# A FIELD TEST OF THE DEGREE OF COEVOLUTION BETWEEN RED ALDER AND *FRANKIA* POPULATIONS

by

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## ABSTRACT

A cross inoculation experiment was set up to examine the degree of coevolution between red alder (*Alnus rubra* Bong.) and *Frankia* populations and to test the competitive ability of red alder/*Frankia* combinations under different field conditions. Seedlings from high and low elevation populations from three watersheds in southwestern B. C. were inoculated with *Frankia* from the parent tree populations and planted into three high and three low elevation planting sites in the U.B.C.M.K. Research Forest. Seedlings were also inoculated with *Frankia* from trees near the planting sites. To examine the effect of neighbours on plant growth, each combination was planted with and without red alder neighbours.

There was a significant interaction between planting elevation, parent and *Frankia* source. On low elevation sites, the final yield of plants inoculated with *Frankia* from their parent's elevation was half that of plants inoculated with *Frankia* from the opposite elevation. There was also an inverse relationship between yield and the proportion of fixed nitrogen in leaves for the different alder/*Frankia* combinations. On high elevation sites, final yield was 3.6 times lower and nitrogen fixation levels were two times higher than on the low elevation sites. On these sites, plants inoculated with *Frankia* from their parents grew significantly more than plants inoculated with *Frankia* from any novel source. These data suggest that *Frankia* can evolve to a less mutualistic state and that expression of this effect depends on environmental conditions. It is predicted that less mutualistic *Frankia* will evolve in situations where the relationship with the host is likely to break down.

The presence of neighbours reduced the growth of plants by half but had no effect on the interaction between plant/*Frankia* combinations. There was no significant difference in plant yield when plants had neighbours from different parent elevations. In terms of productivity, total harvested plant mass per site ranged from 22,000 to 302 grams. Competitive intensity did not vary across sites, except that on the lowest productivity site, where no competitive effect was detectable,

plants with neighbours were 44 % larger than plants without neighbours. On all other sites, the mass of plants with neighbours, relative to the mass of plants without neighbours, decreased over the course of the experiment.

The plants in this experiment were attacked by woolly alder sawfly, *Eriocampa ovata* (L.). The sawflies attacked the fastest growing individuals on low elevation sites, resulting in decreased growth in late summer, 1993. This made observed differences between treatments conservative estimates of potential differences. The only exception to this pattern was that plants with neighbours had a higher degree of herbivore damage than plants without neighbours, confounding the effects of competition and herbivory.

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# GENERAL INTRODUCTION

"Natural selection cannot possibly produce any modification exclusively for the good of another species, though throughout nature one species incessantly takes advantage of, and profits by, the structures of another."

Charles Darwin, 1859

"It appears that we simultaneously know both a great deal and not much at all about mutualism."

Judith Bronstein, 1994

This thesis addresses the interaction between red alder (*Alnus rubra* Bong.) and *Frankia* populations from different watersheds in southwestern British Columbia. The purpose of this work was to gain a preliminary insight into the coevolution of *Frankia* and a host species, at the host species population level. To date, interactions between *Frankia* and its hosts have focused on interactions at the host species or family level. The interpretation of the patterns found between host species have been far from straightforward (reviewed in Benson and Silvester 1993) and so it was felt that interactions occurring within host species may be obscuring the patterns between host species. An understanding of the coevolutionary process between red alder and *Frankia* may lead to a better understanding of the evolution of symbiotic nitrogen fixation in general, which plays a important role in the functioning of terrestrial ecosystems. An experimental approach was used to examine the growth of red alder from different populations when inoculated with different *Frankia* genotypes and planted on a number of field sites. It was assumed that interactions were the result of coevolution between red alder and *Frankia*, at the population level although the experimental approach only provides indirect evidence for coevolution. The competitive ability of different alder/*Frankia* combinations and the intensity of competition between sites were also examined.

## the importance of symbiosis and competition

Mutualism can be defined as an interaction between two organisms that is beneficial to both. Symbiotic interactions occur when organisms live together in a state of mutual influence (Ehrman 1983) and can therefore be a special form of mutualism. These positive interactions between species (in contrast to negative interactions such as competition or parasitism) can produce novel adaptations to problems of acquiring resources and increasing fitness for both partners and therefore have been a source of evolutionary innovation (Atsatt 1988, Margulis and Fester 1991). In a number of cases, the symbionts are now considered part of the host organism (e.g. mitochondria, plastids) while in others, the partners continue to be recognized as separate organisms, which can still live independently from the host. Since symbiosis (or just mutualism) can result in novel adaptations, it plays a key role not only in the fitness of the organisms involved but also in the diversity of communities (Malloch et al. 1980) and the functioning of whole ecosystems (Perry et al. 1989). Although most organisms have mutualistic symbiotic relationships with microbes (Douglas 1995), their importance is often not taken into account in discussions of the ecology of a particular species, the structuring of communities or ecosystem level processes. While it has been noted by a number of authors that studies on mutualism are lacking, Bronstein (1994) has pointed out that there are, in fact, numerous studies on mutualism but they generally only identify the existence of the mutualism. There is still a need to examine the evolutionary and ecological consequences of mutualism and to put it in context with other forms of interactions between organisms and their abiotic and biotic environment.

Unlike mutualism, competition has always been considered in a wider ecological and evolutionary context. One reason for this may be the seemingly logical connection between limited resources and competition which drives natural selection (Keller, 1992), although non-rational reasons (e.g. social biases) have also been proposed (Keddy, 1989). Competition has always been considered a key factor in the ecology of individuals and the structuring of communities (Peters 1991, Kingsland 1985, McIntosh 1985, Worster, 1977) and the importance given to antagonistic

interactions between species receives more attention than positive interactions. For example, the number of pages devoted to the topics of competition and predation is 11 times greater than that devoted to mutualism in ecological texts (May and Serger 1986). Over the last few decades research into competition has addressed a broad range of questions including: its existence (Connell 1983, Schoener 1983), where and under what conditions it occurs (Goldberg and Barton 1992, Grace 1990) and its importance in relation to other processes in life history evolution (Connell 1980, Grime 1977) and community structure (Menge and Sutherland 1987). There has also been an effort to define and measure competition more precisely (numerous chapters in Grace and Tilman 1990, Underwood 1986, Strong et al. 1984). While May (1982) predicted that "empirical and theoretical studies of mutualistic interaction are likely to be one of the growth industries of the 1980s" the concept of competition continues to dominate ecological thinking.

**symbiotic nitrogen fixation**

The evolution of symbiotic nitrogen fixation is one of the most important events to occur in terrestrial ecosystems. On a global scale, nitrogen availability limits net primary productivity (Vitousek and Howarth 1990) and about 60% of all fixed nitrogen comes from symbiotic fixation in terrestrial organisms (Gutschick 1981). Plants which are symbiotic with nitrogen fixing bacteria therefore play a key role in community structure (Chanway et al. 1991) and the development of ecosystems (Wedin and Tilman 1990, Aber 1987, Bradshaw 1983, Crocker and Major 1955, but see Walker and Chapin, 1986). Symbiosis between vascular plants and nitrogen fixing bacteria occurs in 15 vascular plant families, eight of these occurring with the actinomycete *Frankia* (Table 1). However, our understanding of the actinorhizal symbiosis has lagged behind that of the legume/rhizobia symbiosis for a number of reasons. *Frankia* have only recently been isolated (Callaham et al. 1978) and some morphological groups still cannot be isolated. Growth in culture is typically quite slow (Lechevalier and Lechevalier 1990) and the filamentous morphology of *Frankia* makes estimation of biomass problematic (Nittayajarn and Baker 1989). Also, isolation from the soil is generally not considered possible (but see Baker and O'Keefe 1984) making it

difficult to draw any conclusions about the free living nature of *Frankia*. While recent developments in a number of molecular techniques (e.g. papers in Normand et al. 1992) will alleviate some of these problems, work on the bacteria is still hampered by difficulties such as the lack of antibiotic mutants (Mullin and An 1990) making simple assays for strain identification impossible.

It has been argued that taxonomic differences between actinorhizal families are too great to have resulted from a single evolutionary event. This then suggests that the symbiosis with *Frankia* arose a number of times, in the early Cretaceous during the angiosperm radiation, when fixed nitrogen was in short supply (Bond 1983). While this may be true, with nitrogen being in limited supply in many ecosystems, there may presently exist selection pressure for the evolution of new nitrogen fixing symbioses today (Postgate 1981). On the other hand, Gutschick (1981) has suggested the lack of more host species which can be nodulated by nitrogen fixing bacteria results from the competitive inferiority of plants with this ability, resulting in them being restricted to early successional communities or specialized niches. This raises the possibility that while individual fitness may be compromised by the evolution, or breeding, of new nitrogen fixing symbioses, ecosystem productivity could be enhanced. As Harper (1977) has pointed out, characteristics which enhance productivity are not likely to enhance individual fitness. Therefore, management for increased productivity should "be concerned to undo the results of selection for the selfish qualities of individual fitness." There is clearly a need to understand small scale evolutionary trends in actinorhizal plants and their non actinorhizal relatives to gain insight into the evolution of symbiotic nitrogen fixation.

**Table 1**. Vascular plant families with symbiotic nitrogen fixing members and their symbionts. Data from Bond (1983). Plant families follow the classification of Mabberley (1987). Common names are from Smith (1977).

| Plant Family | Common Name | Symbiont |
|---|---|---|
| Ferns | | |
| Azollaceae | mosquito fern | *Anabaena* |
| Gymnosperms | | |
| Cycadaceae | cycads | *Nostoc* or *Anabaena* |
| Stangeriaceae | - | *Nostoc* or *Anabaena* |
| Zaminaceae | Florida arrowroot | *Nostoc* or *Anabaena* |
| Angiosperms | | |
| Gunneraceae | water milfoil | *Nostoc* |
| Fabaceae | pea | *Rhizobium* and relatives* |
| Ulmaceae | elm | *Rhizobium* and relatives |
| Casuarinaceae | beef wood | *Frankia* |
| Myricaceae | sweet gale | *Frankia* |
| Betulaceae | birch | *Frankia* |
| Rosaceae | rose | *Frankia* |
| Coriariaceae | - | *Frankia* |
| Rhamnaceae | buckthorn | *Frankia* |
| Eleagnaceae | oleaster | *Frankia* |
| Datiscaceae | datisca | *Frankia* |

* Includes *Bradyrhizobium*, *Azorhizobium* and *Sinorhizobium*. (Elkan 1992).

## the coevolution of hosts and symbionts

Coevolution is a complementary evolutionary change in closely associated species (Janzen 1980) and leads to a greater degree of specialization between the partners. Among mutualistic interactions, coevolution is considered more likely to occur in symbiotic relationships because of the close physiological link between the partners (Boucher 1982). In symbiotic nitrogen fixing systems there is evidence for a complex physiological integration between the host and symbiont (Djordjevic et al. 1987). This integration probably results in a more efficient symbiosis. While coevolution implies that variation within one of the partners would result in a corresponding degree of variation in the other, Law and Lewis (1983) have argued that there should be less variation in the symbionts than in the host. Their argument is that the host is exposed to more negative interactions than is the symbiont. These negative interactions promote genetic variation and ultimately speciation. The symbionts, on the other hand, experience a much more benign environment within the host, so there is less selection for genetic variation. While Law and Lewis (1983) do provide data showing greater species diversity among hosts than among symbionts, the analysis is only valid if there is an equal degree of knowledge about the taxonomy of hosts and symbionts. This is certainly not the case since the symbionts examined were microbes with few morphological differences, while the hosts were well characterized vascular plants.

The coevolution of hosts and symbionts, resulting in the divergence of either host or symbiont genotypes across spatial or taxonomic scales, can be tested by examining interactions between different genotype combinations. These interactions can be explored by performing cross inoculations and evaluating the performance of the partners. It is expected that coevolution will lead to fitness being greatest in coexistent combinations (i.e. those found in the wild). Since this type of experiment provides no information on changes in gene frequencies or selective pressures in natural populations, it can only be considered indirect evidence of coevolution (sensu Endler 1986). Such experiments have shown fine scale coevolution, within species, in the legume/rhizobia symbiosis (Gabriel and Rolfe 1990) as well as with their conspecifics (Chanway

et al. 1989, 1990, Turkington et al. 1988). This has not generally been found in the actinorhizal symbiosis although experiments have generally been conducted only on a very broad scale (between plant families) with single strains of *Frankia* (see introduction to chapter 1).

## red alder and *Frankia* coevolution

In the Pacific Northwest coniferous trees dominate forest ecosystems (Waring and Franklin, 1978) but major inputs of fixed nitrogen are made by actinorhizal plants, red alder being the dominant actinorhizal species (Bormann et al. 1994). Red alder has been shown to have a strong effect on soil physical and chemical properties (Bormann et al. 1994, Binkley et al. 1994, Rhoades and Binkley 1992). This results in an increased nitrogen status and growth of conifers after the removal of red alder from the site (Brozek 1990) and often, before they are removed (Binkley 1984a,b, Miller and Murray 1978 but see Bollen et al. 1967). Although it has a large latitudinal range, red alder is only found within a few hundred kilometers of the coast at low elevations (below 700 m). Increased interest in the exploitation of red alder for saw logs has prompted a number of studies on genetic variation within the species. Using seedlings from 10 locations across the natural range of the species, planted into two common gardens in Oregon, DeBell and Wilson (1978) demonstrated that trees from southerly, more coastal locations had greater growth and lower survival than trees from more northerly and inland locations, two and 15 years after planting (Lester and DeBell, 1989). Using a greater collection of plants, but restricted to the British Columbia range of the species, Dang et al. (1994) found variation between provenances, but not families within provenances, for a number of ecophysiological traits. Geographic location accounted for a small but significant amount of variation in a number of the variables measured, suggesting genetic variation in drought resistance across a north/south gradient. Elevation only accounted for significant variation in mesophyll conductance. Ager (1987) grew seed collected from a number of populations from different elevations along rivers in Washington and Oregon in a common garden. Plants from low elevation sites, from watersheds closer to the ocean, grew the fastest. This was partly attributable to differences in leaf phenology and biomass allocation, with plants from high

elevation inland sites dropping leaves earlier and allocating more biomass to roots and nodules.

These studies indicate that there is a potential for ecotypic variation in red alder at a number of spatial scales. In chapter 1, coevolution between *Frankia* and *A. rubra* will be examined using a cross inoculation experiment. Since there is some evidence that genetic variation occurs between high and low elevation populations within single watersheds, cross inoculations were made between such populations (Table 2, Figure 1). Although growth conditions can be more easily controlled in the lab, these experiments were performed in the field on a number of sites so that plant performance could be measured under more natural conditions. It is expected that if coevolution has occurred then red alder seedlings should show their best performance when inoculated with *Frankia* from their parent population (familiar strains) and planted at the same elevation as their parents. Referring to the numbers of the treatments in Table 2, my hypotheses are:

a) coevolution of red alder and *Frankia* populations will result in an increase in plant fitness. Plants inoculated with familiar *Frankia* strains (treatments 2, 6, 8 and 12) will perform better, in the absence of neighbours, than plants inoculated with unfamiliar strains (treatments 3, 5, 9 and 11).

b) plant/bacterial combinations will adapt to environmental conditions. On low elevation planting sites, plants from low elevation parents, inoculated with familiar *Frankia* strains (treatment 2) will perform better, in the absence of neighbours, than plants from high elevation parents inoculated with familiar *Frankia* strains (treatment 6) . On high elevation planting sites, the plants from high elevation parents (treatment 12) will perform better than the plants from low elevation parents (treatment 8) when both plant types are inoculated with familiar *Frankia* strains.

c) Coevolution of red alder or *Frankia* to environmental conditions at low and high elevations will result in plants inoculated with *Frankia* from the planting site having a

similar performance to plants inoculated with *Frankia* from the same elevation within the parent plant watershed. On low elevation planting sites, plants inoculated with the site inoculum (treatments 1 and 4) will show a similar performance to plants inoculated with the low elevation *Frankia* from the parent plant watershed (treatments 2 and 5). On high elevation planting sites, plants inoculated with the site inoculum (treatments 7 and 10) will have a similar performance to plants inoculated with the high elevation *Frankia* from the parent plant watershed (treatments 9 and 12).

If coevolution between hosts and symbionts results in a greater efficiency in the mutualistic relationship, then coevolved combinations should be competitively superior, compared with other conspecific combinations. In chapter 2 the competitive ability of these plant /bacterial combinations is examined. This was done by evaluating the performance of plants in the presence of neighbours. The presence of neighbours (treatments 16 - 21 and 22 - 27) should not alter the predictions of the hypotheses (a, b and c) listed above. The effect of different types of neighbours was also examined by exposing plants to red alder neighbours from different elevations within the target plant watershed.

Variation in the intensity of competition is examined in chapter 3. The recent debate among plant ecologists about the conditions under which competition is important can be addressed by examining competitive intensities across different sites. The large variation in growth among the different sites allowed me to examine differences in competitive intensity across a gradient of productivity.

Although experiments test specific processes while trying to control others, in field experiments the degree of control is limited. In this experiment, plants were exposed to herbivore damage, which resulted in defoliation of some plants in 1993. The effect of this damage and the possible induced resistance to further damage are examined in chapter 4. While herbivory was not a consideration in the design of the experiment, its effects cannot be overlooked.

**Table 2**. Summary of treatments used in the field experiments. The designations high and low refer to the elevation for the planting site, parent source of the plant material, inoculum or neighbours. Plants were also inoculated with *Frankia* from trees adjacent to the planting site (site inoculum). Neighbours were three red alder plants, planted on the north, southwest and southeast side of the target plant, 8 cm from its base. Neighbours were always inoculated with site inoculum. Treatments 22 - 27 and 16 - 21 were used to test for the effect of the presence of neighbours on low and high elevation planting sites respectively (chapters 1 - 3). Treatments 13 - 15 were compared to 22 - 24 and 19 - 21 compared to 28 - 30 to test for the effect of neighbour type (Chapter 2) on low and high elevation planting sites respectively. There were 9 replicates per treatment (one from each of three collection watersheds planted on each of three planting sites at each elevation).

| | | | Neighbours | | |
|---|---|---|---|---|---|
| | | | Absent | Present | |
| Planting elevation | Parent source | Inoculum | | High | Low |
| low | low | site | 1 | 13 | 22 |
| | | low | 2 | 14 | 23 |
| | | high | 3 | 15 | 24 |
| | high | site | 4 | | 25 |
| | | low | 5 | | 26 |
| | | high | 6 | | 27 |
| high | low | site | 7 | 16 | |
| | | low | 8 | 17 | |
| | | high | 9 | 18 | |
| | high | site | 10 | 19 | 28 |
| | | low | 11 | 21 | 29 |
| | | high | 12 | 21 | 30 |

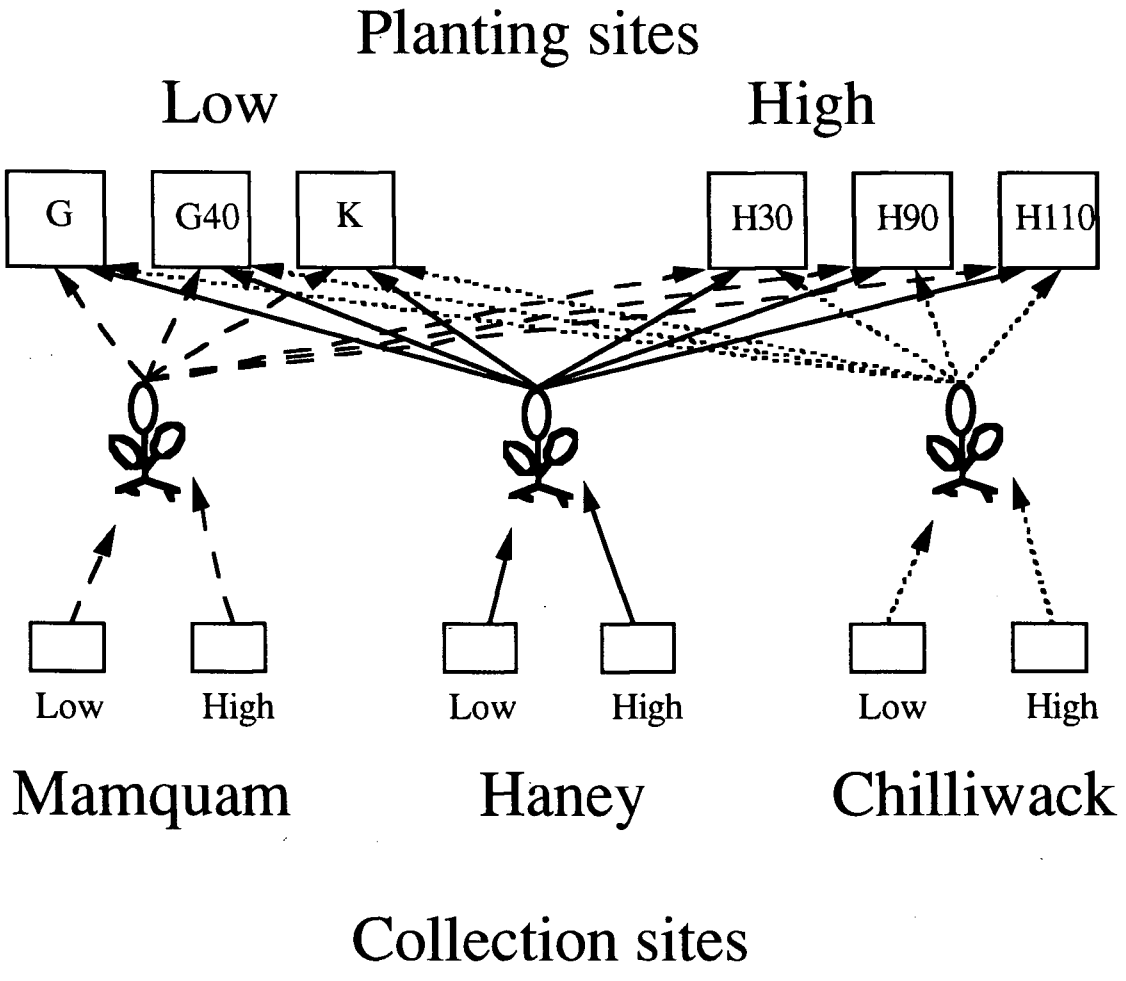


**Figure 1**. Experimental design. Six alder/*Frankia* crosses were made from each collection watershed. Seedlings from high and low elevation populations within each watershed were inoculated with *Frankia* that also originated from either the high or low elevation alder populations in the watershed or from trees adjacent to the planting site (site inoculum, not shown). Each plant/bacteria combination (Table 1) was planted on three high and three low elevation planting sites at the Malcolm Knapp Research Forest near Haney, B. C. Planting site labels refer to the name of the closest road to the site in the research forest.

# CHAPTER ONE

## A Field Test of Coevolution Between *Frankia* and *Alnus rubra* Populations

"My own suspicion is that the universe is not only queerer than we suppose, but queerer than we can suppose."

J. B. S. Haldane

## INTRODUCTION

A number of actinorhizal plant/*Frankia* genotype interactions have been documented in terms of both infectivity (the ability to infect a host) and effectivity (the effect on the performance of the host). These range across physiological groupings of the endophyte and the taxonomic relationship of the hosts. In terms of infectivity, interactions between host and endophyte occur at the host family level, with four recognized cross inoculation groups of *Frankia* across five host families, with varying degrees of promiscuity in the hosts (Baker 1987). Specificity for infectivity does not occur below the family level (Torrey 1990; but see Weber et al. 1987) making the actinorhizal symbiosis more promiscuous than the legume/*Rhizobium* symbiosis. This, along with differences in nodule physiology and development (Torrey 1988) suggest that the actinorhizal symbiosis is less coevolved than is the legume/*Rhizobium* symbiosis.

Although there is no generally accepted species classification of *Frankia* (Benson and Silvester 1993) there are a number of physiologically different groups that can have marked effects on host performance. Only certain *Frankia* strains sporulate within host nodules (termed spore-positive strains). Since the production of spores within nodules reduces nitrogenase activity, spore-positive strains of *Frankia* can be considered inferior symbionts on all hosts (Schwintzer 1990). The spore status of a strain is not influenced by the host or the environmental conditions in which the host is found (Torrey 1987) although the expression and timing of sporulation can be (VandenBosch and Torrey 1983). Another distinct physiological group of *Frankia* are strains which lack hydrogenase.

These strains have only been found in spore-positive *Frankia* and Sellstedt et al. (1986) have found they are less effective than pure cultured spore negative strains, at least in part due to the inefficiency of fixing nitrogen in the absence of hydrogenase.

