	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.46	50.5
14	353	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.36	68.3
14	228	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.37	42.4
14	377	39	28	4.88	5.22	4.63	4.81	39	37	43	33	4.80	5.18	4.53	4.70	0.35	60.5
15	150	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.47	66.3
15	74	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.45	46.9
15	206	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.38	45.1
15	177	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.37	29.1
15	83	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.43	35.6
15	207	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.37	42.6
15	256	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.37	34.5
15	67	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.30	32.9
15	368	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.44	40.0
15	223	24	35	5.06	5.39	5.09	4.69	41	35	37	43	4.77	5.19	4.96	4.17	0.41	64.7
16	3	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.43	63.7
16	243	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.53	57.9

Appendix 4

Coastal Douglas-fir Data

FAM	R	HT2R	HT3R	PHT	HT1	HT2	HT3	PDR	D1R	D2R	D3R	PDBH	DBH1	DBH2	DBH3	NDW	GA9
16	224	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.37	52.9
16	376	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.45	102.0
16	397	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.34	59.4
16	54	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.43	36.1
16	356	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.32	44.3
16	167	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.50	91.9
16	347	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.40	86.4
16	354	44	45	4.48	4.99	4.40	4.04	45	45	45	45	4.13	4.62	4.09	3.68	0.39	69.9

Appendix 5 Radiata Pine

One-tail Spearman Rank Correlation Coefficients

	Taupo (r)	Taupo (p)	Thorp (r)	Thorp (p)	Rotoe (r)	Rotoe (p)	Moer2 (r)	Moer2 (p)	Moer1 (r)	Moer1 (p)	NZ 5 S (r)	NZ 5 S (p)
fam1mGA9	-0.196	0.251	-0.306	0.144	-0.211	0.234	-0.546	0.022	-0.427	0.064	-0.333	0.104
fam4mGA9	0.02	0.473	0.196	0.251	-0.051	0.432	0.015	0.479	0.213	0.232	0.097	0.36
5s4mGA9	-0.108	0.357	-0.04	0.438	-0.117	0.346	-0.363	0.101	-0.099	0.368	-0.141	0.301
5f4mGA9	0.095	0.374	0.209	0.237	0.152	0.302	-0.002	0.497	0.354	0.107	0.171	0.264
1mtotalwt	0.459	0.049	0.235	0.209	0.292	0.155	0.262	0.183	0.35	0.11	0.335	0.102
4mtotalwt	0.381	0.09	0.249	0.196	0.387	0.086	0.29	0.157	0.44	0.058	0.384	0.071
diameter1	0.672	0.004	0.444	0.056	0.55	0.021	0.407	0.074	0.663	0.005	0.573	0.01
diameter2	0.503	0.033	0.329	0.125	0.421	0.067	0.349	0.111	0.495	0.036	0.458	0.037
diameter3	0.466	0.047	0.321	0.132	0.382	0.089	0.371	0.096	0.423	0.066	0.406	0.059
mean3dia	0.563	0.018	0.467	0.046	0.406	0.075	0.413	0.071	0.607	0.011	0.5	0.024
height 1	0.415	0.07	0.244	0.2	0.292	0.155	0.117	0.346	0.328	0.127	0.265	0.161
height 2	0.436	0.06	0.268	0.177	0.262	0.183	0.167	0.284	0.29	0.157	0.275	0.151
height 3	0.374	0.094	0.257	0.187	0.381	0.09	0.271	0.175	0.427	0.064	0.399	0.063
height 4	0.358	0.104	0.213	0.232	0.363	0.101	0.253	0.192	0.446	0.055	0.432	0.047
height 5	0.306	0.144	0.257	0.187	0.363	0.101	0.257	0.187	0.477	0.042	0.424	0.051
height 6	0.301	0.148	0.279	0.167	0.354	0.107	0.27	0.175	0.481	0.041	0.459	0.037
ht2-ht1	0.288	0.159	0.095	0.374	0.02	0.473	0.042	0.444	0.037	0.45	0.132	0.313
ht3-ht1	0.367	0.098	0.226	0.218	0.354	0.107	0.314	0.137	0.543	0.022	0.447	0.041
ht4-ht1	0.22	0.225	0.128	0.332	0.238	0.207	0.191	0.256	0.477	0.042	0.316	0.12
ht5-ht1	0.048	0.435	0.035	0.452	0.059	0.42	0.13	0.329	0.341	0.116	0.133	0.312
ht6-ht1	-0.007	0.491	-0.046	0.438	0.037	0.45	0.13	0.329	0.292	0.155	0.118	0.332
ht3-ht2	0.306	0.144	0.209	0.237	0.345	0.113	0.24	0.205	0.596	0.012	0.377	0.075
ht4-ht2	0.081	0.391	-0.029	0.461	0.169	0.282	0.143	0.313	0.429	0.063	0.203	0.225
ht5-ht2	-0.024	0.467	-0.055	0.426	0.09	0.38	0.068	0.408	0.367	0.098	0.106	0.348
ht6-ht2	-0.178	0.271	-0.117	0.346	-0.042	0.444	0.046	0.438	0.244	0.2	-0.006	0.491
ht4-ht3	-0.389	0.085	-0.473	0.044	-0.451	0.053	-0.187	0.261	-0.073	0.403	-0.309	0.122
ht5-ht3	-0.393	0.082	-0.376	0.093	-0.319	0.133	-0.134	0.324	0.033	0.455	-0.187	0.244
ht6-ht3	-0.235	0.209	-0.108	0.357	-0.134	0.324	0.178	0.271	0.121	0.34	-0.015	0.478
ht5-ht4	-0.244	0.2	-0.134	0.324	-0.051	0.432	-0.07	0.405	0.141	0.316	-0.003	0.496
ht6-ht4	-0.231	0.213	-0.059	0.42	-0.114	0.348	0.242	0.202	0.114	0.348	0.022	0.468
ht6-ht5	-0.291	0.157	-0.013	0.482	-0.137	0.321	0.284	0.162	-0.02	0.473	-0.024	0.465
RGR(ht2-ht1	-0.328	0.126	-0.344	0.114	-0.402	0.077	-0.152	0.303	-0.388	0.085	-0.331	0.105
RGR(ht3-ht1	-0.286	0.161	-0.261	0.184	-0.226	0.219	0.039	0.447	-0.051	0.432	-0.106	0.349
RGR(ht4-ht1	-0.295	0.153	-0.3	0.149	-0.37	0.096	-0.155	0.299	-0.159	0.293	-0.25	0.176
RGR(ht5-ht1	-0.423	0.066	-0.229	0.215	-0.206	0.24	-0.029	0.461	-0.229	0.215	-0.176	0.257
RGR(ht6-ht1	-0.2	0.246	-0.246	0.198	-0.287	0.16	0.063	0.416	-0.027	0.464	-0.104	0.351
RGR(ht3-ht2	-0.102	0.364	-0.049	0.434	0.14	0.317	0.186	0.262	0.298	0.151	0.1	0.356
RGR(ht4-ht2	-0.257	0.188	-0.219	0.226	-0.101	0.365	0.064	0.414	0.05	0.433	-0.088	0.373
RGR(ht5-ht2	-0.392	0.083	-0.124	0.336	-0.124	0.336	-0.029	0.461	-0.038	0.448	-0.119	0.33