Interactions between host and endophyte genotypes, in terms of effectivity, occur at much lower taxonomic levels than those found for infectivity. There is no general trend of greater plant performance with an endophyte naturally found with the host (i.e., coevolution towards an increased mutualistic interaction). In a cross inoculation experiment involving isolated *Frankia* from *Alnus* spp., *Comptonia perigrina* and *Myrica pensylvanica*, *Myrica. gale* was found to have the greatest acetylene reduction (AR) rate when inoculated with the isolate from *M. pensylvanica* (Dillon and Baker 1982) which would suggest coevolution towards increased mutualism. However, the lowest AR rate for the *C. perigrina* isolate occurred in nodules of *C. perigrina* suggesting no coevolution towards an increase in mutualism in the genus. Carpenter et al. (1984) found that although there were no detectable differences in growth, red alder clones had three times higher AR rates (per nodule weight and per plant) when inoculated with isolates from *Alnus. sinnuata* found in the same area, than with isolates from the parent tree. Other studies have found no interaction between host and endophyte genotypes between different host families (Dawson and Sun 1984), between different species of *Alnus* (Prat 1989) and within *Ceonothus velutinus* collected from a number of sites (Nelson and Lopez 1989). The interpretation of interactions between *Frankia* and host plants have also been made more complicated by the fact that ineffective *Frankia* have been isolated from both effective (Hahn et al. 1988) and ineffective nodules (Van Dijk and Sluimer Stolk 1990). In some cases these strains are considered atypical since they are unable to reinfect the original host and variation in infectivity occurs within species (Van Dijk and Sluimer 1994). Also, effectivity has been found to be host dependent in some strains (Kurdali et al. 1988) and host independent in others (Baker et al. 1980).

Fine scale coevolution has been described in the legume/*Rhizobium* symbiosis (Chanway et al. 1991) and is known to develop between different genotypes within the same location over

relatively short periods of time (Chanway et al. 1989). With the exception of the work on resistance to ineffective strains (Hahn et al. 1988, Van Dijk and Sluimer 1994), there has been no study addressing fine scale coevolution between *Frankia* and its hosts. The present study was designed to examine the degree of coevolution between red alder and *Frankia* genotypes found at different elevations within individual watersheds. The hypothesis is that coevolution between partners in a mutualism should lead to greater fitness of the partners in the mutualism. In more antagonistic relationships, such as predation or parasitism, no increase in fitness would be expected (Van valen, 1973). Such an examination not only provides insight into the evolution of mutualism and symbiotic N fixation, but also has practical implications for the management of actinorhizal plants.

To examine the degree of coevolution between red alder and *Frankia* genotypes, I set up a cross inoculation experiment. Seedlings from high and low elevation parent sources within three different watersheds were inoculated with *Frankia* from nodules of either the high or low elevation parent populations within the watershed or from plants adjacent to the planting sites (Table 2, treatments 1 - 12). These combinations were planted into three high and low elevation sites (Figure 1). If the plant/bacteria populations have coevolved at this fine scale then I would expect that plants will show the greatest fitness when planted into their parent's elevation and inoculated with *Frankia* from the parent population. Since it would not be practical to measure the fitness of a tree with life span which exceeds the allowable time of a graduate program by about ten fold, plant growth was measured and assumed to reflect fitness. This is a reasonable assumption, given the shade intolerant nature of red alder (Harrington et al. 1994).

## MATERIALS AND METHODS

### collection of experimental material

Seed and nodules were collected from one low and one high elevation red alder population in each of three watersheds in southwestern British Columbia: the Chilliwack river, the Mamquam river

and the University of British Columbia Malcolm Knapp Research Forest in Haney, B.C. (Table 1.1). These populations contained at least 15 reproductive individuals and seed and nodule collections were made on at least eight trees. Plant age at each collection site was determined by taking cores at breast height from at least five of the dominant trees in the stand. Site index, an estimate of the height of the dominant trees in a stand at age 50, was calculated from standard tables (Harrington and Curtis 1986). Seed, collected in the fall of 1991 from each population, was sown onto wooden flats containing 50:50 mixture of sterilized perlite and peat in a greenhouse in April, 1992. Seedlings were transferred to 2.5 cm diameter by 10 cm deep "conetainers" used to grow tree seedlings (Ray Leach Nursery, Canby, Oregon) which were placed in trays such that the conetainer was held above the bench surface to prevent contamination by unwanted *Frankia*. (This glasshouse was used for a number of experiments with alder. There were only ever a few cases of uninoculated plants becoming accidentally nodulated and these only occurred when the pot the plants were growing in rested directly on the greenhouse bench.) The seedlings were periodically fertilized with a 100 ppm N concentration of a commercial fertilizer (Plant Prod. 20 - 8 - 20).

**Table 1.1.** Description of collection sites. At each watershed red alder seed and nodules were collected from a high and low elevation population. Site index is the predicted height (in meters) of the dominant trees in the stand at a stand age of 50 years.

| | Watershed | | | | | |
|---|---|---|---|---|---|---|
| | Chilliwack | | Haney | | Mamquam | |
| distance from ocean (km) | 110 | | 40 | | 10 | |
| elevation (m) | 100 | 700 | 65 | 540 | 110 | 650 |
| latitude | 49°5' | 49°8' | 49°3' | 49°16' | 49°44' | 49°44' |
| longitude | 121°55' | 121°24' | 122°35' | 122°34' | 123°6' | 123°1' |
| site index (m @ 50 yrs) | 27.6 | 23.0 | 27.5 | 23.0 | 28.8 | 26.5 |
| stand age | 16 | 18 | 24 | 15 | 23 | 17 |

One assumption of this experiment is that the *Frankia* used to inoculate the plants would actually form nodules with these plants, and their effect on plant growth would be measurable throughout the experiment, even though wild *Frankia* may also infect the plants after transplanting. To test this assumption an experiment was devised where plants inoculated using the same procedures described below, would be planted in one of the planting sites, subsamples harvested throughout the course of the main experiments, and the *Frankia* strains identified as belonging to either the wild population or the inoculum. In this way, I would be able to determine how long the *Frankia* from the inoculum would continue to be the dominant strains on a plant. Strains were to be identified using whole cell protein gel electrophoresis (Laemmli 1970, Benson and Hanna 1983). While this identification technique was found to be relatively simple, the major stumbling block in this experiment was the isolation of *Frankia.* Recovery of *Frankia* was less than 1%, using a surface sterilization technique with commercial bleach (p. 47 in Lechevalier and Lechevalier, 1990). As recommended, nodule sections were placed in a number of different media: BAP (Murray et al. 1984) and BAP modified according to Steele et al. (1989), defined propionate media (Baker and O'Keefe, 1984) and Tap Water Agar/NZ (Lechevalier and Lechevalier 1990). Each medium was tested with and without fungicide (either 300 µg/L cyclohexamide and/or 10 µg/L Nystatin). Other isolation techniques, including sucrose density fractionation (Baker et al. 1979, Carpenter and Robertson 1983) and filtration (Benson 1982) were also tried. All techniques and media resulted in an isolation success of less than 1 % for *Frankia*, although vigorous growth of contaminants often occurred when the fungicides were not included. The growth of *Frankia* from nodule fragments was also quite slow, as has been noted by many others (Lechevalier and Lechevalier, 1990), sometimes taking up to a year to get a visible colony. Since isolation was so poor, even preliminary identification of the strains could not be completed and verification of infection in the field could not be performed. This type of experiment was repeated twice in the field and once in the lab in the hope that the isolation success would improve. This should serve as a cautionary note: the difficulty in obtaining isolates, and their slow growth will daunt the most recalcitrant graduate student's attempt to obtain a high isolation success rate, regardless of the

claims of some published reports (e.g., Carpenter and Robertson 1983, Lalonde et al. 1981). For this very reason some very experienced labs no longer perform routine isolations (Dave Myrold, personal communication). Other techniques for examining *Frankia in situ*, such as genetic probes (Myrold and Huss-Dannell 1994) may be more appropriate for the identification of *Frankia* strains but require a higher degree of technical sophistication.

Each seedling therefore was inoculated with an aliquot of crushed alder nodule suspension, instead of a pure culture of a single strain, originating from one of three sources: the low or high elevation population within the parent watershed, or from mature stands adjacent to the site the seedlings were being planted into (hereafter referred to as site inoculum). At least 10 nodules were collected from each seed tree and from trees around the planting sites in mid July. Nodules were stored in a refrigerator at ca. 5° C until they were used. To prepare the inoculum, nodules were soaked in water overnight, disinfected with 50% commercial bleach for five minutes and rinsed three times in distilled water. They were then crushed in distilled water with a sterile mortar and pestle. Seedlings were inoculated by injecting 1 ml of the crushed nodule suspension into the rooting zone with a syringe. To ensure nodulation, seedlings were inoculated three times: 1 week before transplanting (July 15/92), 3 weeks after transplanting (August 10/92), and in April 1993. For the first inoculation a suspension of 16.7 mg fresh weight of nodules per ml was used (100 times the concentration used by Huss-Dannell 1991). For subsequent inoculations a concentration of 1.67 mg/ml was used. Prior to initial inoculation seedlings from each population were sorted by size and then randomly assigned to each treatment.

Since the spore status of nodules has a strong effect on a *Frankia* strain's effectivity and infectivity (Schwintzer 1990, Torrey 1987) the spore status of the nodules used for inoculation was determined. Nodules were collected in November 1993 from the populations used to inoculate the plants. Hand cut sections were stained with Fabril's reagent (Noel 1964) and examined at 1000x magnification under a light microscope (Figure 1.1). A number of samples were sent to Christa Schwintzer at the University of Maine to confirm the presence of sporangia. At least 20 nodules

were examined in each population and up to five sections containing vesicles per nodule were examined for the presence of sporangia. With the exception of the low elevation population from the Mamquam watershed, all populations contained only spore negative nodules. The frequency of spore-positive nodules in the low elevation Mamquam population was 20%. All nodules examined contained numerous vesicles and therefore were considered effective.

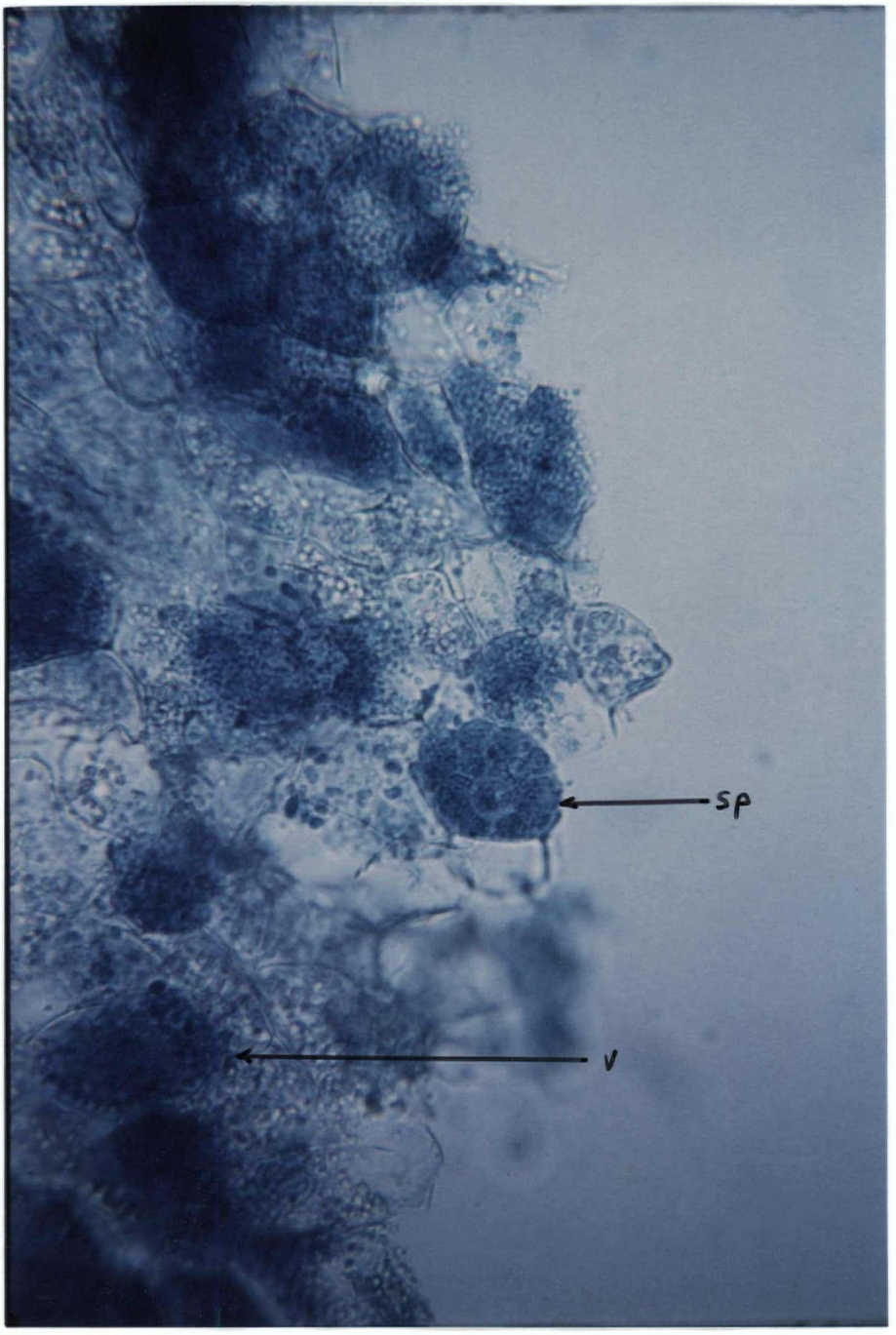


**Figure 1.1**. Red alder nodule section showing cells filled with *Frankia* sporangia (sp) and vesicles (v). Note that the spores within the sporangia are much smaller than the vesicles.

**field procedures**

A replicate of each treatment (Table 2) from each watershed was planted into each of three low and high elevation planting sites (Table 1.2, Figure 1). There were 9 replicates per treatment combination (one from each of three collection watersheds on each of three planting sites). Although the effects of neighbours will be dealt with in later chapters, data from plants with neighbours are included in this chapter to increase sample sizes for the statistical analysis. Only the presence (Table 2. treatments 16 - 21, 22 - 27) and absence (treatments 1 - 12) of neighbours is considered in this chapter. The neighbouring seedlings were from the parent populations from the same watershed as the target plants and the same elevation as the planting site. All sites were located within the U.B.C. research forest near Haney, B. C. (Table 1.1). The original selection criteria was to pick disturbed sites on which red alder is naturally found. The low elevation sites were to be below 150 m and the high elevation sites above 500 m elevation. Unfortunately, one of the low elevation sites had to be discarded shortly before planting was to begin due to a scheduled aerial herbicide spraying on the same cutblock. Site K was then picked as an alternative although it did not meet the selection criteria in that it was over 150 m in elevation. One low and one high elevation site, G and H30, were adjacent to the Haney plant collection sites and so the low and high elevation inoculum for the Haney plants are the same as the G and H30 site inoculum. All the sites had been dominated by coniferous forests and harvested. A bulldozer was used to clear all vegetation from the sites and remove the organic soil layer in early July, 1992. Plots were laid out using a 2 m spacing. To ensure a homogeneous rooting environment, 30 cm diameter x 30 cm deep holes were dug at each plot and the soil sieved through a 1 cm mesh back into the holes. Seedlings were dibbled into the center of the hole. Planting was done from July 21 to 23 with treatments randomly assigned to the plots. Plants were approximately 4 cm tall at the time of planting. Neighbours were three plants on the north, southwest and southeast side of the target plants, planted at a distance of 8 cm from the bas of the target plant. For the first month after planting, seedlings were watered and any dead plants were replaced with pre-inoculated plants kept

in the greenhouse. During the experiment weeds were mowed on up to a weekly basis using a gas powered grass trimmer. Also, all vegetation within the diameter of the crown was removed from around the base of the plants on a monthly basis.

**Table 1.2.** Description of planting sites. Planting site names refer to the name of the road nearest the site in the U.B.C.M.K. Research Forest. Organic matter (OM) and total nitrogen (N) are expressed in percent; phosphorus (P) and potassium (K) are in ppm. Parent material was determined from ecosystem classification maps (Klinka, 1976).

| | Planting sites | | | | | |
|---|---|---|---|---|---|---|
| | Low elevation | | | High elevation | | |
| | G | G40 | K | H30 | H90 | H110 |
| elevation (m) | 65 | 85 | 250 | 530 | 650 | 550 |
| parent material | glacial fluvial | veneer moraine | blanket moraine | colluvial | blanket moraine | blanket moraine |
| harvest date | 1990 | 1985 | 1987 | 1966 | 1982 | 1972 |
| soil chemistry | | | | | | |
| pH | 5.7 | 5.7 | 5.6 | 6.0 | 5.4 | 5.8 |
| OM | 11.1 | 16.1 | 13.1 | 6.0 | 19.0 | 19.0 |
| total N | 0.27 | 0.38 | 0.18 | 0.10 | 0.20 | 0.17 |
| P | 15.0 | 13.0 | 10.0 | 13.7 | 14.0 | 5.0 |
| K | 46.7 | 55 | 19 | 15.3 | 31 | 80 |

**data collection**

Height and diameter measurements were made on a monthly basis during the growing seasons on all plants, including neighbours. These data were converted to total dry mass estimates for each plant using a linear regression of (height x diameter$^2$) to mass of harvested plants ($r^2 = 0.964$). Leaf phenology data were recorded in the spring of 1993 and 1994 by counting the number of flushed buds on each plant on a weekly basis. A bud was considered flushed when the leaf had extended 5 mm beyond the bud scale. The date at which 50% of the leaves on a plant had burst bud was determined by plotting the proportion of burst buds over time and interpolating a curve using Cricket Graph version 1.3 (Cricket Software, Malvern, PA) for each plant. Relative growth rates (RGR) were calculated on a seasonal and monthly basis in 1993 and 1994. Seasonal RGRs were determined for each plant by taking the log transformed estimate of the plant mass in October 1993 and at the time of harvest in 1994 and subtracting the estimated log mass of the plants in the spring of each year before budflush occurred. Monthly RGR values were calculated from the log mass estimates between months divided by the period in days between measurements. For the spring monthly RGR values, the initial time period was calculated as the date at which 50% of the buds had flushed.

Plants were harvested between Aug. 2 and Aug. 11, 1994. Since red alder, like many Betulaceae, sheds leaves in mid summer, leaf litter was collected from around the base of each plant and included as part of the leaf mass. For plants with neighbours, the leaf litter was assigned to each plant as a proportion of the stem mass of the plant, relative to the stem mass of all the plants in the plot (target plant and neighbours). The stems were cut ca. 1 cm from the base of the plant and the leaves and shoots bagged separately. The roots were harvested by digging a trench, with a 16" radius centered at the base of the target plant, to a depth of 40 cm. The remaining soil was then carefully worked away from the roots. Any roots encountered during the digging of the trench, that exceeded the trench boundary, were dug until the root was less than 2 mm in diameter and then pulled by hand from the soil. On the site with the largest plants, site G, eight plants were harvested

by excavating the entire root system. The mass of roots harvested by each method on this site was compared using ANCOVA to determine the proportion of roots actually harvested. The analysis was performed on ln transformed root mass data with ln shoot mass data as the covariate. Roots were stored in plastic bags in a refrigerator for up to two weeks until they were cleaned. All other tissue was put in paper bags and dried to a constant mass at 65° C on the same day it was harvested. Roots were washed using a hose and nozzle before being dried. Nodules were separated from the roots and washed again in the lab before being dried. Allocation to nodules, roots, stems and leaves was calculated on a percent total dry mass basis.

### $\partial^{15}N$ analysis

Differences in ratios of naturally occurring nitrogen isotopes were used to estimate differences in the proportion of fixed nitrogen in the harvested leaf tissue. To reduce costs, plants inoculated with site inoculum and plants with neighbours were not analyzed. The total dried leaf mass from each plant was first crushed and mixed by hand and a subsample (ca. 2 grams) was then ground in a ball mill and sent for analysis of $\partial^{15}N$ (i.e., the ratio $^{15}N$ to $^{14}N$ relative to a standard sample) at the Department of Oceanography, U.B.C. as outlined in Ehleringer and Osmond (1991). The percent nitrogen fixed (Ndfa) was calculated using the formula

$$Ndfa = (X - Y)/(X - C)$$

where X is the $\partial^{15}N$ from a non-nitrogen fixing plant, Y is $\partial^{15}N$ from the alder sample and C is the $\partial^{15}N$ from the nitrogen fixing plant grown under N-free conditions (Domenach et al. 1989). Grasses and rushes were collected from around the perimeter of each site (*Holcus lanatus* on the low elevation sites and a *Fescue* sp. and *Juncus spp.* on the high elevation sites) a week before the harvest of the experimental material for the non-nitrogen fixing plant samples. The percent nitrogen fixed by plants from each site was calculated using the $\partial^{15}N$ for the grasses on that site. I assumed a value of -0.3 for the $\partial^{15}N$ of red alder when growing under N-free conditions (published in Binkley et al. 1985).

## soil analysis

Soil samples were collected from the planting sites in May, 1993 for chemical analysis (Table 1.2). Approximately 100 $cm^3$ of soil was collected to a depth of 10 cm at a point 50 cm south of each plot. Samples from each site were bulked, air dried and chemical analysis performed on the < 2 mm fraction. pH was measured on a 1:1 soil water suspension and percent organic matter by loss on ignition according to McKeague (1981). NPK analysis was performed by Pacific Soil Analysis Inc. (Richmond, B.C.) according to the standard methods described in Page (1982). Nitrogen was analyzed as total nitrogen using a Technicon Auto-analyser, on a semi-micro Kjedahl digest. Phosphorus availability was determined colourmetrically using the ascorbic acid method, on a 1:10 soil to Bray extract. Potassium availability was measured using a Perkin-Elmer atomic absorption spectrophotometer on a 1:5 soil to ammonium acetate extract.

## statistical analysis

Data were analyzed using the software package JMP version 2.0.2 (SAS Institute Inc.) which uses an "effective hypothesis test" (i.e., means model, Shaw and Mitchell-Olds 1993) in cases of unbalanced designs. For unbalanced models with random effects, a synthetic denominator is used to estimate F ratios. When the denominator of the F ratio is not the same as the denominator for a balance designed, the proportion of the mean square of each effect used to construct the synthetic denominator is given in the ANOVA table. The assumption of homogeneity of variance was tested by a visual examination of the residuals and in cases of heterogeneous variances, data were transformed before analysis. Unless otherwise stated, post-hoc tests were performed using a Ryan's Q test (Day and Quinn, 1989).

The data were analysed to determine if there was any evidence for genetic variation between the plant populations sampled and to determine if variation between populations within watersheds was greater than between watershed variation. To test for plant ecotypic variation, data were analyzed using the following mixed nested model:

$$Y_{ijkl} = \mu + E_i + S_{j(i)} + W_k + E_iW_k + W_kS_{j(i)} + P_{l(k)} + E_iP_{l(k)} + S_{j(i)}P_{l(k)} + e_{m(ijkl)} \quad 1.1$$

where $\mu$ is the mean of all data; E is the effect of planting elevation; S is the effect of planting site within each planting elevation; W is the effect of the parent population watershed; P is the effect of the parent population within each watershed and e is the experimental error. All effects are random except for planting elevation which is fixed at two levels. For this analysis the parent population is considered one of six randomly sampled populations within three randomly sampled watersheds. When interaction effects were found to be nonsignificant they were dropped from the model and the analysis was repeated to increase the degrees of freedom of the error (Winer 1971: page 378). To avoid a type 2 error during this procedure, the alpha value for the test was set at 0.20 as recommended by Winer (1971). Ecotypic variation in spring phenology and final biomass was examined. Both the day on which the first bud on a tree flushed (budflush) and when 50% of the leaves on a tree had flushed ($BF_{50}$) were analyzed using all the target plants in the study. It was assumed that trees with and without neighbours did not vary their spring phenology. To examine ecotypic variation in final biomass, the presence and absence of neighbours was added to the model as a fixed factor, not interacting with any other factors.

Interactions between alder and *Frankia* populations were tested using a mixed model ANOVA

$$Y = \mu + E_i + P_j + I_k + E_iP_j + E_iI_k + P_jI_k + E_iP_jI_k + N_l + S_{m(i)} + W_n + e_{o(nmlkji)} \quad 1.2$$

where E is planting elevation (high or low), P is the effect of elevation of the population fixed at two levels (high or low), I is the effect of source of *Frankia* inoculum (site, high or low), N is the effect of presence or absence of neighbours, S is the effect of planting site within each planting elevation and W is the effect of watershed of the plant collection. All factors are fixed except for watershed and planting site. In this model, population source is considered a fixed effect whereas in the model for ecotypic variation (equation 1.1), it is considered a random effect. This is justified on the ground that for ecotypic variation, it can be considered that six out of any number of populations were sampled, but when plant/bacterial interactions are considered, the populations are

sampled as either high or low elevation and are therefore fixed at two levels. Unless stated, tests of significance were performed using the mean square error as the denominator of the F ratio. Dependent variables (Y) used in the analysis were final plant mass, estimated mass in October 1992 and 1993, RGR in 1993 and 1994, and final mass allocation to leaves, stems, roots and nodules. The same model was used to examine variation in percent fixed nitrogen in leaf tissues. However, in this analysis there were only two inoculum levels and the neighbour effect was dropped since data were not collected from all plants (see above).

## RESULTS

### root harvesting

On site G, where a subsample of eight transplants was harvested by excavating the entire root systems and the remaining plants were harvested by trenching, there were no detectable differences between the two harvesting techniques (Figure 1.2). ANCOVA showed no interaction between harvesting technique and ln shoot mass on ln root mass and subsequently no effect of harvesting technique on ln root mass when ln shoot mass was accounted for (Table 1.3). Since plants on this site were the largest in the experiment, it is unlikely that root trenching would have resulted in a greater bias in the estimation of root mass on any of the other sites. So, while trenching around the roots obviously resulted in the loss of some fine root material, the effect on total root mass was not detectable and the root mass values reported can be considered actual total root dry mass.

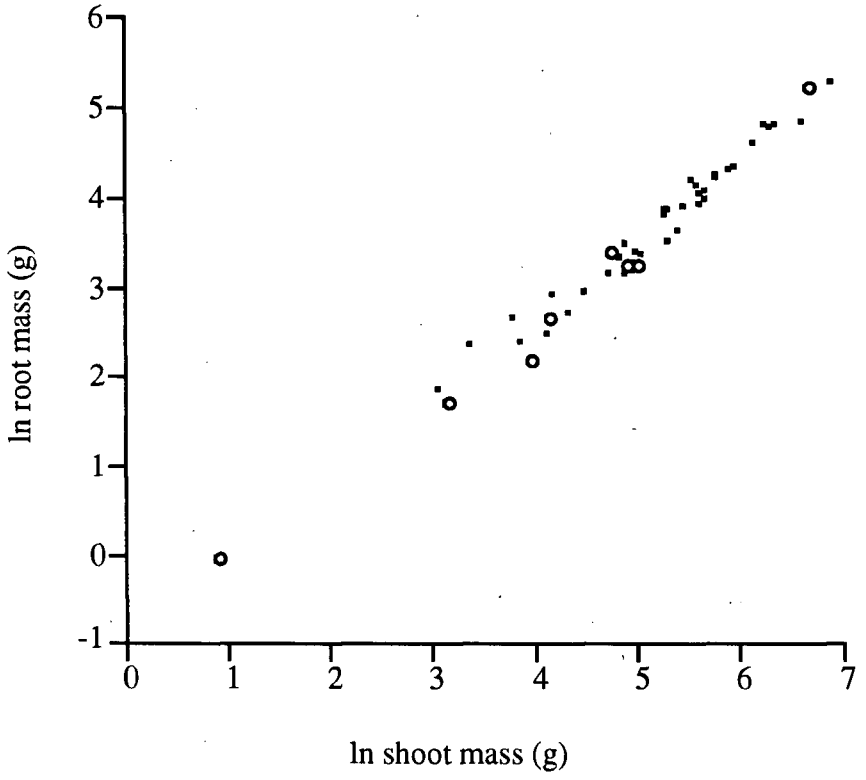


**Figure 1.2.** Effect of root harvesting techniques on root mass. Relationship between ln root mass and ln shoot mass (in grams) for plants harvested by trenching (solid points) and by total root excavation (hollow circles) on site G.

**Table 1.3.** Analysis of the effect of root harvesting technique on root mass. ANCOVA analysis for the effect of root harvesting technique (trenching) on ln root mass with ln shoot mass as a covariate. The model was first run with an interaction term (A) to test for differences in slope before testing for the effect of trenching (B). 37 plants were harvested by trenching and eight by excavating the whole root mass.

| Source | DF | SS | F | P |
|---|---|---|---|---|
| A | | | | |
| shoot | 1 | 38.442 | 1400.177 | 0.000 |
| trenching | 1 | 0.005 | 0.178 | 0.675 |
| trenching x shoot | 1 | 0.000 | 0.002 | 0.965 |
| Error | 41 | 1.146 | | |
| B | | | | |
| shoot | 1 | 39.971 | 1491.310 | 0.000 |
| trenching | 1 | 0.042 | 1.552 | 0.220 |
| Error | 42 | 1.149 | | |

**plant ecotypic variation**

No significant differences were detected between plants from different watersheds or populations within watershed in terms of date of budflush, $BF_{50}$ or final mass (Table 1.4). Although not statistically significant, final plant mass varied by a factor of 1.7 among populations (Table 1.5), with plants from high elevation populations being 28% larger on average than plants from the corresponding low elevation site within the same watershed. The difference between high and low elevation populations was likely due to the effect of inoculation (see below). There was a possibly significant effect (i.e., $0.05 < p < 0.10$) of populations on spring phenology in 1994 ($p = 0.09$ for budflush, $p = 0.07$ for BF50). However the differences only varied by up to 4.5 days, the only apparent pattern being that the plants from the Mamquam watershed flushed their buds earlier than plants from the other watersheds (Table 1.5).

Planting elevation and sites within elevations did however have a significant effect on spring phenology and final mass. In 1993, leaves started to flush on March 12, with the last tree flushing on June 2, a span of 81 days. Trees on low elevation sites started flushing 11 days earlier (85.3 +/- 14.5 vs. 96.1 +/-13.7 days (mean +/- 1 s)) and took 8 fewer days (16.1 +/- 8.7 vs 24.1 +/- 10.7 days) to complete bud flushing (Figures 1.3, 1.4). A similar pattern occurred in 1994, with budflush starting March 6, the last tree starting to flush on April 19, with a 9 day difference between low and high elevation sites. Except for the day of first flushed bud in 1993, there were no interaction effects between planting elevations or sites and plant source for any of the dependent variables (Table 1.4). There was a significant interaction between planting sites and parent watersheds for the date of first bud flushed in 1993. Plants from all watersheds flushed their first buds, on average, later on the higher elevation sites but plants from the Mamquam watershed showed more differences between sites than plants from either Chilliwack or Haney (Figure 1.5).

The coefficients of variation (CV) showed that planting elevation, and sites within elevations accounted for at least 80% of the variation not attributable to experimental error for all dependent

variables examined (Table 1.4). The effect of sites was significant for all dependent variables. Plants on site G always flushed their first buds and 50% of all their buds before plants on any other site, followed by site G40 and site K (Figure 1.4). There were no significant differences in the budflush data between the high elevation sites (Figure 1.4). The pattern in budflush characteristics was reflected in the final plant mass data (Table 1.5), plants on sites flushing buds earlier being significantly larger at the time of harvest.

The relationship in spring phenology between years for individual plants was weak, although significant, with the date when 50% of the bud flushing on a plant in 1993 accounting for 26.4% of the variation in the date when 50% of the buds on a plant flushed in 1994 (Figure 1.6). However, when planting sites were examined individually the relationship usually accounted for much less variation suggesting that the relationship in spring phenology between years is more an effect of differences between sites than between individual plants. The lower range of values seen in 1994, compared to 1993, may have resulted from warmer spring conditions in 1994, causing plants to flush their buds over a shorter period of time.

**Table 1.4.** ANOVAs for ecotypic variation. Analysis on the Julian date of spring budflush and day on which 50% of the buds on a tree had flushed ($BF_{50}$) in 1993 and 1994 and ln mass of harvested plants. Planting site (Site) is nested within planting elevation (Elevation) and population is nested within collection watershed. Nonsignificant interactions were dropped from the model (equation 1.1) and the analysis repeated. Analysis of mass was performed with neighbours presence and absence included as an effect. The test denominator is the proportion of the mean square of each effect used to construct the synthetic denominator of the F ratio for an unbalanced design with random effects. When no test denominator is given, the mean square of the error was used as the denominator of the F ratio. CV is the coefficient of variation for each effect.

| Source | MS | DF | F Ratio | Test denominator | P | CV |
|---|---|---|---|---|---|---|
| 1993 | | | | | | |
| Budflush | | | | | | |
| Elevation | 6719 | 1 | 3.280 | 0.963S* + 0.037E | 0.1439 | 40.1 |
| Site (S) | 2122 | 4 | 5.729 | | 0.0177 | 43.3 |
| Watershed (W) | 374 | 2 | 1.367 | 0.097WS* + 0.003E | 0.3520 | 1.2 |
| Watershed·Site | 371 | 8 | 2.337 | | 0.0198 | 15.7 |
| Population (P) | 63 | 3 | 0.399 | | 0.7542 | -2.4 |
| Error (E) | 158 | 226 | | | | 158.8 |
| $BF_{50}$ | | | | | | |
| Elevation | 16677 | 1 | 8.045 | 0.960S* + 0.040E | 0.0468 | 126.0 |
| Site (S) | 2153 | 4 | 15.823 | | 0.0000 | 50.1 |
| Watershed | 28 | 2 | 0.411 | 1.013P* - 0.013E | 0.6968 | -0.5 |
| Population (P) | 69 | 3 | 0.506 | | 0.6783 | -1.7 |
| Error (E) | 136 | 232 | | | | 136.0 |
| 1994 | | | | | | |
| Budflush | | | | | | |
| Elevation | 3246 | 1 | 16.061 | 0.718S* + 0.282E | 0.0133 | 44.3 |
| Site (S) | 268 | 4 | 8.048 | | 0.0000 | 7.4 |
| Watershed | 193 | 2 | 2.655 | 1.009P* - 0.009E | 0.2180 | 1.8 |
| Population (P) | 72 | 3 | 2.170 | | 0.0929 | 1.2 |
| Error (E) | 33 | 192 | | | | 33.3 |
| $BF_{50}$ | | | | | | |
| Elevation | 1166 | 1 | 3.981 | 0.718S* + 0.282E | 0.1124 | 12.7 |
| Site | 395 | 4 | 12.090 | | 0.0000 | 11.4 |
| Watershed | 205 | 2 | 2.587 | 1.009P* - 0.009E | 0.2233 | 1.9 |
| Population (P) | 79 | 3 | 2.410 | | 0.0683 | 1.4 |
| Error (E) | 32 | 192 | | | | 32.7 |
| ln(final mass) | | | | | | |
| Elevation | 100 | 1 | 4.224 | 0.869S* + 0.131E | 0.1074 | 1.1 |
| Site (S) | 26 | 4 | 12.781 | | 0.0000 | 1.0 |
| Watershed | 1 | 2 | 0.287 | 0.993P* + 0.007E | 0.7691 | -0.1 |
| Population (P) | 4 | 3 | 1.901 | | 0.1317 | 0.1 |
| Neighbours | 29 | 1 | 13.635 | | 0.0003 | 0.3 |
| Error (E) | 2 | 153 | | | | 2.1 |

* For a balanced design, the MS of this effect would be the denominator of the F ratio.

**Table 1.5.** Spring phenology and final mass by population. The date of budflush (BF), the date when 50% of the leaves on a tree flushed but ($BF_{50}$) and mass data are given as the mean + standard deviation (sample size) for each collection population. Mass is in grams and values are based on pooled neighbour presence and absence treatments. Budflush is measured by Julian date. Mortality is given as a percent.

| | Chilliwack | | Haney | | Mamquam | |
|---|---|---|---|---|---|---|
| | Low | High | Low | High | Low | High |
| 1993 | | | | | | |
| BF | 87.1 + 13.2 (38) | 89.2 +14.6 (39) | 92.6 + 15.4 (41) | 90.5 + 12.2 (41) | 90.7 + 17.2 (41) | 92.9 +17.2 (43) |
| $BF_{50}$ | 94.7 + 14.8 (38) | 96.8 + 15.0 (39) | 97.0 + 15.9 (41) | 97.3 + 13.9 (41) | 95.1 + 16.0 (41) | 98.1 + 17.2 (43) |
| 1994 | | | | | | |
| BF | 80.4 + 9.1 (34) | 83.0 + 10.3 (30) | 81.1 + 6.4 (36) | 82.5 + 7.1 (33) | 79.5 + 7.05 (35) | 78.6 + 6.5 (35) |
| $BF_{50}$ | 85.3 + 9.6 (34) | 86.0 + 6.4 (30) | 85.7 + 5.5 (36) | 86.0 + 5.5 (33) | 83.7 + 8.2 (35) | 81.6 + 6.4 (35) |
| final mass | 93.4 + 112.9 (25) | 162.9 + 202.3 (23) | 131.8 + 197.1 (28) | 167.4 + 290.7 (31) | 98.4 + 113.4 (28) | 121.2 + 166.4 (30) |
| mortality | 27.8 | 33.3 | 33.3 | 16.6 | 22.2 | 11.1 |

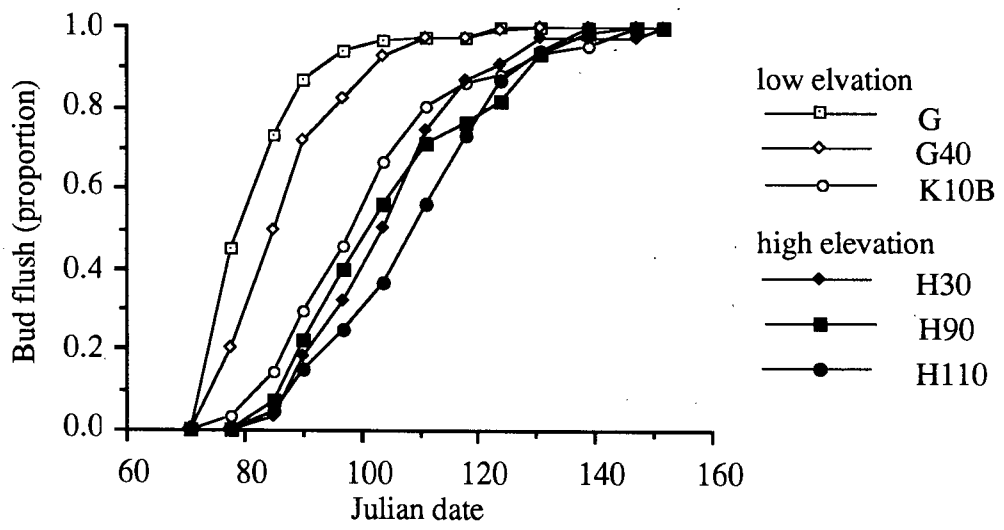


**Figure 1.3**. Cumulative mean proportion of buds flushed per tree on each planting site over time in 1993. Hollow symbols represent low and solid symbols high elevation planting sites. Julian date is the day since January 1 of the year.

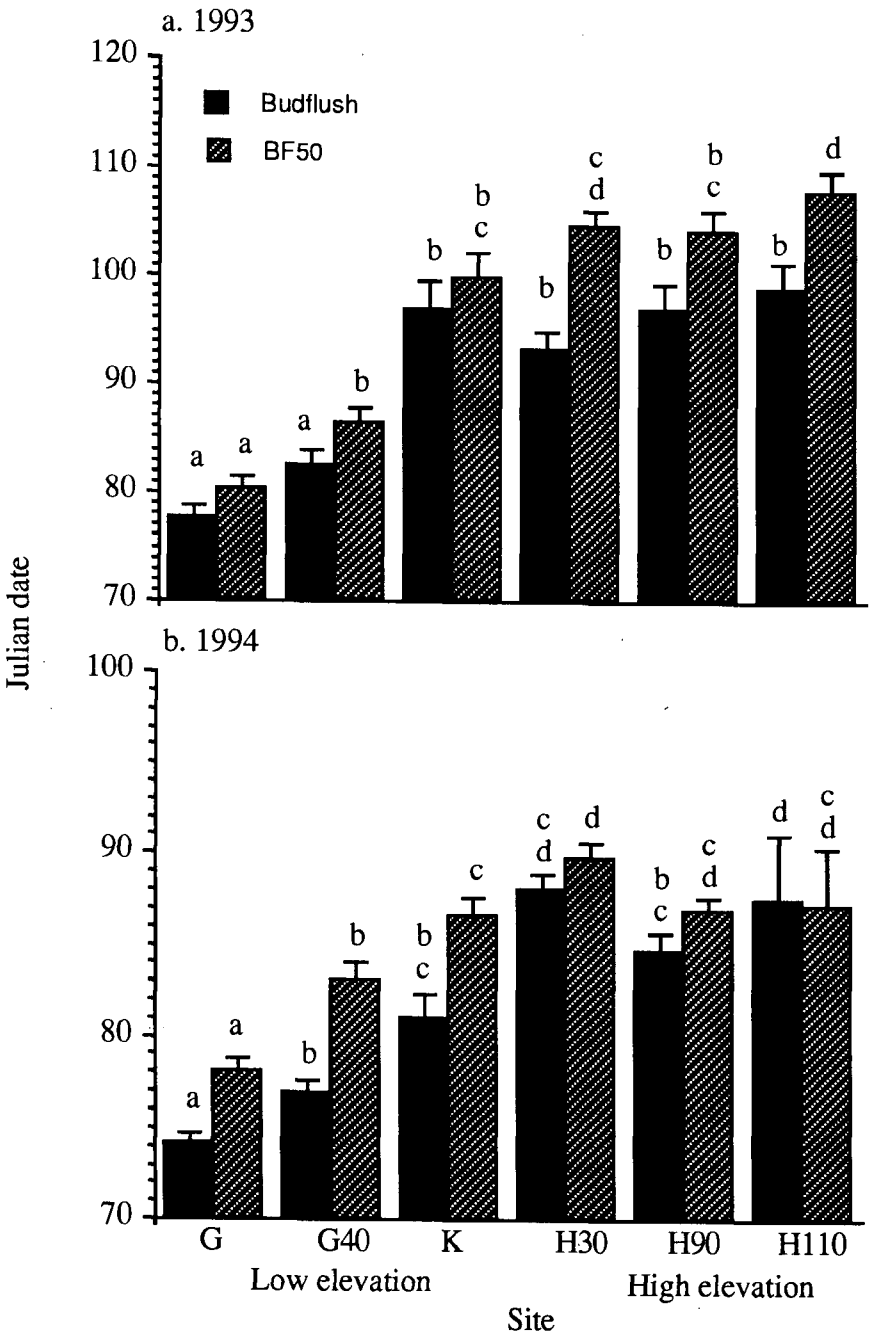


**Figure 1.4**. Spring phenology of transplants on each planting site. Julian date of budflush and date at which 50% of the buds on a plant flushed (BF50) in a) 1993 and b) 1994 on each planting site. Values are means (+ 1 SE). Letters above bars indicate significant differences at alpha = 0.05 between sites, according to a Ryan's Q test, for each dependent variable.

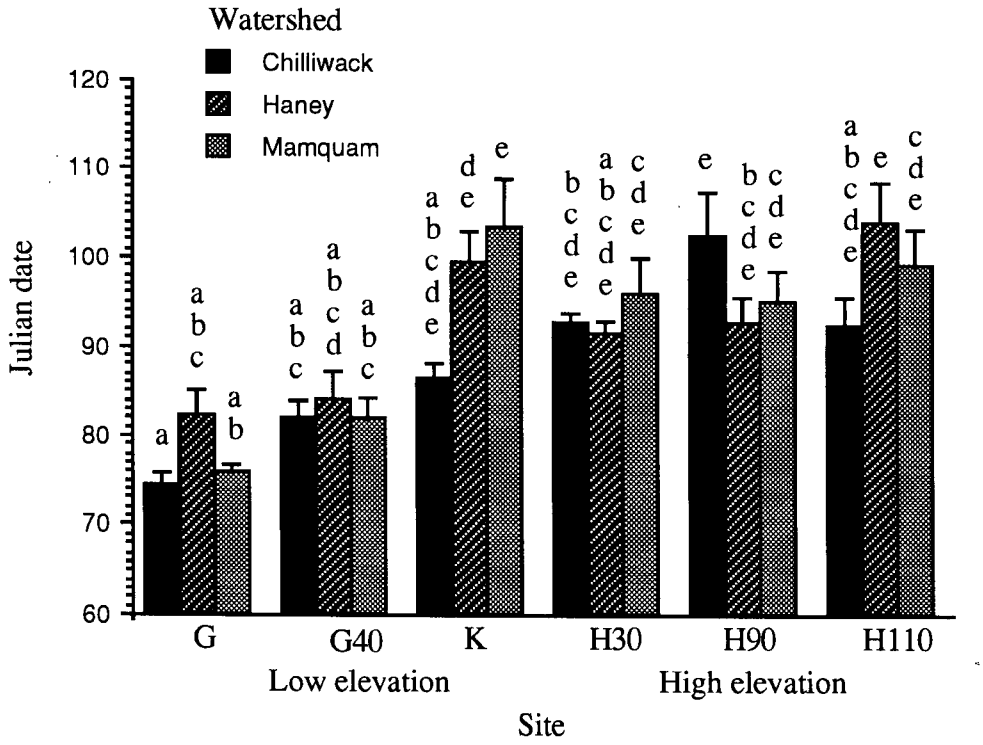


**Figure 1.5**. Mean Julian date of budflush for plants from each collection watershed planted on each site in 1993. Letters above bars indicate significant differences at alpha = 0.05 according to a Ryan's Q test. Error bars are one standard error.

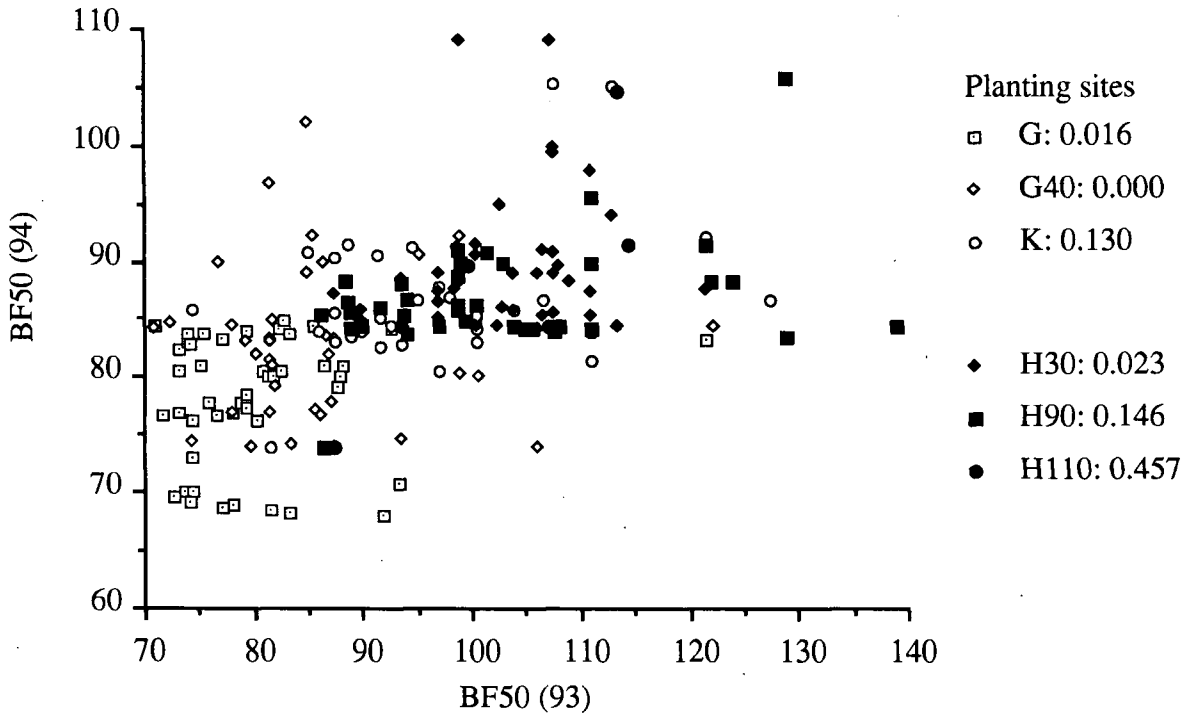


**Figure 1.6**. Relationship between Julian date when 50 percent of the buds on a tree had flushed in 1994 (BF50 (94)) and 1993 (BF50 (93)). Points are data for individual trees on each planting site. Values next to the planting site symbols are the $r^2$ value of the linear regression for the site. The $r^2$ for the whole data set is 0.264.