Appendix 5 Radiata Pine

One-tail Spearman Rank Correlation Coefficients

	Taupo (r)	Taupo (p)	Thorp (r)	Thorp (p)	Rotoe (r)	Rotoe (p)	Moer2 (r)	Moer2 (p)	Moer1 (r)	Moer1 (p)	NZ 5 S (r)	NZ 5 S (p)
RGR(ht6-ht2	-0.369	0.097	-0.304	0.145	-0.224	0.22	0.072	0.403	-0.137	0.32	-0.14	0.302
RGR(ht4-ht3	-0.515	0.03	-0.325	0.129	-0.515	0.03	-0.23	0.214	-0.33	0.125	-0.441	0.044
RGR(ht5-ht3	-0.623	0.009	-0.391	0.083	-0.514	0.03	-0.285	0.161	-0.468	0.046	-0.477	0.031
RGR(ht6-ht3	-0.423	0.066	-0.398	0.079	-0.474	0.044	-0.227	0.218	-0.334	0.122	-0.378	0.074
RGR(ht5-ht4	-0.387	0.086	-0.147	0.308	-0.201	0.245	0.065	0.413	-0.059	0.42	-0.088	0.372
RGR(ht6-ht4	-0.179	0.27	0.072	0.404	0	0.5	0.143	0.313	-0.072	0.404	0.027	0.46
RGR(ht6-ht5	-0.308	0.142	0.085	0.386	-0.08	0.393	0.228	0.217	-0.22	0.225	-0.042	0.439
RGR(D2-D1)	-0.363	0.101	-0.309	0.141	-0.279	0.167	-0.135	0.323	-0.365	0.1	-0.261	0.165
RGR(D3-D1)	-0.185	0.263	-0.138	0.319	-0.185	0.263	-0.104	0.362	-0.36	0.103	-0.185	0.247
RGR(D3-D2)	-0.08	0.392	-0.025	0.466	-0.297	0.152	-0.036	0.451	-0.23	0.214	-0.25	0.178
RGR(vol2-vo	0.409	0.073	0.222	0.223	0.235	0.209	0.114	0.348	0.425	0.065	0.255	0.171
RGR(vol3-vo	0.544	0.022	0.355	0.107	0.421	0.067	0.256	0.189	0.612	0.01	0.408	0.058
RGR(vol3-vo	0.181	0.268	-0.02	0.473	0.261	0.184	0.113	0.351	0.391	0.084	0.272	0.154
upstem dw	0.311	0.139	0.192	0.255	0.351	0.109	0.227	0.217	0.492	0.037	0.422	0.052
upneedle dw	0.326	0.128	0.361	0.102	0.414	0.071	0.295	0.153	0.482	0.04	0.423	0.051
r	-0.266	0.179	-0.24	0.205	-0.073	0.403	-0.037	0.45	-0.073	0.403	-0.074	0.393
p	0.211	0.234	0.187	0.261	0.057	0.423	-0.053	0.429	-0.018	0.476	0.01	0.485
b1	-0.139	0.318	-0.152	0.302	-0.187	0.261	-0.015	0.479	0.064	0.414	-0.062	0.41