**alder/*Frankia* interactions: yield**

Sites within elevations, parent elevation, the presence of neighbours and the interaction between planting elevation, parent elevation and inoculum source were all significant sources of variation in final harvested plant mass in 1994 (Table 1.6). Although planting elevation accounted for more variation than sites within planting elevation, mean plant mass within elevations varied three fold between sites, was significantly different between sites within planting elevations and was positively correlated to survivorship (Table 1.7). Plants without neighbours were twice as large as plants with neighbours, 175.3 +/- 234.0 vs. 82.5 +/-113.0 grams (mean +/- standard deviation) averaged across all treatments (neighbour effects are the subject of chapter 2). On low elevation planting sites, plants inoculated with *Frankia* from their parents were half as large as plants inoculated with *Frankia* from the corresponding population within the parent watershed (Figure 1.7), a trend opposite to what was expected. The site inoculum had the same effect as the low elevation inoculum in that plants from low elevation parents were just as small, and plants from high elevation parents were just as large, as when inoculated with low elevation *Frankia* from the parent watershed. On high elevation sites, plants from low elevation parents were twice as large when inoculated with *Frankia* from low elevation hosts and plants from high elevation parents were 1.5 times as large when inoculated with *Frankia* from high elevation parent plants. Again, the effect of *Frankia* from the planting sites was similar to *Frankia* from the high elevation sites within the parent plant watersheds. A similar overall pattern was observed in plants with neighbours although the differences weren't as pronounced. These patterns were observed quite early in the experiment using estimates of mass from height and diameter measurements. By the end of 1992 (i.e. the first growing season, on low elevation sites, plants inoculated with *Frankia* from an elevation which was not the same as the parent plant were twice as large as plants inoculated with *Frankia* from the same elevation as the parent plant (Figure 1.8) although the differences were not significant (Table 1.8). By the end or the 1993 growing season (Figure 1.9) the pattern was the

same as for final yield, with a statistically significant interaction between planting elevation, parent elevation and inoculum source (Table 1.9) as was found with the final yield.

**Table 1.6.** Analysis of the effect of alder/*Frankia* interactions on final plant yield. Analysis of variance on ln transformed total plant mass for the effects of (planting) elevation, parent (elevation), inoculum source, neighbour (presence or absence), planting site and plant collection watershed. All factors are fixed except for planting site and watershed. Planting site is nested within elevation. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to equation 1.2 for the full model.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 103.12 | 1 | 4.5168* | 0.0990 |
| parent | 10.98 | 1 | 5.2997 | 0.0227 |
| elevation x parent | 0.11 | 1 | 0.0539 | 0.8168 |
| inoculation | 0.62 | 2 | 0.3025 | 0.7394 |
| elevation x inoculation | 3.14 | 2 | 1.5162 | 0.2230 |
| parent x inoculation | 0.89 | 2 | 0.4282 | 0.6525 |
| elevation x parent x inoculation | 6.86 | 2 | 3.3095 | 0.0393 |
| neighbours | 27.01 | 1 | 13.0316 | 0.0004 |
| site | 26.30 | 4 | 12.6919 | 0.0000 |
| watershed | 1.27 | 2 | 0.6105 | 0.5444 |
| Error | 2.07 | 146 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

**Table 1.7**. Differences in yield, growth, mortality and biomass allocation between planting sites. Mean total plant biomass (in grams), percent mortality, relative growth rate (RGR in g/g/yr) and percent allocation to plant parts. Letters after mean values indicate significant difference at alpha = 0.05 according to Ryan's Q test using the MS error from the ANOVA models (equation 1.2). Significance tests performed on ln transformed mass data and arcsine transformed data for allocation measures.

| | Low elevation | | | High elevation | | |
|---|---|---|---|---|---|---|
| | G | G40 | K | H30 | H90 | H110 |
| mass | 318.8a | 149.6b | 86.1c | 45.6c | 77.3bc | 28.2d |
| mortality | 0 | 16.9 | 22.2 | 11.1 | 22.2 | 72.2 |
| RGR | | | | | | |
| 1993 | 1.77a | 1.74a | 1.49ab | 1.24b | 1.54ab | 0.42c |
| 1994 | 1.17ab | 1.34b | 1.04a | 1.19ab | 1.28b | 1.20ab |
| Allocation | | | | | | |
| nodules | 1.01bc | 0.94bc | 1.97a | 1.45bc | 1.37b | 0.97c |
| roots | 18.9a | 20.27a | 25.0b | 24.13b | 25.02b | 29.10c |
| stem | 44.9a | 38.3b | 36.13b | 32.76b | 33.86b | 30.22b |
| leaves | 34.89 | 40.49 | 36.89 | 41.23 | 39.76 | 41.96 |

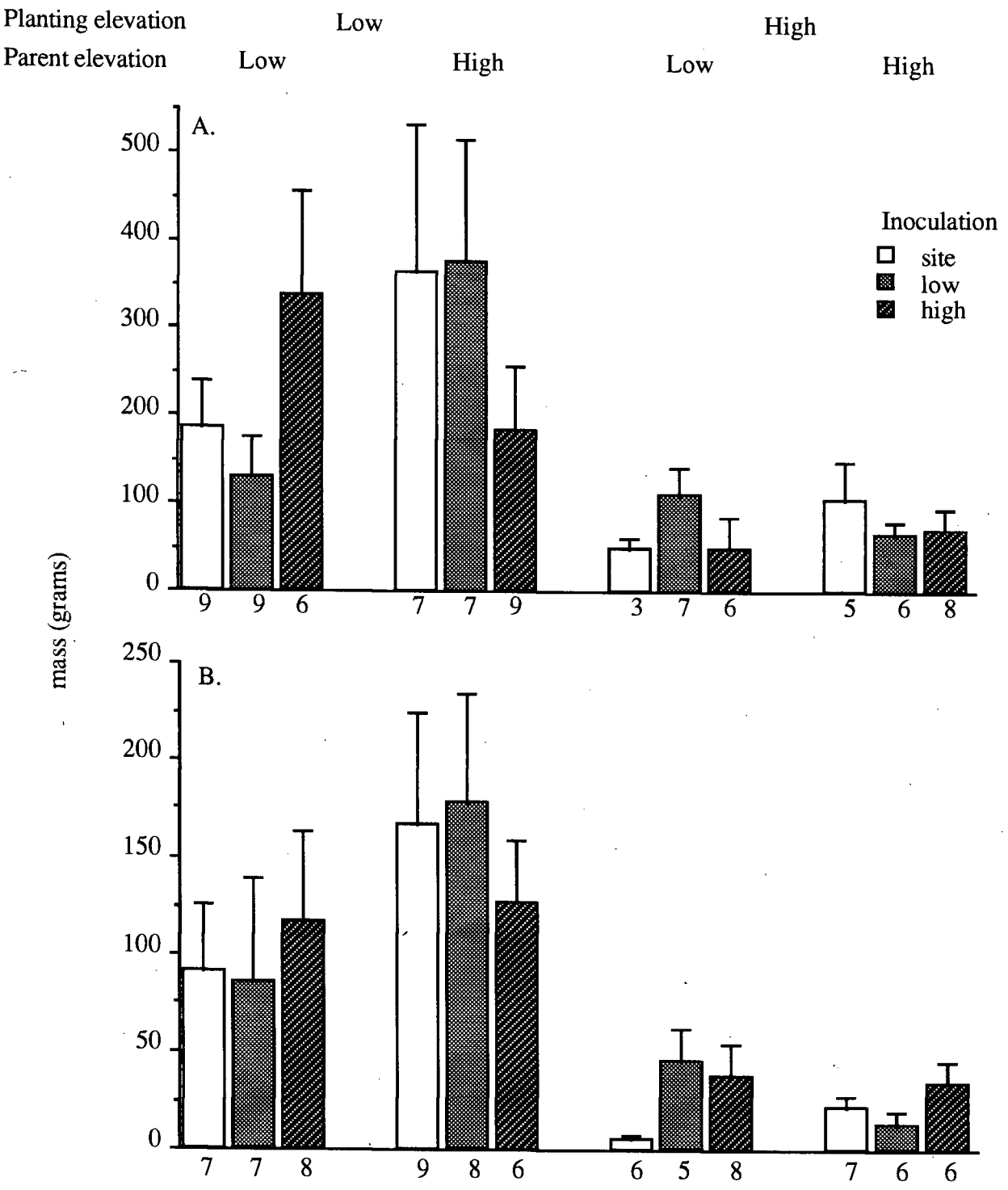


**Figure 1.7**. Effect of alder/*Frankia* combinations on final yield. Mean final dry mass in 1994 of plants A) without and B) with neighbours on low and high elevation planting sites, from low or high elevation parent populations and inoculated with *Frankia* from the planting site or the low or high elevation population within the parent plant watershed. Values are means (+ 1 SE). Numbers below bars are sample sizes.

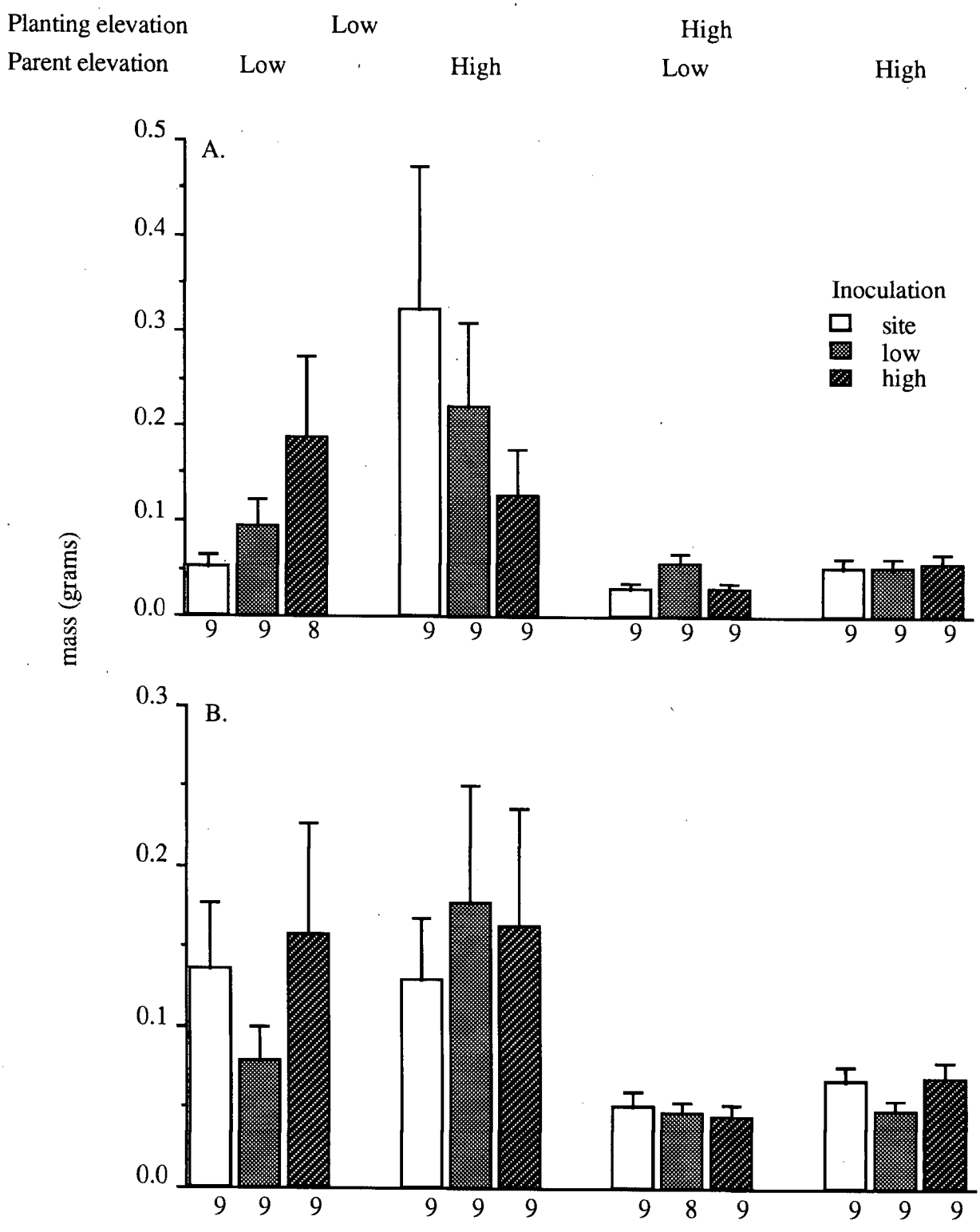


**Figure 1.8**. Effect of alder/*Frankia* combinations on yield in the first growing season. Estimated dry mass at the end of the 1992 growing season for plants A) without and B) with neighbours. Refer to Figure 1.7 for further details.

**Table 1.8**. Analysis of the effect of alder/*Frankia* interactions on plant yield in the first growing season. Analysis of variance on ln transformed estimate of mass at the end of the 1992 growing season. The error mean square is the denominator of the F ratio for all effects, except elevation. See Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 23.55 | 1 | 1.4749* | 0.2914 |
| parent | 8.42 | 1 | 13.6339 | 0.0003 |
| elevation x parent | 0.44 | 1 | 0.7078 | 0.4012 |
| inoculation | 0.16 | 2 | 0.2581 | 0.7728 |
| elevation x inoculation | 0.11 | 2 | 0.1701 | 0.8437 |
| parent x inoculation | 0.17 | 2 | 0.2819 | 0.7547 |
| elevation x parent x inoculation | 0.92 | 2 | 1.4839 | 0.2293 |
| neighbours | 1.06 | 1 | 1.7171 | 0.1916 |
| site | 15.98 | 4 | 25.8114 | 0.0000 |
| watershed | 0.54 | 2 | 0.8675 | 0.4216 |
| Error | 0.619 | 194 | | |

* Synthetic denominator = $0.999MS_{site} + 0.001MS_{error}$
For a balanced design the denominator is the site effect mean square.

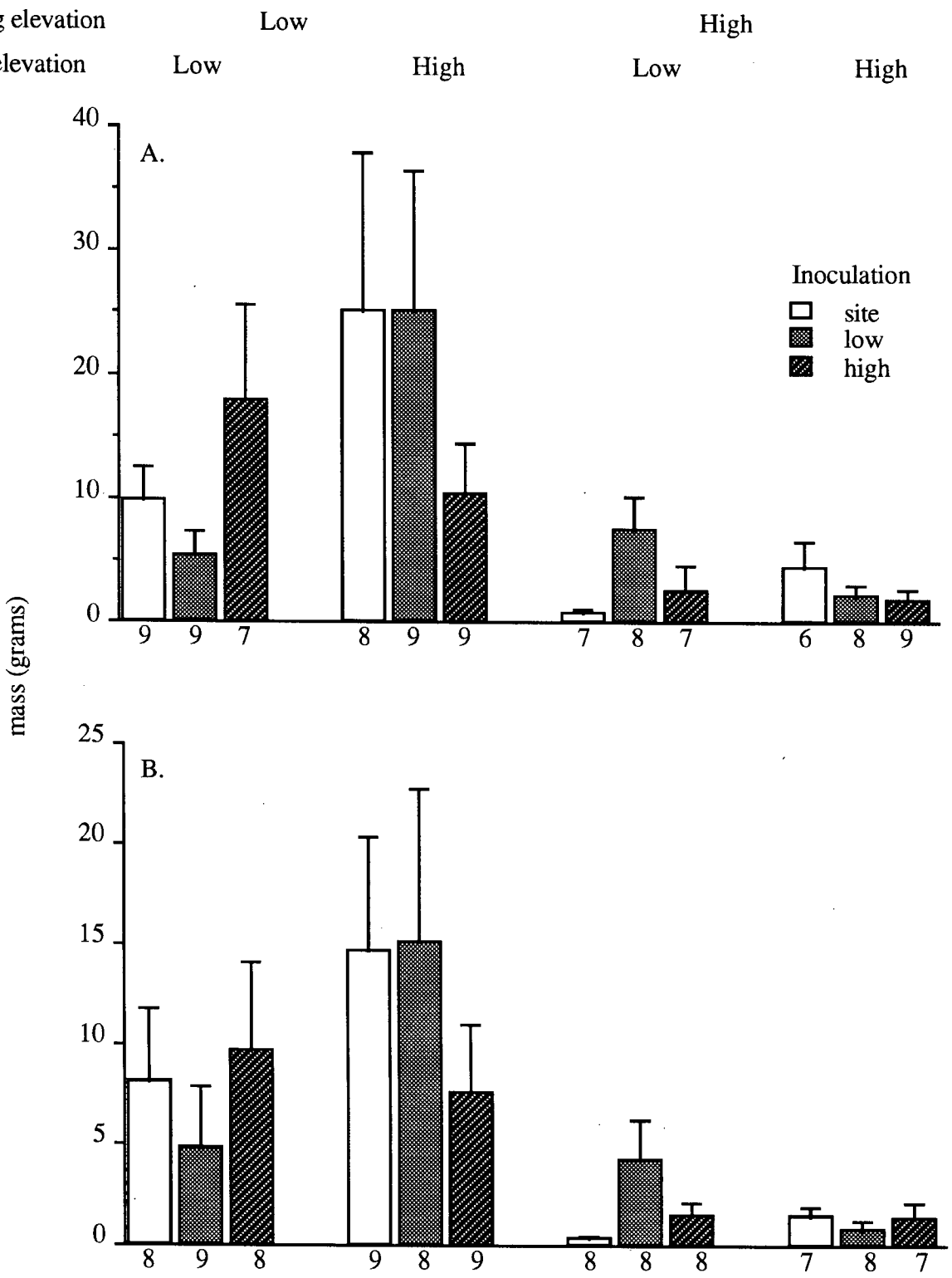


**Figure 1.9**. Effect of alder/*Frankia* combinations on yield in 1993. Estimated dry mass of at the end of the 1993 growing season for plants A) without and B) with neighbours. Refer to Figure 1.7 for further details.

**Table 1.9**. Analysis of the effect of alder/*Frankia* interactions on plant yield in 1993. Analysis of variance for ln transformed estimate of mass at the end of the 1993 growing season. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 186.59 | 1 | 3.5124* | 0.1341 |
| parent | 10.84 | 1 | 4.9850 | 0.0268 |
| elevation x parent | 1.68 | 1 | 0.7720 | 0.3808 |
| inoculation | 0.07 | 2 | 0.0339 | 0.9666 |
| elevation x inoculation | 7.24 | 2 | 3.3288 | 0.0381 |
| parent x inoculation | 2.37 | 2 | 1.0887 | 0.3389 |
| elevation x parent x inoculation | 12.24 | 2 | 5.6308 | 0.0043 |
| neighbours | 16.37 | 1 | 7.5310 | 0.0067 |
| site | 53.92 | 4 | 24.8034 | 0.0000 |
| watershed | 0.53 | 2 | 0.2451 | 0.7829 |
| Error | 2.17 | 174 | | |

* Synthetic denominator = $0.985MS_{site} + 0.015MS_{error}$
For a balanced design the denominator is the site effect mean square.

### alder/*Frankia* interactions: relative growth rates

Since it was assumed that there was no significant difference in plant size at the start of the experiment, no analysis on RGR was performed for the first growing season. The results of ANOVA on RGR in 1993 and 1994 revealed different patterns of variation compared to what was found for plant yield. Most of the variation in RGR between plants in both years occurred between sites (Tables 1.10 and 1.11). In 1993, the RGR of plants was significantly higher on two of the low elevation sites, G and G40, and significantly lower on site H110, than on the remaining sites, as was found for final plant mass (Table 1.7). There was a significant interaction between planting elevation, parent source and inoculum, as was found for final yield (Figure 1.9), but the pattern of variation was different (Figure 1.10). On low elevation planting sites, low elevation plants with site inoculum had the highest RGR. In 1994 differences in mean RGR between sites did not relate to differences in final yield. Plants on sites G40 and H90, had significantly higher RGRs than plants on site K (Table 1.7). The greatest RGR in 1994 occurred in treatments with the lowest final yields. This may have resulted from decreasing RGR with increasing plant size. RGR changed with plant mass, and was more variable at smaller initial masses (Figure 1.11). Changes in RGR over time between treatments could then be affected by changes in plant size over time. The lack of differences in RGRs between alder/*Frankia* combinations, compared to differences in final yield, indicates that differences in final yield were the result of differences in growth in 1992 i.e., the first growing season and these early differences in growth resulted in differences in final yield.

The variation in RGR across elevations and sites could be partially accounted for by differences in the date of budflush. Plants on low elevation sites started flushing buds an average of 11 and nine days earlier in 1993 and 1994, respectively, than plants on high elevation sites. There was an inverse relationship found between the relative growth rate and the date when 50% of the buds on a plant had flushed in 1993 ($r^2 = 0.300$) and 1994 ($r^2 = 0.181$).

**Table 1.10**. Analysis of the effect of alder/*Frankia* interactions on plant growth in 1993. Analysis of variance on alder relative growth rates (RGR g/g/yr) in 1993. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 16.70 | 1 | 3.5419* | 0.1328 |
| parent | 0.21 | 1 | 0.5984 | 0.4403 |
| elevation x parent | 0.08 | 1 | 0.2272 | 0.6342 |
| inoculation | 0.08 | 2 | 0.2190 | 0.8035 |
| elevation x inoculation | 1.56 | 2 | 4.3825 | 0.0139 |
| parent x inoculation | 0.10 | 2 | 0.2832 | 0.7537 |
| elevation x parent x inoculation | 1.78 | 2 | 4.9735 | 0.0079 |
| neighbours | 3.04 | 1 | 8.5176 | 0.0040 |
| site | 4.78 | 4 | 13.3826 | 0.0000 |
| watershed | 0.024 | 2 | 0.0672 | 0.9350 |
| Error | 0.36 | 173 | | |

* Synthetic denominator = $0.985MS_{site} + 0.015MS_{error}$
For a balanced design the denominator is the site effect mean square.

**Table 1.11.** Analysis of the effect of alder/*Frankia* interactions on plant growth in 1994. Analysis of variance on alder RGR (g/g/yr) in 1994. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 0.0123 | 1 | 0.0414* | 0.8478 |
| parent | 0.0942 | 1 | 0.9324 | 0.3358 |
| elevation x parent | 0.3920 | 1 | 3.8786 | 0.0508 |
| inoculation | 0.2904 | 2 | 2.8734 | 0.0597 |
| elevation x inoculation | 0.1047 | 2 | 1.0363 | 0.3574 |
| parent x inoculation | 0.0101 | 2 | 0.0998 | 0.9050 |
| elevation x parent x inoculation | 0.0353 | 2 | 0.3487 | 0.7062 |
| neighbours | 0.0600 | 1 | 0.5932 | 0.4424 |
| site | 0.3309 | 4 | 3.2736 | 0.0133 |
| watershed | 0.0379 | 2 | 0.3753 | 0.6878 |
| Error | 0.1011 | 146 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

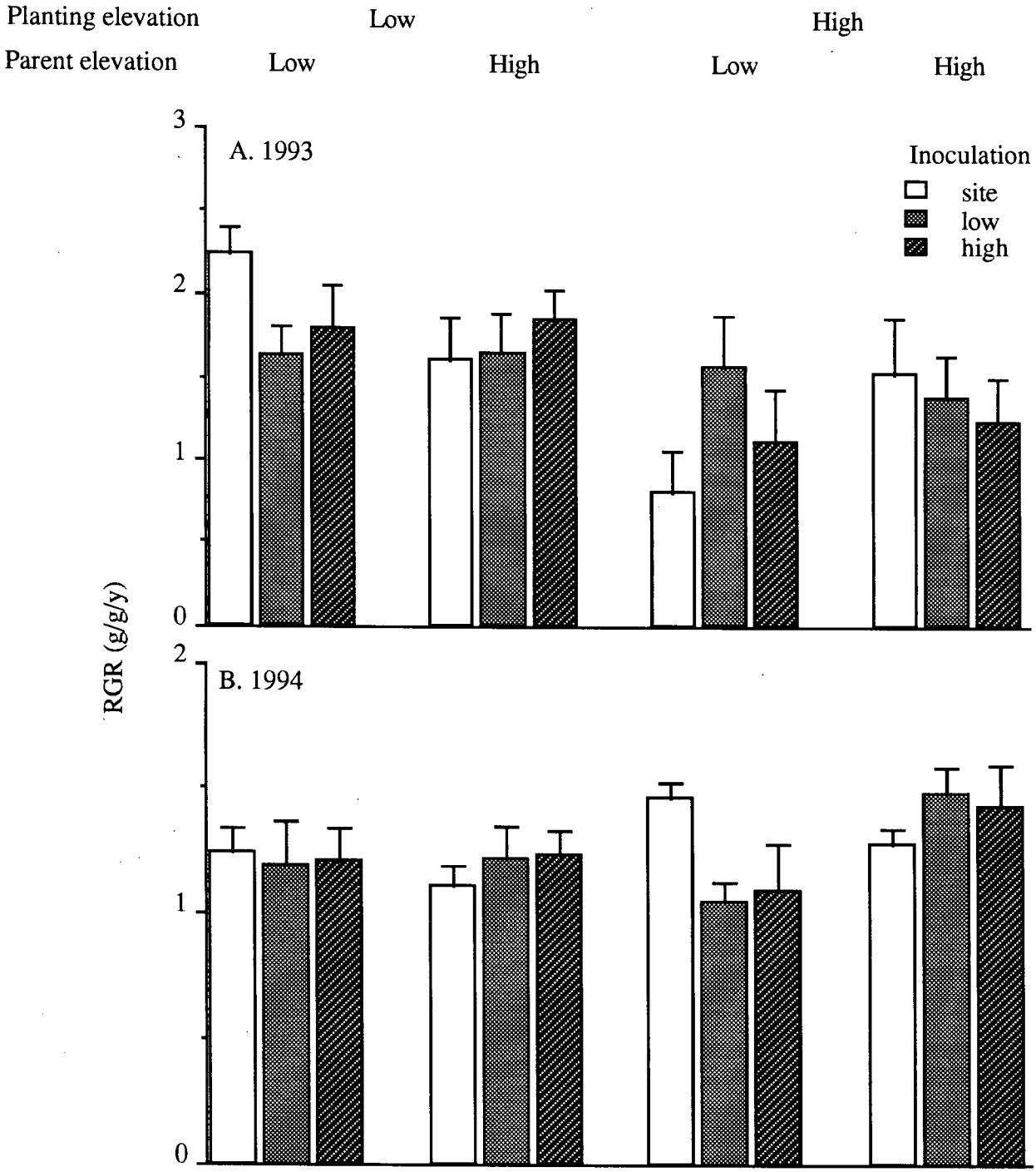


**Figure 1.10.** Effect of alder/*Frankia* combinations on mean relative growth rate (RGR) per year of plants without neighbours in A) 1993 and B) 1994. Error bars are one standard error. Refer to Figure 1.7 for further details.

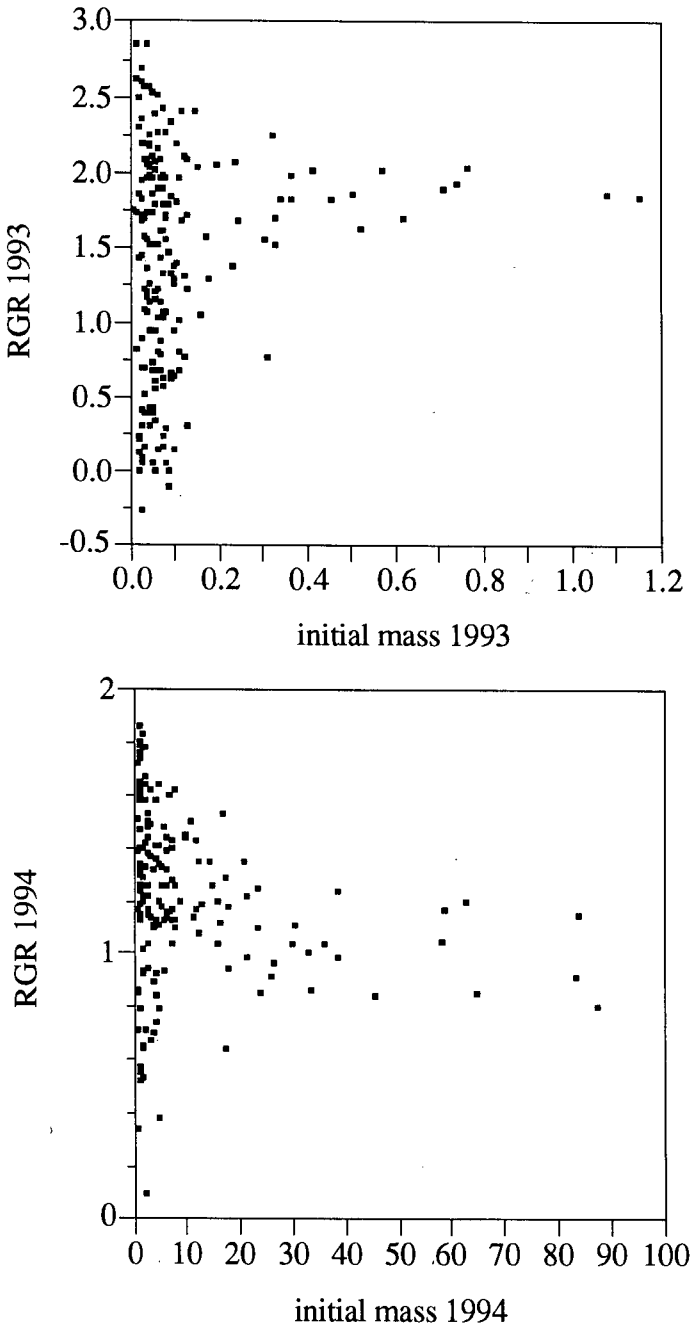


**Figure 1.11.** Relationship between yearly relative growth rate in 1993 (RGR 1993) and 1994 (RGR 1994) and the mass of plants (in grams) at the beginning of the growing season for all plants.

**mortality**

Overall, of the 270 transplants put into the field, 52 died, resulting in a mortality rate of 24.1%. Of the total mortality, 34.6% and 42.3 % were recorded during the spring census in 1993 and 1994, respectively, indicating that 76.9% of the mortality occurred during the winter. Plants on low elevation sites had a 12.9% mortality, which was significantly lower than the mortality rate on high elevation sites (35.2%, Table 1.7). Fifty percent of the total mortality occurred on site H110. Mortality among populations ranged from 11.1% to 33.3% with no significant difference between high and low elevation populations (Table 1.5).

**biomass allocation**

The effect of the different inoculums on plant sources when planted on the different planting elevations could not be explained by differences in biomass allocation to nodules, roots, stems and leaves. Mean biomass (+/- standard deviation) allocation to leaves, stems, roots and nodules was 38.7 +/- 7.6, 37.2 +/- 9.8, 22.6 +/- 6.5 and 1.3 +/- 0.8 %, respectively. Most of the variation was attributable to planting sites (Tables 1.12 - 1.15). For stem and leaf allocation analysis, a number of extreme values made the variances non-homogeneous and could not be corrected using transformations, so the analysis violates the assumption of homogeneity of variances. Plants on the site with the lowest productivity, site H110, had the lowest allocation to nodules and the greatest allocation to roots (Table 1.7). Plants on the most productive site, G, had the lowest allocation to roots and the greatest allocation to stems. There was a possibly significant ($p = 0.068$) three factor interaction effect on root allocation (Table 1.13) which was not related to differences in plant size (Figure 1.12). Plants from high elevation parents, on high elevation sites allocated significantly more biomass to leaves (42.2%) than plants from low elevation parents on low elevation sites (36.2%). These changes in leaf allocation were accompanied by corresponding (although non significant) changes in stem allocation. Allocation tended to vary with mass (Figure 1.13) with increasing allocation to stems and decreasing allocation to roots as plant size increased. Since there

were no data for allocation patterns over time, it is unclear whether these allocation patterns resulted from, or caused differences in plant size. For all plant parts, extremes in allocations were found at small total plant mass.

**Table 1.12.** Analysis of the effect of alder/*Frankia* interactions on nodule dry mass allocation. ANOVA for percent nodule allocation. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 0.0042 | 1 | 0.4565* | 0.5351 |
| parent | 0.0009 | 1 | 0.6827 | 0.4101 |
| elevation x parent | 0.0028 | 1 | 2.1162 | 0.1481 |
| inoculation | 0.0000 | 2 | 0.0118 | 0.9882 |
| elevation x inoculation | 0.0001 | 2 | 0.0769 | 0.9576 |
| parent x inoculation | 0.0033 | 2 | 2.538 | 0.0872 |
| elevation x parent x inoculation | 0.0015 | 2 | 1.1412 | 0.3225 |
| neighbours | 0.0003 | 1 | 0.2142 | 0.6443 |
| site | 0.0104 | 4 | 7.8158 | 0.0000 |
| watershed | 0.0003 | 2 | 0.2238 | 0.7997 |
| Error | 0.0013 | 135 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

**Table 1.13.** Analysis of the effect of alder/*Frankia* interactions on root dry mass allocation. ANOVA for percent root allocation. The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 0.07707 | 1 | 4.7949* | 0.0818 |
| parent | 0.00375 | 1 | 0.7769 | 0.3796 |
| elevation x parent | 0.00027 | 1 | 0.0558 | 0.8136 |
| inoculation | 0.00084 | 2 | 0.1743 | 0.8403 |
| elevation x inoculation | 0.00009 | 2 | 0.0177 | 0.9825 |
| parent x inoculation | 0.00427 | 2 | 0.8842 | 0.4154 |
| elevation x parent x inoculation | 0.01327 | 2 | 2.7487 | 0.0675 |
| neighbours | 0.00193 | 1 | 0.3993 | 0.5285 |
| site | 0.02105 | 4 | 4.3600 | 0.0024 |
| watershed | 0.00178 | 2 | 0.3692 | 0.6919 |
| Error | 0.00482 | 139 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

**Table 1.14.** Analysis of the effect of alder/*Frankia* interactions on stem dry mass allocation. ANOVA for percent stem allocation. Note: variance were not homogeneous due to a number of extreme values (Figure 1.13). The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 0.17915 | 1 | 5.3676* | 0.0765 |
| parent | 0.00175 | 1 | 0.1961 | 0.6585 |
| elevation x parent | 0.01871 | 1 | 2.0949 | 0.1499 |
| inoculation | 0.02474 | 2 | 2.7700 | 0.0660 |
| elevation x inoculation | 0.01132 | 2 | 1.2674 | 0.2846 |
| parent x inoculation | 0.01946 | 2 | 2.1793 | 0.1168 |
| elevation x parent x inoculation | 0.02368 | 2 | 2.6521 | 0.0739 |
| neighbours | 0.01429 | 1 | 1.6001 | 0.2079 |
| site | 0.03747 | 4 | 4.1955 | 0.0030 |
| watershed | 0.00278 | 2 | 0.3108 | 0.7333 |
| Error | 0.00893 | 146 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

**Table 1.15.** Analysis of the effect of alder/*Frankia* interactions on leaf dry mass allocation. ANOVA for percent leaf allocation. Note: variance were not homogeneous due to a number of extreme values (Figure 1.13). The error mean square is the denominator of the F ratio for all effects, except elevation. Refer to Table 1.6 for further details.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 0.02691 | 1 | 2.0621* | 0.2151 |
| parent | 0.00333 | 1 | 0.5319 | 0.4670 |
| elevation x parent | 0.03933 | 1 | 6.2764 | 0.0133 |
| inoculation | 0.01862 | 2 | 2.9705 | 0.0544 |
| elevation x inoculation | 0.00187 | 2 | 0.2980 | 0.7428 |
| parent x inoculation | 0.00327 | 2 | 0.5214 | 0.5948 |
| elevation x parent x inoculation | 0.0076 | 2 | 1.2120 | 0.3006 |
| neighbours | 0.0187 | 1 | 2.9845 | 0.0862 |
| site | 0.0142 | 4 | 2.2664 | 0.0649 |
| watershed | 0.00144 | 2 | 0.2295 | 0.7952 |
| Error | 0.00627 | 145 | | |

* Synthetic denominator = $0.857MS_{site} + 0.143MS_{error}$
For a balanced design the denominator is the site effect mean square.

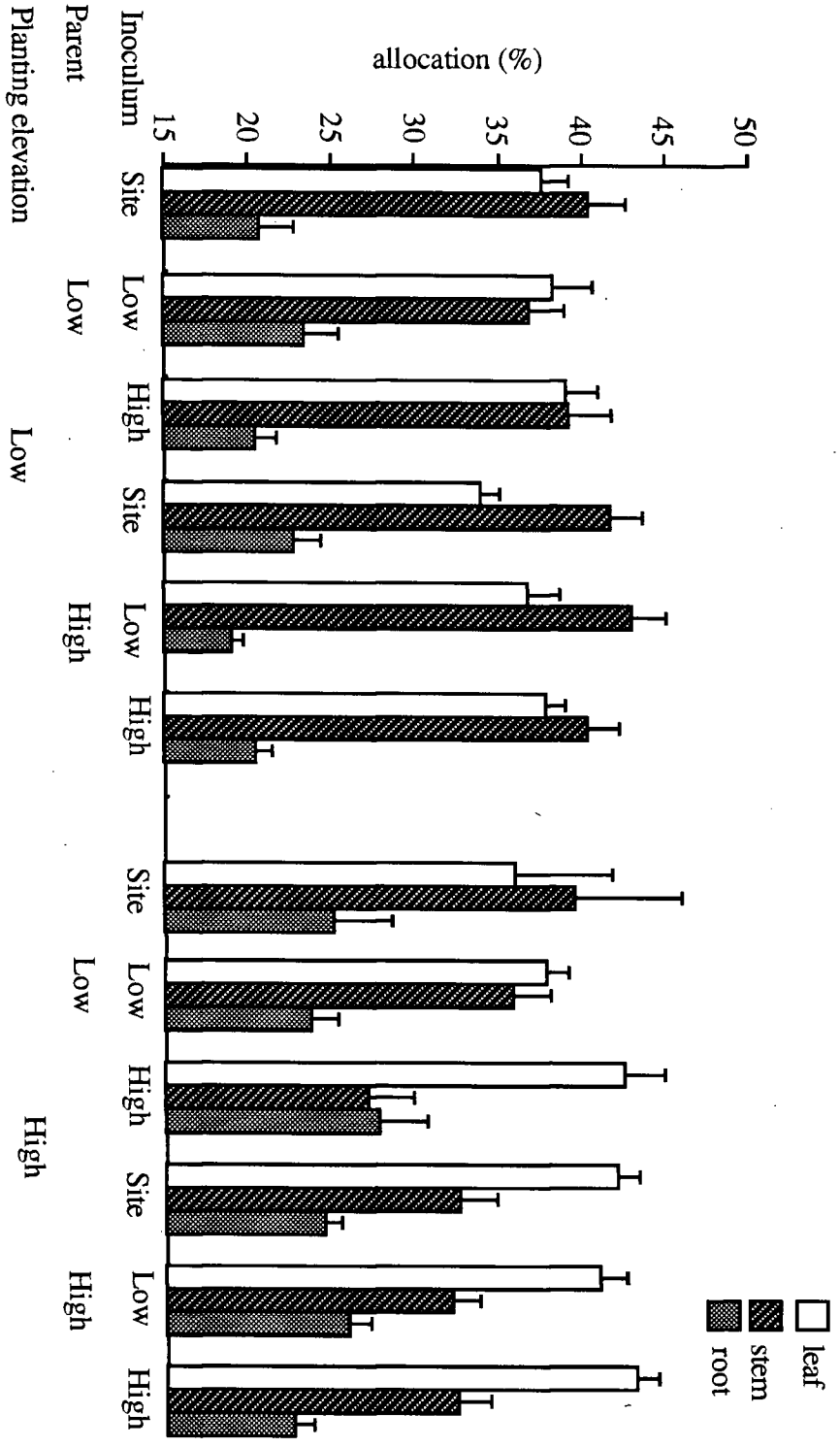


**Figure 1.12.** Mean (+ 1 SE) allocation (% of total dry mass ) to roots stems and leaves for plants on low and high elevation planting sites from low or high elevation parent populations, inoculated with *Frankia* from the planting site or the low or high elevation population within the parent plant watershed.

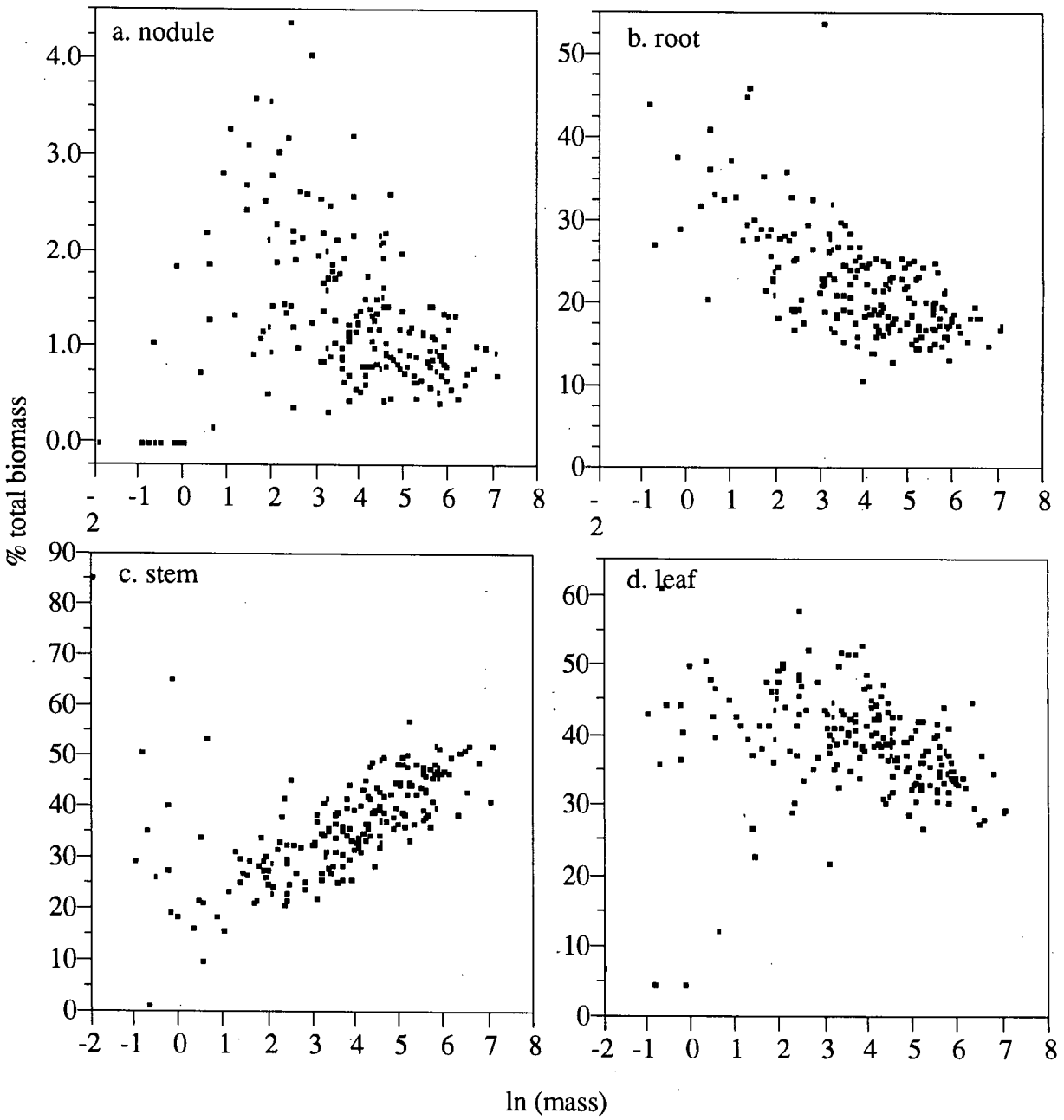


**Figure 1.13.** Relationship between biomass allocation to a) nodule, b) root, c) stem, and d) leaf tissues and ln transformed final plant mass in grams. Y axis is in percentage of the total biomass.

## $\partial^{15}N$ analysis

$\partial^{15}N$ values were negative for all samples and ranged from -2.4 to -0.8 for the alder samples and -4.6 to -1.4 for the samples from the non-nitrogen fixing species. On site H110 the $\partial^{15}N$ values for the non-nitrogen fixing samples were less negative than the alder samples which may have resulted from the two plant types accessing different pools of soil nitrogen. Values from this site were therefore excluded from the analysis since they result in Ndfa values greater than 100%. On all other sites, the $\partial^{15}N$ values for the alder samples were significantly higher than the values for the non-fixing species. The mean Ndfa was 53.05 and ranged from -77.5 to 97.7 %. The mean Ndfa for plants on low elevation sites (38.3 +/- 33.6%) was almost half that of plants on high elevation sites (72.7 +/- 17.6 %). Plants inoculated with *Frankia* from their parent's population had a lower proportion of fixed nitrogen on low elevation planting sites (Figure 1.14), while on high elevation sites plants inoculated with low elevation inoculum had a higher percentage of fixed nitrogen in their leaves (80.2 +/- 10.5 %) than plants inoculated with high elevation inoculum (65.8 +/- 20.5 %). Given that the biomass allocation to nodules did not change across treatments, this indicates that on low elevation planting sites, plants inoculated with familiar *Frankia* populations had less effective nodules. Also, plants on high elevation sites had more effective nodules than plants on low elevation sites and low elevation inoculum was more effective than high elevation inoculum. An examination of the residuals after ANOVA showed a decreasing variance in Ndfa with treatment mean. Since this systematic heterogeneity of variance could not be removed using transformations, the statistical analysis is not included.

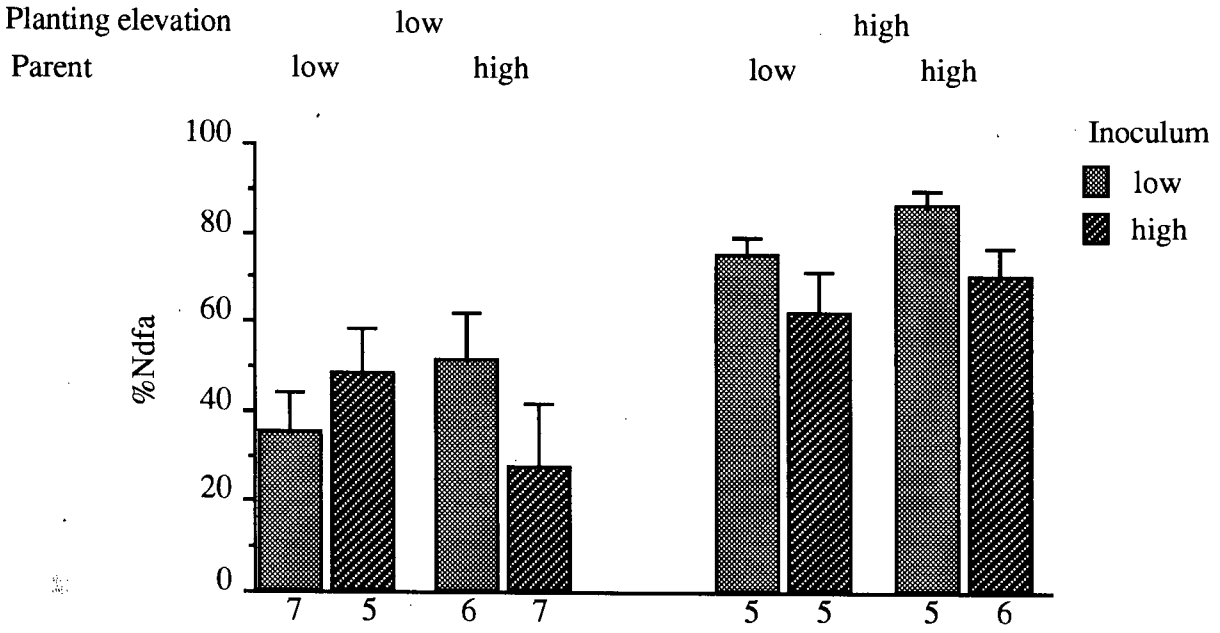


**Figure 1.14.** Effect of planting elevation, parent plant source and high or low elevation *Frankia* inoculum from the parent plant watershed on the percent fixed nitrogen in leaf tissue (Ndfa) at time of harvest of plants without neighbours. Values are mean with error bars of one standard error.

## DISCUSSION

### plant ecotypic variation

There was little evidence for ecotypic variation between the plant populations in terms of growth or spring phenology. Differences between the low and high elevation sites in the date of budflush indicate different climatic conditions vary enough across the elevation range of red alder in this region to have an effect on spring phenology. The 11 and nine day difference in budflush in 1993 and 1994 was somewhat less than what was expected according to Hopkin's law, which states that there should be a 4 day difference for every 400 ft change in elevation (Campbell 1974).There was however, no difference in spring phenology for plants coming from different elevations, a finding which is consistent with the findings of Ager et al. (1993). Wright (1976) summarized phenology data for a number of conifer species in the Pacific northwest and found large differences between seedlots, but elevation and latitudinal trends between seedlots were not pronounced. No elevation trend was found in spring phenology for *Pseudotsuga menziesii* (Campbell and Sugano 1979), and Ager (1993) has suggested this is due to the cool spring climate of the Pacific northwest. Another possible reason for a lack of genetic variation on spring phenology may be the generally low degree of genetic variation in pioneering, patchily distributed species such as red alder (Loveless and Hammrick 1984). A lack of variation in dehardening and budflush have also been found for red alder provenances growing in Britain (Cannell et al. 1987). Provenance trials on *A. glutinosa* growing across Europe (Dewald and Steiner 1986) and *Betula* spp. in Scotland (Billington and Pellham 1991) show strong trends in spring phenology for provenances from different regions although the trends are not explainable by any gradient in latitude, longitude, elevation or distance from the ocean.

The lack of variation in yield between high and low elevation provenances was not expected. The interaction with inoculum source indicates that genetic variation does exist among plant populations however. Although Ager (1987) found genetic variation in growth across elevation gradients

within watersheds, the variation did not occur in all watersheds. Also, the response of different red alder genotypes to different environmental conditions has been found to vary within families (Hook et al. 1987). The amount of genetic variation within red alder populations does not seem to be constant. Like other species which colonize newly disturbed sites, red alder is likely to go through periods of small population sizes within any one area, creating a high degree of genetic variation among populations but a low degree within populations (Loveless and Hammrick 1984).

### alder/*Frankia* interactions

These results indicate a fine scale interaction between alder host and *Frankia* endophyte genotypes and suggests that actinorhizal symbioses can exhibit a similar degree of coevolution between populations as is found in the legume/*Rhizobium* symbiosis. Other cross inoculation studies performed to date have been inconsistent in that no host/endophyte interactions have been detected (Dawson and Sun 1984, Nelson and Lopez 1989, Prat 1989) or when they were detected, they did not result from greater effectiveness when plants were inoculated with *Frankia* from their own taxa (Carpenter et al. 1984, Dillon and Baker 1982, Weber et al. 1989). A possible reason for this inconsistency is that interactions occurring at a finer scale of resolution obscured interaction effects at higher levels where the test were performed. The hierarchical nature of biological systems makes decisions about the scale at which to study phenomena a non trivial problem (O'Neill et al. 1986). Also, past cross inoculation studies have used single pure cultures from each host tested. While the use of pure cultures reduces the possibility of contamination by other microbes, the difficulty (and in some cases impossibility) of isolating and growing *Frankia* in pure culture suggests that the use of pure cultures introduces unwanted selection of strains. Use of a collection of crushed nodules from a stand as opposed to a single pure culture as an inoculum could have a number of effects on host/endophyte interactions. A number of strains have been found within a single *Alnus* stand (Benson and Hanna 1982) and even within a single nodule, using whole cell protein gel electrophoresis (Gardes and Lalonde 1987) but not isozyme electrophoresis (Faure-Raynaud et al. 1991). Using three different isolates on eight *Alnus* species, Prat (1989) found that while one

strain consistently resulted in greater growth when applied as a single strain, a mixture of the strains produced greater plant growth than single strain inoculations. It is therefore possible that the crushed nodules used in this study had a greater effect on plant growth than single strains would have. Although the nodules used for inoculation in this experiment were surface sterilized, it is likely that other soil microbes were present in the inoculum since surface sterilization was usually not completely effective in removing contaminants during attempts to obtain pure cultures. Other bacteria have been shown to act as "helpers" to increase nodulation in actinorhizal (Molina et al. 1994) and legume (Postgate 1982) nodules. Both increased (Chatarpaul et al. 1989) and decreased (Rojas et al. 1992) growth of nodulated *Alnus* have also been shown in the presence of other soil microbes.

My results present a complex picture of plant/*Frankia* interactions within a host species which is distributed across relatively short distances. The expression of these interactions depends on environmental conditions and therefore can be considered a plastic response. On high elevation sites, low elevation plants had the greatest growth when inoculated with *Frankia* from their parents, supporting the idea of coevolution towards increased mutualism. At low elevations, where overall plant yield was greater, the results were opposite to the expected pattern. Plants inoculated with *Frankia* from their parent's elevation were smaller than wild seedlings growing in the same region (personal observation) indicating that these seedlings were stunted (as opposed to the seedlings inoculated with *Frankia* from a different elevation being stimulated). Since the strains which caused stunting in coexistent plant genotypes did not do so in novel plant genotypes, their effects are more indicative of coevolution towards parasitism than mutualism. Although a number of environmental conditions change across an elevation gradient, one likely factor affecting the expression the alder/*Frankia* interactions found here, was total soil nitrogen (Table 1.2). Low elevation planting sites had almost twice as much total nitrogen on average as high elevation sites. Consequently, the amount of fixed nitrogen in leaves was twice as high on high elevation sites. This suggests the plant's demand for fixed nitrogen (and the need to form a symbiotic relationship with *Frankia*) may determine the expression of the interaction between alder and *Frankia*

genotypes.

Differences in growth between sites were correlated to differences in allocation patterns, but differences between treatments were not. Allocation to roots was greater on high elevation planting sites. Although this may have resulted in lower growth and is consistent with the prediction that plants allocate more mass to roots when growing in nutrient poor conditions (Chapin 1980), it is also possible that higher root allocation is the result of small plant size. Since mean plant size changed between sites, and allocation patterns changed with plant size, it is impossible to conclude whether higher root allocation is a cause or effect of plant size. This problem is not always noted (Hook et al. 1990, Shainsky et al. 1992). One way to overcome this problem would be to perform sequential harvests as was done by Arnone and Gordon (1991), who found a rapid decrease in root allocation over time in red alder. The existence of extreme values, the relationship between mass and allocation pattern found here, and the general problem of analyzing ratios (Green 1979) rendered interpretation of the analysis difficult. The allocation values found here are in general agreement with other studies on red alder (Elliot and Taylor 1981, Hook et al. 1990) and *A. incana* (Huss-Dannell and Ohlsson 1992).

Assuming that the constant nodule allocation across treatments found here represents equal access to plant resources for *Frankia* across treatments, then on low elevation sites coexistent *Frankia* can be considered "cheaters" in their mutualistic relationship since they provide less benefit (in terms N fixation and plant growth) to their partners for the same investment made by the host. Theoretical analysis predicts there should be strong selection for cheating in situations were there is a large disparity in the life span of the partners, the partner with the shorter life span evolving a cheating strategy (Boucher 1982). Mutualism is often assumed to involve an initial investment by both partners, leading to future benefits (Axlerod and Hamilton 1981) although other scenarios are possible (Connor 1995). If investments are made by both organisms, then it is possible for one organism to cheat in the relationship by not providing an investment, yet receiving a benefit. There will be selection for cheating since a cheating strategy increases fitness of the cheater (Rosenberg

1992). Since symbionts will have a shorter generation time than their hosts, they will be more likely to evolve a cheating strategy first.

Another prediction from the theoretical study of mutualism is that there is a strong incentive for cheating (defecting) when the likelihood that the partnership will break down increases (Axlerod and Hamilton 1981). In this as in other cross-inoculation studies, *Frankia* were collected from mature trees. As red alder stands mature there is often a reduction in the rate of nitrogen fixation (Binkley et al. 1994) as the tree's nitrogen demand decreases and more fixed nitrogen can be obtained from the soil. Since increased soil N can inhibit the formation of nodules (Granhall et al. 1983) the probability of a breakdown in the partnership will increase as stands mature. Therefore *Frankia* in mature red alder stands could be under selection pressure to evolve a cheating strategy in order to gain access to plant resources, before the relationship with the host trees breaks down, and maximize its net gain in resources. Infection of seedlings by such strains would result in slower growing, and presumably competitively inferior plants. In this study, the depressive effect of coexistent *Frankia* occurred on low elevation sites, which had 1.75 times higher total soil nitrogen. Since red alder only reproduces sexually and seeds germinate on mineral soil, seedlings are unlikely to encounter *Frankia* strains from their parents and so the conditions created in this experiment (the exposure of plant to *Frankia* from their parents) may not occur in nature.

I predict that in actinorhizal species where the breakdown of the relationship between plant and microbe is unlikely, cheating *Frankia* will not evolve. Such situations would occur where the actinorhizal plant is part of the climax community and has little effect on the nitrogen status of the soil. For example, in boreal ecosystems, a number of *Alnus* shrubs form a persistent part of the understory where their effect on soil nitrogen availability can be insignificant (Wurtz 1995). *Alnus* shrubs are also persistent in some tundra communities (Wilson et al. 1985). Dillon and Baker (1982) found *Myrica gale* (sweet gale), which persists in bog communities (which are generally low in available nitrogen) had the highest acetylene reduction (AR) rate when inoculated with *Frankia* from *M. pennsylvanica*, compared to inoculation with *Frankia* from two other host genera.

This suggests coevolution towards increased mutualism. The lowest AR rate, in plants inoculated with the strain from *Comptonia perigrina*, which has a more ephemeral life history (Schwintzer 1989), occurred in *C. perigrina*, indicating evolution of an antagonistic relationship. Plants which reproduce vegetatively are also more likely to encounter their parents' *Frankia* strains. Plants under these situations are more likely to evolve strategies to recognize and inhibit cheating strains.

The fate of *Frankia* strains after the loss of hosts is unclear. Red alder stands are short lived (50 to 60 years; Harrington et al. 1994) and give way to non actinorhizal species. The *Frankia* strains would then have to go through a free living state which may drive the evolution of cheating strains in a different direction. Little is known about the free living status of *Frankia*, although there is some evidence that *Frankia* can adapt to local soil conditions. Sheppard et al. 1989 found that *Alnus glutinosa* and *A. rubra* inoculated with *Frankia* from peat had better growth and nodulation, compared to plants inoculated with *Frankia* from mineral soil, when the plants were grown in peat. I found almost no *Frankia* in second growth conifer stands in the region this study was conducted but relatively high numbers of *Frankia* associated with non actinorhizal species in harvested areas (Markham and Chanway 1996), indicating that *Frankia* do react to changes in non actinorhizal vegetation.

The evolution-towards-cheating hypothesis can also be used to explain the results of a number of other studies. There is at least one other report of *Frankia* from *A. rubra* having a depressive effect when inoculated onto *A. rubra*. duCros et al. (1984) found that when *A. glutinosa*, *A. cordata* and *A. rubra* were inoculated with a pure strain of *Frankia* from *A. rubra*, two out of three *A. rubra* provenances showed significantly lower plant height as compared to uninoculated plants. The *A. cordata* and *A. glutinosa* provenances were unaffected by the inoculation treatment. Cole et al. (1990) have also found that when red alder is planted on previous red alder sites, growth is stunted compared to red alder planted on previous Douglas-fir (*Pseudotsuga menzeisii)* stands. Although they provide data showing decreased soil pH and phosphorus availability on the alder to alder site, part of the decrease in growth could be due to differences in *Frankia* genotypes on the two sites,

with the *Frankia* on the site previously occupied by alder, evolved to a less effective state.

Spore positive *Frankia* can also be considered another type of cheating *Frankia* since they sporulate at the expense of nitrogenase activity and are consequently more infective than spore-negative strains (Schwintzer 1990). Although the distribution of the two strain types has been explained by host selection and differential survival in the soil, it is also compatible with the predictions outlined above. In northeastern North America, both spore types are found on *A. incana* ssp. *rugosa*. Spore-negative nodules dominate disturbed areas while spore-positive nodules are more likely to be found in stream side habitats subject to flooding and where soil pH is less than 4.0 (Holman and Schwintzer 1987). Low pH can inhibit nodule development (Sheppard et al. 1989), depending on the host species (Zitter and Dawson 1992), and therefore increase the chances that the symbiosis will break down. Similarly, flooding and the anaerobic conditions in the stream side habitat may also inhibit nodule activity (Silvester et al. 1988). It has been found, by determining the age of nodules, that spore-positive strains become more prominent on *A. glutinosa* over time in stands in the Netherlands (Van Dijk 1978) which may indicate that spore positive strains evolve from spore negative strains. Weber (1986) found that *A. incana* was normally nodulated by spore-positive strains except were it had invaded an old field. He suggested that only spore negative strains can live saprophytically and are usually dominant on sites previously devoid of actinorhizal plants. This supports the suggestion that the free living state of *Frankia* selects against cheating strains.

The existence of ineffective strains of *Frankia* (Baker et. 1980, Hahn et al. 1988, Lechevalier et al. 1983) also indicates their ability to act as parasites. Some of these strains have been isolated from effective nodules yet are ineffective when inoculated on the original host species. Ineffective strains have been found to dominate the soil in some waterlogged habitats in the Netherlands yet *A. glutinosa* found on these sites never seem to have nodules with these strains (Van Dijk and Sluimer-Stolk 1990). This is likely the result of the low survivorship of seedlings which are not resistant to these strains and also suggests that these strains survive in the soil as free living

saprophytes. The existence of lower resistance to these ineffective strains of *A. glutinosa* from Germany and *A. nitida* from Pakistan suggest that alder is able to evolve resistance to these strains.

When similar types of cross inoculation studies have been performed using the legume symbiosis, performance of plants is generally found to be greatest when inoculated with the familiar bacterial genotype (Lie et al. 1987, Chanway et al. 1989, Parker 1995). There is however evidence for virulent *Rhizobium* mutants and resistant host plants (Djordjevic et al. 1987). Also, ineffective *Rhizobium* strains have been found to be common in some field situations (Hagerdorn 1979, Holding and King 1963).

*Frankia* strains from the planting sites were more similar, in terms of their effect on alder growth, to strains from the same elevation in different watersheds, than to strains in different elevations from the same watershed. This suggests that strains from different areas are subject to similar selection across an elevation gradient. For this study elevation was originally chosen as a source of potential genetic variation because a number of environmental factors vary across elevations. The factors that have resulted in the functionally different *Frankia* strains, or the effects they have at different elevations are unknown.

# CHAPTER TWO

## The competitive ability of alder/*Frankia* combinations

## INTRODUCTION

In chapter one the effect of different *Frankia* populations on the performance of different alder populations was examined under different environmental conditions. The hypothesis being tested was that alder growth would be best when plants were inoculated with their coexistent *Frankia* and planted into their parent's elevation. One would also expect that such coexistent combinations would grow better in the presence of neighbours than non-coexistent combinations. There is evidence from other systems that the effect of soil microbes on plant growth will alter both the intraspecific (Shumway and Koide 1995, Allsopp and Stock 1992) and interspecific (Grime et al. 1987, Fitter 1977) competitive ability of plants, which in turn can have effects on symbiotic interactions (Turkington et al. 1988).

To test the competitive ability of alder/*Frankia* combinations, inoculated plants were grown with other alder neighbours inoculated with the site inoculum. The poorer growth of plants in the coexistent combinations on the low elevation sites, documented in chapter one, meant that the prediction of neighbour effects should be altered. Specifically, one would predict that combinations that produce the largest plants in the absence of neighbours (chapter one) would also produce the largest plants in the presence of neighbours. Since red alder is shade intolerant (Harrington et al. 1994), only treatments resulting in the largest plants should produce competitively successful individuals. The null hypothesis being tested is that the presence of neighbours does not affect the interactions between alder and *Frankia* genotypes.

The type of competitor should also affect the performance of the alder/*Frankia* combinations. To test this, plants from low elevation parents on low elevation sites and plants from high elevation parents on high elevation sites were planted with neighbours from low and high elevation parents (treatments 13 - 15, 22 - 24 and 19 - 21, 28 - 30 in Table 1). The null hypothesis was that plant

performance does not change with neighbour type. The original prediction was that plant growth would be poorer when neighbours were inoculated with coexistent *Frankia* and planted onto their parent's elevation. This was based on the assumption that ecotypic variation resulted in plants being adapted to both their parent's elevation and *Frankia* strains. Since this was not the case (Chapter one), the prediction was changed. Plant/*Frankia* combinations which produced the best growth in the absence of neighbours should also have the best growth in the presence of neighbours, and when used as neighbours, should result in the greatest reduction in growth of the target plant (i.e., plant that receives the effect of neighbours). Specifically, on low elevation sites, the growth of plants from low elevation parents with plants from high elevation parents as neighbours, is likely to be poorer than with plants from low elevation parents as neighbours. Since plants from low elevation parents had poor growth when inoculated with site inoculum on high elevation sites, plants from high elevation parents on high elevation planting sites are expected to have greater growth when grown with plants from low than with high elevation parents as neighbours.

## MATERIALS AND METHODS

Experimental procedures are as described in Chapter one. Neighbour plants were always from the same watershed as the target plant and were always inoculated with site inoculum. The neighbours were three individual seedlings planted on the north, southwest and southeast side of the target plant at a distance of 8 cm from the base of the target plant. As all other vegetation was weeded in a regular bais, it was assumed that neighbour effects are solely due to the presence of these three alder neighbours.

In order to examine the effect of the presence of neighbours on the interaction between plant and bacterial genotypes the following ANOVA model was run on the final biomass data:

$$Y = \mu + E_i + P_j + I_k + E_iP_j + E_iI_k + P_jI_k + E_iP_jI_k + N_l + N_l E_i + N_lP_j + N_lI_k + N_lE_iP_j + N_lE_iI_k + N_l P_jI_k + N_lE_iP_jI_k + S_{m(i)} + W_n + e_{o(nmlkji)} \quad 2.1$$

where Y is the mass of an individual plant, $\mu$ is the mean mass of all plants, E is the effect of planting elevation, P is the effect of the elevation of the population, I is the effect of the source of *Frankia* inoculum, N is the effect of the presence or absence of neighbours, S is the effect of the planting site within each planting elevation, W is the effect of the watershed of the plant collection and e is the error term. All factors are fixed except for watershed and sites. This is the same model that was used to test for interactions between alder and *Frankia* in Chapter 1 (equation 1.2) except that in this model the neighbour effect has been crossed with plant elevation, plant population and inoculum, giving a four factor factorial design with two random factors. This model only tests for the effect of neighbour presence (treatments 22 - 27, 16 - 21) or absence (treatments 1 - 12) and not neighbour type. For this analysis, on low elevation planting sites, the neighbours were from low elevation parents and on high elevation planting sites, neighbours were from high elevation parents. While this may seem like confounding elevation and neighbour type, this procedure was chosen since it mimics what would happen in an on-site removal experiment i.e., low elevation genotypes inoculated with *Frankia* from the planting site would be the neighbours on low elevation sites and high elevation genotypes inoculated with *Frankia* from the sites would be the neighbours on high elevation sites.

The intensity of competition was calculated for each alder/*Frankia* genotype combination. This was done by comparing plant/*Frankia* combinations from the same watershed on the same planting site with and without neighbours. Competitive intensity was calculated as the relative competitive intensity, RCI

$$RCI = (mass_{absent} - mass_{present})/mass_{absent}$$

where $mass_{absent}$ is the mass of plants in the absence of neighbours and $mass_{present}$ is the mass of plants in the presence of neighbours. A relative measure of competitive intensity is preferred over an absolute measure since differences in plant $mass_{absent}$ between treatments can obscure neighbours' effects (Goldberg and Scheiner 1993, Grace 1995).

To test the effect of neighbour type on the competitive ability of plants, the neighbour genotype was varied. On low elevation sites, plants from low elevation parents with neighbours from low elevation parents (treatments 22 - 24) were compared to plants from low elevation parents with neighbours from high elevation parents (treatments 13 - 15). On high elevation sites, plants from high elevation parents with neighbours from high elevation parents (treatments 19 - 21) were compared to plants from high elevation parents with neighbours from low elevation parents (treatments 28 - 30). In order to account for variation due to inoculation, sites and parent plant watersheds, the comparison was made using the following model:

$$Y = \mu + Nt_i + I_j + Nt_iI_j + S_k + W_l + e_{m(ijkl)} \qquad 2.2$$

where Nt is the effect of neighbour type. All other variables were described in equation 2.1. A separate model was run on high and low elevation sites so the site effect S was not nested within elevations.

## RESULTS

Plants with neighbours generally showed the same trend in mean yield per treatment as plants without neighbours (Chapter 1, Figure 1.7). The four factor ANOVA showed that the three factor interaction between planting elevation, parent source and inoculum did not interact with neighbour presence (Table 2.1) indicating that the presence of neighbours did not affect the interaction between alder and *Frankia* genotypes. There was however a possible interaction ($p = 0.07$) between planting elevation, inoculum and neighbour presence. There was a greater difference in growth between plants with than without neighbours on high elevation sites, when inoculated with site inoculum, compared to plants inoculated with high elevation inoculum regardless of the parent plant elevation (Figure 2.1).

**Table 2.1** Analysis of the effect of planting elevation, plant parent elevation, inoculum source and neighbours (presence/absence), planting site and plant collection watershed. on plant yield (equation 2.1). Dependent variable is the ln transformed final plant mass. Planting site is nested within planting elevation. All factors are fixed except for planting site and watershed. The error mean square is the denominator of the F ratio for all effects, except elevation.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 92.363 | 1 | 4.277* | 0.1052 |
| parent | 9.434 | 1 | 4.708 | 0.0318 |
| elevation x parent | 0.293 | 1 | 0.146 | 0.7030 |
| inoculation | 0.250 | 2 | 0.125 | 0.8829 |
| elevation x inoculation | 2.016 | 2 | 1.006 | 0.3683 |
| parent x inoculation | 0.294 | 2 | 0.1465 | 0.8639 |
| elevation x parent x inoculation | 6.637 | 2 | 3.312 | 0.0394 |
| neighbours | 32.134 | 1 | 16.037 | 0.0001 |
| elevation x neighbours | 0.758 | 1 | 0.3781 | 0.5396 |
| parent x neighbours | 0.032 | 1 | 0.016 | 0.9003 |
| elevation x parent x neighbours | 2.857 | 1 | 1.4258 | 0.2345 |
| inoculation x neighbours | 4.719 | 2 | 2.3552 | 0.0988 |
| elevation x inoculation x neighbours | 5.341 | 2 | 2.666 | 0.0732 |
| parent x inoculation x neighbours | 3.319 | 2 | 1.656 | 0.1947 |
| elevation x parent x inoculation x neighbours | 3.228 | 2 | 1.6107 | 0.2036 |
| site | 25.704 | 4 | 12.828 | 0.0000 |
| watershed | 0.903 | 2 | 0.451 | 0.6380 |
| error | 2.003 | 135 | | |

* Synthetic denominator = $0.827MS_{site} + 0.173MS_{error}$
For a balanced design the denominator is the mean $square_{site}$.

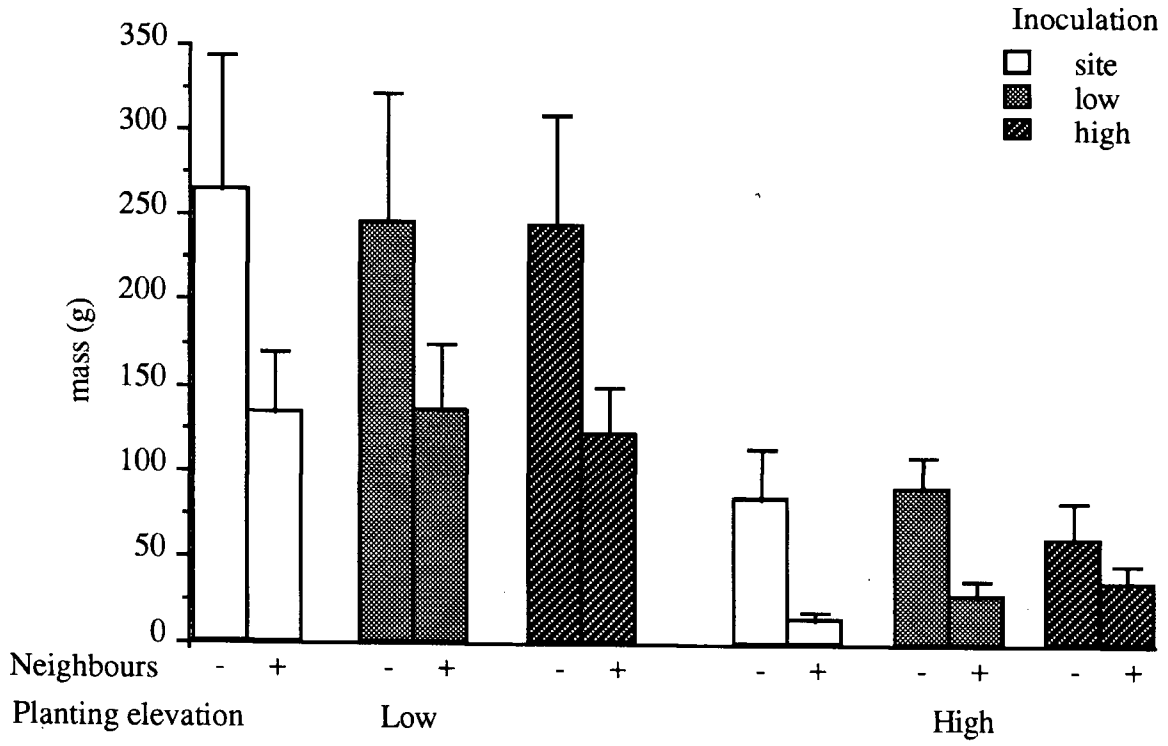


**Figure 2.1.** Effect of planting elevation, target plant inoculum and neighbour presence on final mean total dry plant biomass of target plants. Bars are means (+ 1 SE). Neighbour presence and absence are denoted by + and -, respectively. Neighbours were from the same watershed as the target plant and the same elevation as the planting site. Neighbours were inoculated with the site inoculum. Plants from high and low elevation parents have been pooled.

**competitive intensity**

When plants with and without neighbours were paired by common watershed source, parent elevation, inoculum and planting site, there was a significant ($p = 0.033$) although weak ($r^2 = 0.067$) relationship between ln final mass for plants with vs. without neighbours. There were 40 out of 108 cases where plants could not be paired due to mortality of the matching plant. In 20 out 68 cases, plants with neighbours were larger than plants without neighbours. This results in negative RCI values which were not relative to the largest plant in the pair. Consequently, this resulted in a highly skewed distribution of RCI with extreme negative values. To overcome this problem, the competitive intensity was calculated relative to the largest plant in the pair. To distinguish this index from RCI, I call this the relative neighbour effect (RNE). It varies from -1 to 1 and is negative when plant mass is greater in the presence of neighbours and positive when plant mass is greater in the absence of neighbours. RNE varied from -0.121 to 0.943 across different alder/*Frankia* combinations on low and high elevation planting sites with a mean of 0.310 and a high degree of variability ($s = 0.670$). Values tended to be higher on high elevation planting sites (mean 0.373 +/- 0.689 standard deviations) compared to low elevation sites (0.260 +/- 0.675) with lower values occurring in plants inoculated with high elevation *Frankia* (Figure 2.2). On low elevation planting sites, treatments that resulted in the highest final yields (Figure 1.7) had a higher RNE, indicating that alder/*Frankia* combinations that resulted in greater plant yield were more strongly affected by the presence of neighbours. Since ratio variables such as RNE and RCI may be affected by autocorrelations (Green 1979), no statistical tests were performed and data should be interpreted with caution (see chapter 3 for further discussion).

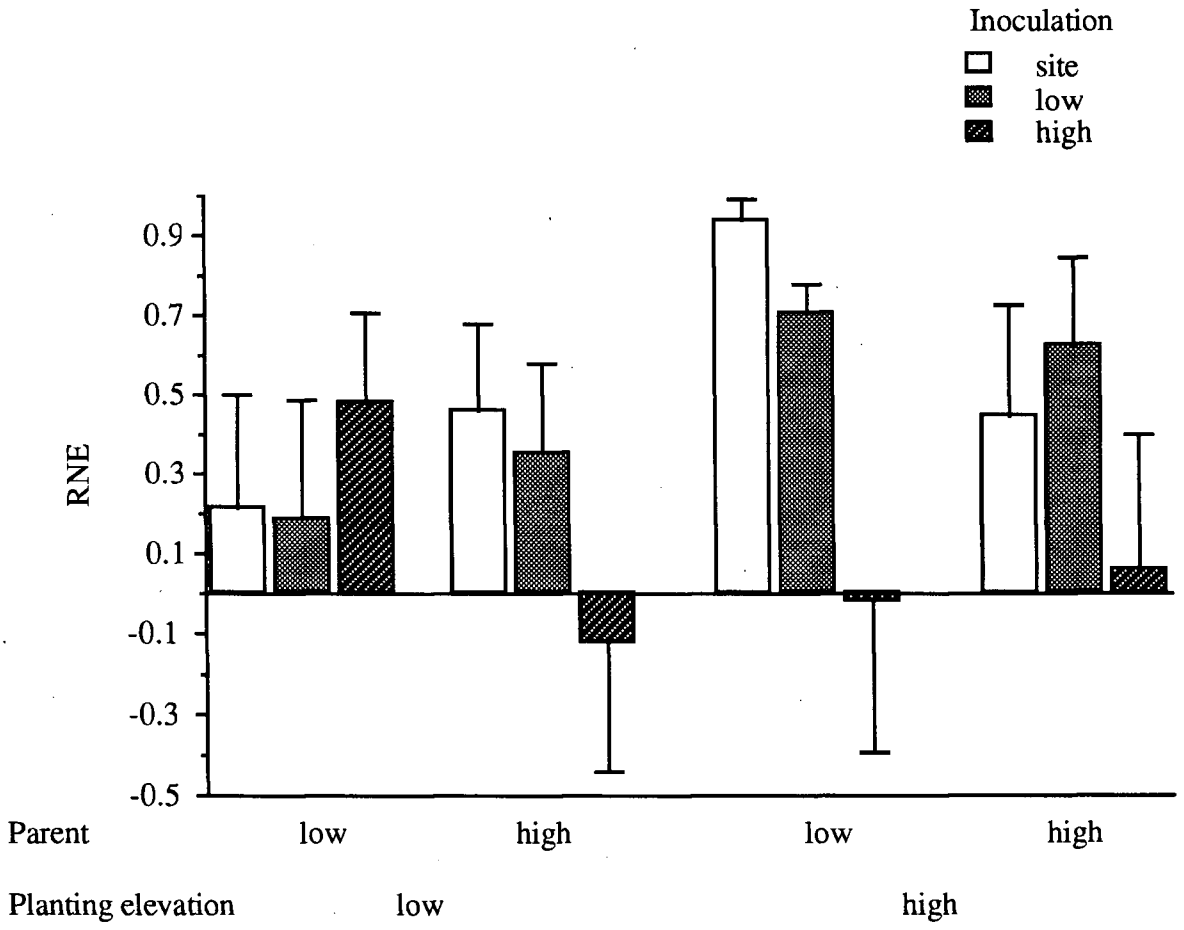


**Figure 2.2.** Effect of planting elevataion, parent plant elevation and inoculum on competitive intensity. Mean (+ 1 SE) relative neighbour effect (RNE) based on final dry biomass of plant genotypes with different inoculation treatments on low and high elevation planting sites. Neighbours were from the same watershed as the target plant and the same elevation as the planting site. Neighbours were inoculated with the site inoculum.

**neighbour type**

Although there were no statistical differences in plant mass for plants with different neighbour types (Table 2.2) there were some consistent trends (Figure 2.3). On low elevation sites, plants from low elevation parents with neighbours from high elevation parents were 30% larger than low elevation plants with low elevation neighbours. This is opposite to the predicted trend. Given the poorer growth of plants from low elevation parents as opposed to high elevation parents when grown without neighbours it was expected that plants would be more negatively affected, in terms of growth, by high elevation neighbours. However differences in growth occurred only during the first growing season (Table 2.3) when there was little difference in growth between plants with or without neighbours (Chapter 3) so are unlikely due to the competitive effects of neighbours.

On high elevation sites, plants from high elevation parents with neighbours from low elevation parents were 2.0 times the size of plants with neighbours from high elevation parents. This was the result of a greater relative growth rate of plants with low elevation neighbours in 1994 (Table 2.3) and was expected since high elevation plants, inoculated with site inoculum grew relatively well and should therefore have a large impact on plant growth when used as neighbours. Therefore while the null hypothesis cannot be rejected according to the statistical test, on high elevation planting sites the trends are as predicted.

**Table 2.2.** Analysis of the effect of neighbour type on plant yield. ANOVA for the effect of neighbour type on ln transformed dry mass (equation 2.2) on low and high elevation planting sites. On low and high elevation sites, target plants were from low and high elevation parent plants, respectively. The error mean square is the denominator of the F ratio for all effects.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| Low elevation | | | | |
| neighbour type | 2.037 | 1 | 0.8078 | 0.3749 |
| inoculation | 2.240 | 2 | 0.8882 | 0.4205 |
| neighbour type x inoculation | 1.591 | 2 | 0.6308 | 0.5381 |
| watershed | 0.636 | 2 | 0.2524 | 0.7783 |
| site | 20.007 | 2 | 7.9334 | 0.0014 |
| error | 2.522 | 35 | | |
| High elevation | | | | |
| neighbour type | 2.922 | 1 | 1.8991 | 0.1809 |
| inoculation | 2.546 | 2 | 1.6546 | 0.2122 |
| neighbour type x inoculation | 2.206 | 2 | 1.5395 | 0.2582 |
| watershed | 0.616 | 2 | 1.0555 | 0.6743 |
| site | 7.390 | 2 | 4.8027 | 0.0176 |
| error | 1.539 | 24 | | |

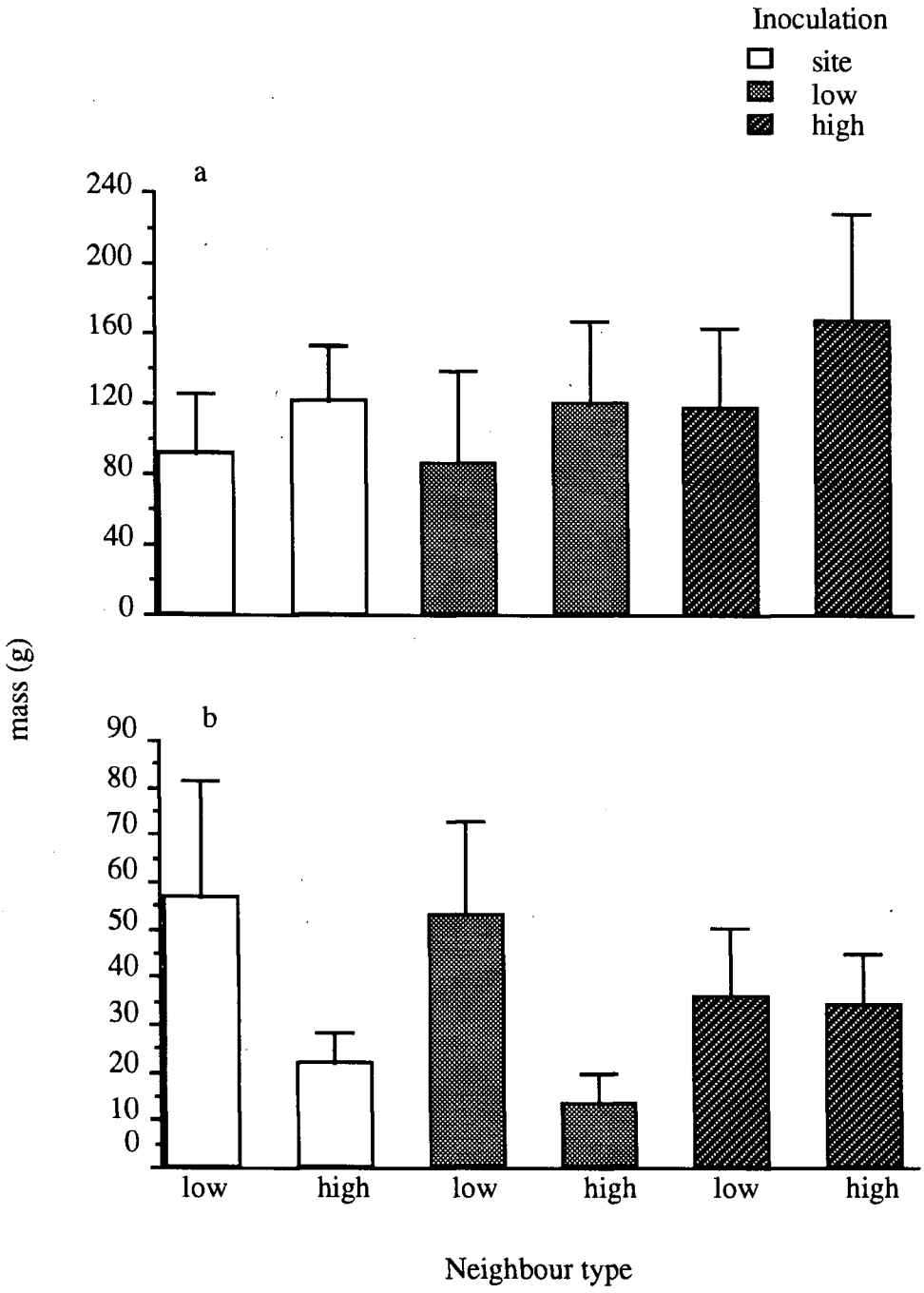


**Figure 2.3.** Effect of neighbour type and inoculum on mean (+ 1 SE) plant yield of a) plants from low elevation parents on low elevation sites, b) plants from high elevation parents on high elevation sites. All neighbouring plants are inoculated with site inoculum. Neighbours were from the same watershed as the target plant and the same elevation as the planting site. Neighbours were inoculated with the site inoculum.

**Table 2.3.** Estimated plant growth for plants with high and low elevation neighbours on high and low elevation planting sites. Values are mean (standard deviation) total dry mass (g) per plant at the end of the 1992 growing season in grams, and relative growth rate (RGR) in 1993 and 1994 in g/g/yr. On low and high elevation sites, target plants were from low and high elevation parent plants, respectively.

| planting elevation | low | | high | |
|---|---|---|---|---|
| neighbour type | low | high | low | high |
| 1992 | 0.140 (0.162) | 0.162 (0.164) | 0.040 (0.024) | 0.054 (0.026) |
| RGR 1993 | 1.55 (0.63) | 1.55 (0.65) | 0.99 (0.91) | 1.03 (0.71) |
| RGR 1994 | 1.19 (0.29) | 1.07 (0.29) | 1.40 (0.22) | 1.19 (0.35) |

## DISCUSSION

In general, treatments resulting in superior plant performance in monoculture also produced larger plants in the presence of neighbours. This is to be expected given the shade intolerant nature of red alder (Harrington et al. 1994). Shade intolerance implies organisms are strongly affected by the presence of neighbours, in terms of competition for light. As competition for light is asymmetrical (Thomas and Weiner 1989), the competitive advantage will be had by the largest individual. This asymmetry of competition also implies that size is a good measure of fitness since the largest plants are more likely to survive and reproduce. The correlation between size and fitness has been documented for a number of plant species (Weiner and Solbrig 1984). Therefore, the coexistent alder/*Frankia* combinations used in this study were likely less fit than other alder/*Frankia* combinations on low elevation planting sites. As discussed in chapter 1, the poor growth of alder with coexistent *Frankia* on low elevation planting sites can be explained as the result of a cheating strategy in *Frankia*. As the presence of neighbours did not affect the interaction between alder and *Frankia* genotypes, this explanation cannot be discounted on the grounds of competitively superior coexistent combinations. It would seem then that, depending on the environmental conditions, the relationship between red alder and *Frankia* coevolves to produce less fit plants, although it is not clear how likely a seedling will be exposed to *Frankia* strains which infected its parent. If they are exposed to these strains, selection should eliminate these combinations through competitive exclusion and there should be selection for plants which are resistant to infection by these strains.

The high degree of variation in competitive intensity and plant growth with different neighbours found here may mask some potentially important neighbour effects. Generally, models of individual plant growth based on neighbour presence or size and distance account for a small proportion of variation in plant size, and models that do are usually in situations where a lack of soil resources means that competition is primarily symmetrical (Firbank and Watkinson 1987). The almost significant interaction of neighbour presence and inoculum on high but not low elevation sites may be a consequence of the performance of the neighbours at the different elevations. On

low elevation sites, the effect of neighbour presence was tested using plants from low elevation parents inoculated with the site inocula. As these site inoculated, low elevation plants, on low elevation sites showed relatively poor growth in the absence of neighbours (Chapter 1) their small effect on the growth of other plants is to be expected. This however does not explain why plants on low elevation sites were larger when grown with neighbours of a different parent population - high elevation parents (which were larger in monoculture and therefore more likely to suppress their neighbours growth). Neighbour effects aren't necessarily negative and high elevation plants inoculated with site inoculum on low elevation sites had a high nitrogen fixation rate when grown without neighbours (chapter 1) which could benefit the target plant when they were used as neighbours. On high elevation sites, neighbour presence was tested using plants from high elevation parents inoculated with site inoculum as neighbours. Since these plants showed relatively good growth when grown in the absence of neighbours (chapter 1), their effect on the growth of other plants, especially those with poorer growth, should be strong. Hence the highest competitive intensity was found for the plants showing the poorest growth in the absence of neighbours (plants from low elevation parents inoculated with site inoculum, planted on high elevation sites). The effect of neighbours on plants from high elevation parents on high elevation planting sites was reduced (although not significantly) when the plants were grown with the relatively poor growing neighbours of low elevation parents as expected.

How, or if *Frankia* strains on one plant affect the likelihood that neighbouring plants may be nodulated by those strains is unknown. Actinorhizal plants can certainly discriminate and prevent nodulation by *Frankia* strains from different host families (Baker 1987) and ineffective strains from the same host family (Van Dijk and Sluimer-Stolk 1990). Also, actinorhizal plants support relatively large free living *Frankia* populations in the soil (Markham and Chanway 1996, Smolander 1990, Smolander and Sarsa 1990). It is therefore possible that a tree or root supporting one *Frankia* strain may increase the possibility of other plants or roots being infected by that strain. In this experiment, the close proximity of the neighbouring plants (8 cm) and the high concentration of the inoculum may have resulted in some cross contamination. Since neighbours

were always inoculated with site inoculum, cross contamination would only be a problem when the target plants were inoculated with *Frankia* from the parent plant watersheds.

Some plants benefitted from the presence of neighbours (i.e., there was a net facilitation effect). Facilitation is a generally overlooked phenomenon in plant communities (Bertness and Hacker 1994). Although a number of studies have measured competitive intensity relative to plant performance in monoculture when facilitation occurs (Belcher et al. 1995, Turkington et al. 1993), the problem of biased estimates of competitive intensity due to extreme negative values has not been addressed. The approach adopted here of making effects of neighbours relative to the larger plant in the pair offers a simple solution to the problems of measuring both the positive (facilitation) and negative (competition) effects of neighbours.

# CHAPTER THREE

## An Analysis of Differences in Competitive Intensity Between Planting Sites

"And do not substitute the pathetic squabble of academic dispute for the voice of nature."

Voltaire

## INTRODUCTION

Over the past decade there has been an active debate about the conditions under which competition is important in structuring plant communities. This debate has grown out of contrasting views of what it means to be a good competitor. Expanding on r-K selection theory (MacArthur and Wilson 1967), Grime (1977) proposed three basic life history strategies: ruderal, stress-tolerant and competitive. It was predicted that these different strategies would dominate in different environmental conditions and stages of succession. Along a productivity gradient, Grime predicts that the intensity of competition will decrease as productivity decreases. This change is accompanied by a change in the dominant life history strategies. Grime assumes that competition for different resources are "interdependent" leading to a "unified competitive ability." Plants with a high maximal relative growth rate are competitively superior under all conditions, although they will not dominate under conditions they cannot tolerate (high disturbance and low resource levels). The concept of a unified competitive ability has been given some physiological basis (Chapin et al. 1993) and other models of community structure have also proposed that there is a tradeoff between competitive ability and stress tolerance. For example, the centrifugal organization model, proposed for a number of plant communities, states that all plants are able to live on sites with low levels of abiotic stress, but only certain individuals will occupy the high stress sites (Keddy and MacLellan 1990). These stress tolerant species are competitively excluded on the low stress sites. The facilitation model of succession proposes that only certain individuals can occupy sites in early succession (Connell and Slayter 1977), and once they alter the site conditions these species are

competitively excluded by later successional species. The idea of a unified competitive ability, however, has been challenged by Tilman (1987) who proposed that there is a tradeoff in plants' ability to tolerate low levels of different resources (Tilman 1982). Competitive ability under this model depends on tolerating low resource levels whereas Grime sees it as the ability to capture resources before competitors do. According to Tilman, a consequence of the tradeoff in competitive ability is that competition will be equally important at high and low productivity sites, only the resources competed for will change. While differences between Grime's and Tilman's view of competition are partly semantic (Grace 1991, Goldberg 1990), they still differ in the prediction of the intensity of competition and its importance in structuring communities under different abiotic conditions.

Both Grime and Tilman have framed their hypotheses about the intensity of competition in terms of interspecific competition. There is no reason to believe why the same arguments wouldn't apply to intraspecific competition. Since plants in the experiments of this present study were grown with and without neighbours at a number of planting sites across the natural elevation range of red alder, the results offer an opportunity to examine changes in competitive intensity between sites and to correlate these with differences in site productivity. Planting sites accounted for the single largest source of variation in plant survival and yield (Chapter 1). Also, since plant mass could be accurately measured non-destructively using height and diameter measurements, competitive intensity could be examined on the same plants over time. The purpose of this chapter therefore is to examine differences in competitive intensity between sites and relate those differences to differences in productivity between sites.

## MATERIALS AND METHODS

Experimental procedures are outlined in Chapter one. To examine the intensity of competition across sites, plants without neighbours (Table 1, treatments 1 - 12) were compared to plants with neighbours (treatments 16 - 27). Given the high variation in plant mass between sites, an absolute measure of competitive intensity is not appropriate for comparisons between sites. To control for

differences in mass between sites, a relative measure of competitive intensity can be used (Grace 1995). However, since in some cases the mass of plants with neighbours was greater than mass of plants without neighbours, competitive intensity needs to be calculated relative to the largest plant in the pair. Competitive intensity therefore was calculated as the relative neighbour effect, RNE (the mass of plant without neighbours minus the mass of plants with neighbour divided by the larger of the two values; see chapter two). This is the same as the more conventional relative competitive intensity, RCI, when the performance of plants without neighbours is greater than the performance of plants with neighbours. However, when the performance of plants without neighbours is less than the performance with neighbours, RNE has a minimum value of -1 whereas RCI has no minimum value. RNE was calculated on mass and on yearly relative growth rate (RGR) data (chapter one). Means of untransformed values for plants with and without neighbours on each site were used to calculate RNE based on mass and on log transformed values used for RGR. Dead trees were considered as missing from the data set. Since mortality was not affected by the presence of neighbours (Chapter 1) this should not bias estimates of competitive intensity. To compare the effects of competition to values presented in Gurevitch et al. (1992) the effect size was calculated for each site as the difference in mean mass of plants with and without neighbours divided by the pooled standard deviation. This was calculated on the ln transformed mass data to obtain a pooled standard deviation.

Productivity was calculated as the total mass of all transplants (target plants and neighbours) harvested from each site. Therefore, site productivity is a function of the size and number of surviving plants on each site.

## RESULTS

Total harvested alder mass varied 74 fold over planting sites (Table 3.1). The low productivity on site H110 was a function of both high mortality (82.2 %, Table 1.7, chapter one) and low growth of the surviving plants on the site. Two of the low elevation sites, G and G40, had the greatest productivity due to greater growth of plants on these sites (chapter one). Plant mass without

neighbours varied 24 fold across sites, following the same trend as productivity (Figure 3.1). The mass of plants with neighbours varied by a factor of 10 across sites. On all but the two lowest productivity sites, K and H110, the effect size was greater than 0.5 (Table 3.1). Only on sites with the highest effect sizes, G and H90, was there a significant difference between the ln mass of plants with and without neighbours. The RNE, based on mean values for plants with and without neighbours on each site was 0.372 +/- 0.422 (mean +/- one standard deviation). Based on seasonal RGR, RNE values were much lower and less variable in 1993, 0.179 +/- 0.061, and 1994, -0.018 +/- 0.125 compared to RNE based on plant mass. Also, the RNE varied between sites between years. The two lowest productivity sites, H110 and K, had the highest RNE values in 1993 and the lowest in 1994. The competitive intensity based on RNE did not vary with site productivity, except that the lowest RNE, in terms of final mass and RGR in 1994 was found at the lowest productivity (Table 3.1).

**Table 3.1.** Plant productivity and effect of neighbours across planting sites. Productivity is the total dry mass of trees planted on each site. The probability that the mass of plants with and without neighbours is equal, p, is based on t-tests of ln transformed final dry mass values. The effect size, $\partial$, is the difference between the mean ln mass of plants with and without neighbours divided by the standard deviation. The relative neighbour effect, RNE, is based on mean untransformed mass and yearly relative growth rate data in 1993 (RGR 1993) and 1994 (RGR 1994). $Mass_{N+ @0.435}$ is the mean mass of plants with neighbours when the mean ln mass of plants without neighbours on the site is 0.435 grams (line a in Figures 3.2 and 3.3). $T_{@0.435}$ is the day, from January 1, 1993, on which the ln mass of plants without neighbours is 0.435 grams.

| | Planting sites | | | | | |
|---|---|---|---|---|---|---|
| | G | G40 | H90 | H30 | K | H110 |
| productivity (g) | 22200 | 11400 | 6100 | 4090 | 3190 | 302 |
| p | 0.037 | 0.173 | 0.011 | 0.137 | 0.446 | 0.629 |
| $\partial$ | 0.724 | 0.519 | 1.02 | 0.525 | 0.296 | 0.252 |
| RNE | | | | | | |
| mass | 0.552 | 0.332 | 0.694 | 0.670 | 0.425 | -0.441 |
| RGR 1993 | 0.065 | 0.159 | 0.208 | 0.196 | 0.221 | 0.226 |
| RGR 1994 | 0.120 | -0.029 | 0.110 | 0.017 | -0.152 | -0.171 |
| $Mass_{N+ @0.435}$ | 2.90 (2.18) | 2.94 (4.10) | 2.46 (3.82) | 1.38 (1.50) | 2.28 (3.35) | 18.8 (24.5) |
| $T_{@0.435}$ | 159.3 | 213.2 | 270.9 | 341.2 | 248.9 | 577.0 |

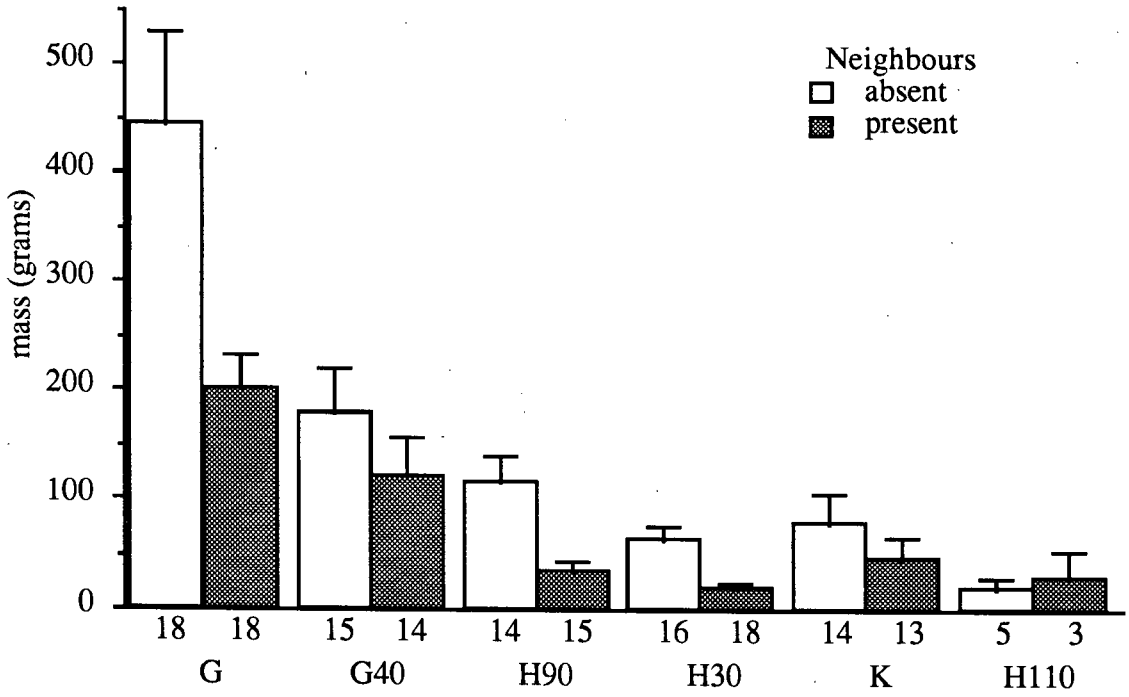


**Figure 3.1**. Final mean plant final dry mass of plants with (target plants) and without neighbours on each planting site. Values are means (+ 1 SE). Values below bars are sample sizes. Sites are arranged from high to low productivity. Sites G, G40 and K were the low elevation sites.

There are two problems associated with the calculation of relative competitive intensity. The first is the problem of spurious correlations involved in the use of ratio data (Jackson and Sommers 1991, Kenny 1991). Even if two variables, X and Y are uncorrelated, a correlation can exist between X/Y and Y. Site productivity, as measured here is a function of plant size. Since plant size is used to calculated RNE, a spurious correlation could exist between site productivity and RNE. A second problem with calculating the intensity of competition at final harvest is related to the fact that plants in this experiment were not allowed to grow to maturity. Plants on lower productivity sites may not show the same degree of competitive intensity as plants at higher productivity sites because they have not reached the same level of crowding.

To overcome the problems of using an absolute measure of competitive intensity when plant sizes are different, and a relative measure when plants on higher productivity sites are at a more advanced developmental stage, the mass of plants with neighbours can be compared between sites when the mass of plants without neighbours is equal. This can be done by making the comparison between sites at different times (plant mass on more productive sites is estimated at an earlier period in the experiment and compared to plant mass of less productive sites at a later period). Plant mass with and without neighbours was calculated from the height and diameter measurements made on a monthly basis throughout the growing season (chapter 1). Figure 3.2 shows the mean of the ln transformed mass estimates for plants without neighbours on each planting site. The change in ln mass over time is fairly linear for most sites. The ln mass of plants with neighbours is graphed against the ln mass of plants without neighbours in Figure 3.3. Each y value for a site in Figure 3.2, corresponds to an x value in Figure 3.3. Comparing the mass of plants with neighbours at the same mass of plants without neighbours removes the need for using a relative competitive index. An examination of the changes in the mass of plants with neighbours as the mass of plants without neighbours increases, will also show if sites are following the same trajectory of competitive intensity as plants grow in size. All sites do not follow the same trajectory (Figure 3.3). In the early stages of the experiment (at small plant masses) the mass of plants with

neighbours tends to be larger than the mass of plants without neighbours (i.e., facilitation is occurring). This occurs on all but the most productive site, G, where the initial estimated mass of the plants was out of the range where facilitation occurred on the other sites (below a ln mass of plants without neighbours of -3 g). Plants on the four mid productivity sites, G40, H90, H30, and K, all follow the same trajectory of decreasing mass of plants with neighbours compared to mass of plants without neighbours. This decrease was also observed on the highest productivity site, G, but occurred at a much larger plant size, indicating that the same intensity of competition took longer to develop. This is also reflected in the lower RNE based on RGR in 1993 for this site. On the lowest productivity site, H110, there was an increase in the mass of plants with neighbours, relative to the mass of plants without neighbours over the course of the experiments, even at plant sizes where there was a net competitive effect on the other planting sites.

To test differences in the mass of plants with neighbours when plants on each site were at the same stage of growth, the mass of each plant was calculated when the mean ln mass of plants on each site was the maximum mean ln mass on the lowest productivity site, H110, i.e., 0.435 grams (line a in Figures 3.2 and 3.3). Although this means comparisons were made at a small plant size (ca. 15 cm in height), this is the maximum size for comparing all sites. It also seems unlikely that by estimating plant mass at later dates, that a greater difference between the remaining sites would be found. The dates between which the 0.435 gram value was reached for each site was first determined from Figure 3.2. The slope of the line between the two dates was then determined for each site and used to calculated the date on which the mean ln mass was 0.435 grams. The ln mass of individual plants was then calculated using individual ln mass estimates to calculate the mass on the date when the mean ln mass was 0.435 grams. The backtransformed mean mass of plants with neighbours at a constant ln mass of 0.435 grams for plants without neighbours is given in Table 3.1. Plants with neighbours on site H110 were 6 times larger than plants on all other sites but there was no significant differences in ln mass between sites ($p = 0.369$) according to a one way ANOVA.

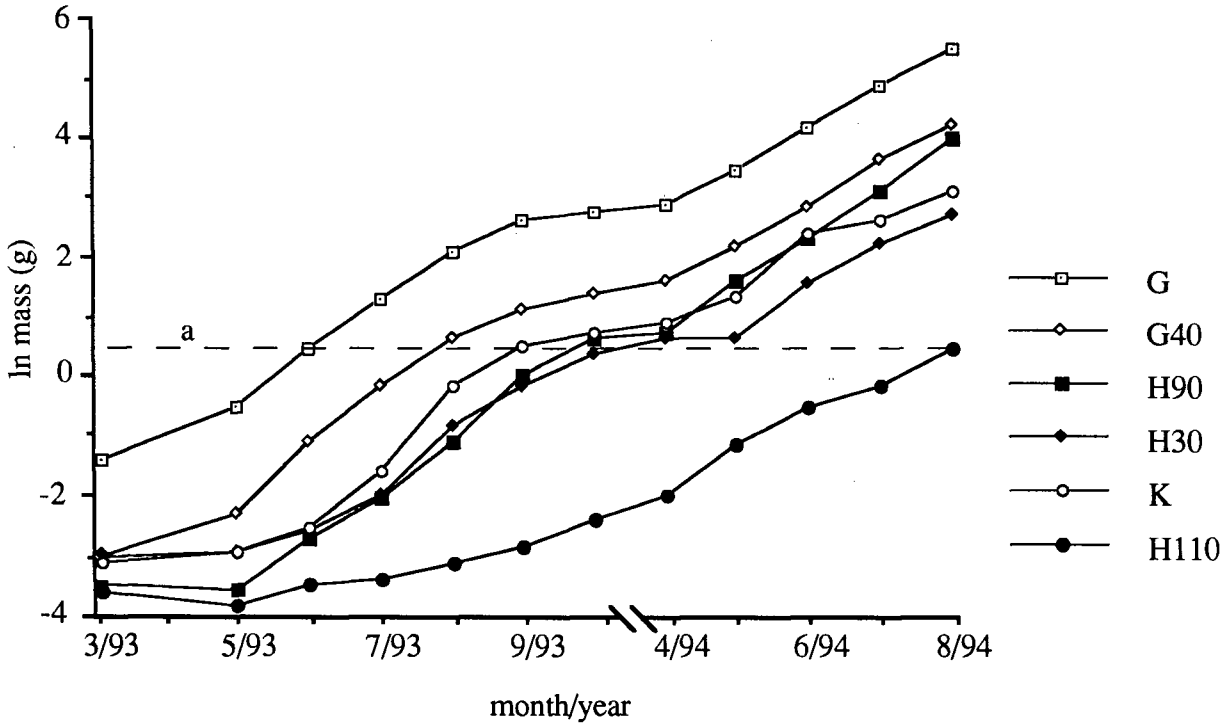


**Figure 3.2** Mean ln dry mass of plant without neighbours on each planting site over time. Sites are listed in order of decreasing productivity. Low elevation sites have hollow and high elevation sites have solid symbols. Line a indicates mean ln mass for each site at which the mass of individual plants with neighbours was calculated (0.435 grams).

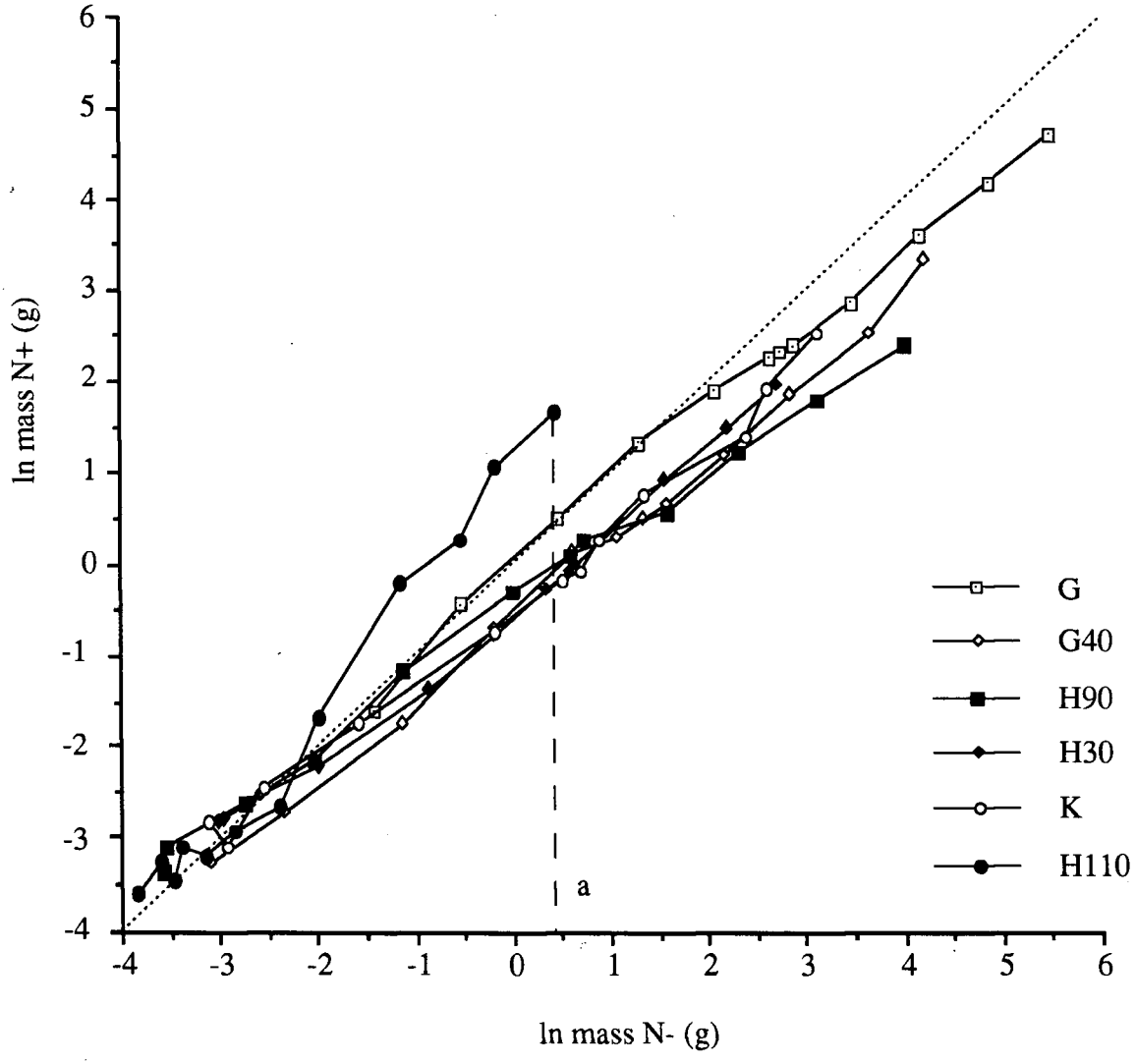


**Figure 3.3.** Mean ln mass of plants with neighbours , N+, vs plants without neighbours, N-, over the course of the field experiment. Each point along each line is the estimated mass of plants with and without neighbours at a different point in time over the course of the experiment. Values above the dotted line indicate a net facilitation effect and values below, a net competition effect. Effects of neighbours at constant plants sizes between sites can be made by comparing plant mass in the presence of neighbours (ordinate) at a constant mass of plant in the absence of neighbours (abscissa). Line a delineates the largest plant mass were the mass in the presence of neighbours for all sites can be compared.

## DISCUSSION

The data presented here indicate that within a large range of site productivity competitive intensity is not correlated to productivity. However, on the lowest productivity site, H110, there was net competitive effect due to the presence of neighbours. These data therefore do not support Tilman's hypothesis of no change in competitive intensity across a productivity gradient. Since the decrease in competitive intensity only occurred on one site, these data do not provide enough evidence to falsify Tilman's hypothesis and it may be possible that the reduction in competitive intensity on site H110 was not a function of the productivity of the site but due to some other factor particular to that site.

On the lowest productivity site, growth was enhanced in the presence of neighbours, indicating that facilitation, and not competition, is occurring. Facilitation is generally believed to be important where abiotic conditions are harsh (Bertness and Hacker, 1994) and has been found to occur in a number of other competition experiments (Belcher et al. 1995, Wilson and Tilman 1995, Turkington et al. 1993, Wilson and Keddy 1986). Its existence creates a problem for the measurement of relative competitive intensity since changes in growth due to the presence of neighbours are not made relative to the largest plant. This results in an index with no minimum value and a maximum value of one, creating a skewed distribution. Comparisons and estimates of means are then strongly affected by a few extreme negative values. This problem of measuring neighbour effects is easily overcome by adopting the RNE index presented here. The interaction between competition and facilitation is not addressed by either Grime or Tilman and is generally not a component of models of community structure. One model has proposed that the degree of benefit one plant receives from another is inversely proportional to the degree of niche overlap (Hunter and Aarssen 1988). This is unlikely in this case since neighbours are conspecific. Rather, the assertion by Belcher et al. 1995 that facilitation occurs at extremely low productivity is a more likely explanation of facilitation for the data presented here. Their model also suggests that competition will be equally intense over a broad range of productivity, which is also consistent

with these findings. It may be that competitive intensity is not proportional to productivity but rather there is some minimal threshold level of productivity needed for a competitive effect. Such a pattern would agree with Grime's prediction that competition is less intense at low productivity but not Tilman's prediction that there is a change in the resources competed for along a productivity gradient.

Examining the mass of plants with neighbours, relative to the mass of plants without neighbours, over time, offers a method of examining the effects of neighbours independent of plant size. Here, the effect of neighbours was similar for most plants over the course of the experiment, although plants on the most and on the least productive sites followed different trajectories from the remaining sites. Although there are strong arguments for the use of relative measures of competition (Goldberg and Scheiner 1993, Grace 1995), the problems associated with analyzing ratio data have not been addressed by workers in this field.

The effect sizes (differences between mean performances of plants with and without neighbours divided by the pooled standard deviation) found here are at the high end of the range of plant competition studies reviewed by Gurevitch et al. (1992). This study differs from those reviewed in a number of ways however. First, this study deals with intraspecific competition, which is likely to be stronger than interspecific competition (Harper 1977) which was reviewed by Gurevitch et al.(1992). Second, this is not a field study as defined by Gurevitch. Rather, it is a garden experiment where density and wild vegetation are under control.

While the published data addressing competition over productivity gradients has been presented as being somewhat equivocal (Goldberg and Barton 1992, Turkington et al. 1993) I believe a close examination lends more support to the idea of decreasing competitive intensity at low productivity. In two mesocosm studies, the relative competitive intensity has been reported as not varying across the productivity gradient while the absolute competitive intensity does (Campbell and Grime 1992, Turkington et al. 1993). Since a relative measure of competitive intensity is clearly preferable when plants of different sizes are compared (Grace 1995, Goldberg and Schiener 1993) these data would

at first glance appear to falsify Grime's prediction of decreased competition with decreased productivity. Turkington et al. (1993) feel that an absolute measure of competitive intensity is the "most appropriate measure of competitive suppression" because RCI obscures competitive effects relative to changes caused by nutrients and disturbance. However they did not measure absolute reduction relative to these other factors. When this was done using the data of Campbell and Grime (1992) the effect of competition, relative to the effect of nutrient supply and disturbance, decreased as nutrient supply increased and disturbance decreased. Competition in this instance was measured as the difference in yield between monocultures and mixtures relative to the difference between maximum yield for the species in the matrix and the yield in mixture (page 245 in Campbell et al. 1991). This is a measure of the importance of competition (Weldon and Slauson 1986), not its intensity. Also, by definition, the importance of competition will decrease with increasing disturbance and decreasing nutrient supply when the maximum plant mass is the mass at the lowest disturbance and highest nutrient level in the matrix. I therefore think that relative competitive intensity is the most useful measure to look at in these mesocosm studies. While there is no difference in the relative competitive intensity in terms of plant yield in Campbell and Grime's mesocosm and percent cover in Turkington et al. (1993) for most species, the situation for the smallest species, in terms of mass and stature, is different. The smallest species in Campbell and Grime, *Desmazeria rigida*, suffers from high competitive intensity at all levels in the matrix. Both *Poa annua* (the next smallest species in Campbell and Grime) and *Trifolium repens* (the smallest species in Turkington et al.) show decreased relative competitive intensity as disturbance increases and nutrients decrease. Since plant height and mass are strong predictors of plant performance (Gaudet and Keddy 1988) these smaller plants are more likely to be affected by neighbours (show higher competitive intensities) and have less of an effect on other plants (resulting in lower competitive intensities on their neighbours). It may therefore only be useful to test hypotheses about competitive effects using the smaller species in a community since the larger species will be mostly unaffected by the presence of their smaller neighbours. If one species displaces another, the effect of competition is certainly intense, but only for the species that gets displaced. These

mesocosm studies can therefore be considered examples of relative competitive intensity increasing with productivity.

A number of studies using natural productivity gradients have found that competition increases as productivity increases, falsifying Tilman's prediction of no change in competitive intensity over a productivity gradient. Competitive intensity was measured in terms of reproductive output (Reader and Best 1989) or plant yield (Reader and Buck 1986, Wilson and Keddy 1986). When both natural and artificial productivity gradients were compared in the same experiment, both produced a significant effect on competitive intensity but the effect of the natural gradient was stronger (Kadmon 1995). Using a removal experiment, Belcher et al. (1995) reported no difference in the relative competitive intensity across a biomass gradient. However, their data show a number of cases were plant mass is less with than without neighbours. Given the problems this creates, I calculated the RNE for their data. There was a significant positive correlation between RNE and ln plot biomass ($r^2 = 0.198$, $p = 0.049$) indicating competition is less intense on lower productivity plots.

There are a number of studies in which there is clearly no difference in the effect of neighbours over a productivity gradient. Gurevitch et al. (1992) compared the reduction of plant biomass in the presence of neighbours in low (desert and Arctic communities) and high (prairies, meadows and old fields) productivity communities using meta analysis (effect size is the dependent variable). They found no significant difference between these two site types although no actual productivity values were compared. Using a soil nutrient gradient, Wedin and Tilman (1993) found no difference in the competitive displacement of species at different levels of nutrient availability. The difference in plant response in their study varied seven fold over a soil nitrogen gradient of 120 fold, compared to Campbell and Grime's mesocosm study which showed a change of $10^4$ times in plant mass with a change in productivity of 74 fold between sites. While the differences in growth on Wedin and Tilman's gradient are large, they may not be large enough to produce differences in competitive intensity. Using the same reasoning, Kadmon (1995) compared his results to the those

of Wilson and Shay (1990) who found no effect of standing crop on competitive intensity. When the competitive intensity in Kadmon's data was compared over the same range of standing crop as in Wilson and Shay's, there was still a significant effect of standing crop on competitive intensity in Kadmon's data. It is therefore possible that the effect of productivity on the intensity of competition changes with the environmental conditions or the species present.

The different predictions from Grime's and Tilman's hypotheses about life history evolution have forced plant ecologists to formulate more precisely what they mean by competition (e.g., importance vs. intensity, response vs. effect) and to perform experiments under varied conditions. There have also been tests performed examining the underlying assumption of the mechanisms of competition proposed by the two theories (Shipley and Peters 1990). The predictions about competition along a productivity gradient focus competition within differing abiotic conditions. Ultimately, understanding of community structure will depend on putting competition in a context not only with productivity, but with other processes such as herbivory (Lubchenco and Gaines 1981, Reader 1992, Goldberg 1992), recruitment (Menge and Sutherland 1987) and interactions with symbionts (Eissenstat and Newman, 1990, Grime et al. 1987).

# CHAPTER FOUR

## The Effect of Herbivore Damage By Woolly Alder Sawfly On the Growth of Transplants And Its Interaction With Imposed Treatments

## INTRODUCTION

Experiments are designed to examine the effects of a specific set of imposed or controlled treatments. In order to ensure that unimposed treatments do not vary systematically with imposed treatments, techniques such as stratification and randomization are used. In this way, interactions which are not the focus of the experiment can be ignored. In conducting field experiments however, organisms may be exposed to conditions and interactions over which the experimenter has no control, and which may interact systematically with the treatments. Such uncontrolled effects can confound results and lead to false interpretations of the data. Field experiments therefore require careful monitoring of potentially confounding treatments.

The focus of this study was the interaction between alder and *Frankia* genotypes and its effect on growth and competitive ability. However, in the summer of 1993, woolly alder sawfly, *Eriocampa ovata* L., attacked transplants on some sites, with total defoliation occurring on some plants by mid summer. Casual observation indicated that the herbivory was neither evenly nor randomly distributed so it was decided that the herbivore damage should be monitored to determine how it influenced the experimental data collected. The purpose of this analysis was to determine if herbivore damage had an effect on plant growth and if so, was the effect of herbivory equally distributed across treatments? Herbivore damage was much less severe in 1994. This suggests that the plants may have developed some resistance to herbivory. The development of herbivore resistance the year after herbivore damage has been found in other woody species (Matson et al. 1988). To examine the existence of herbivore resistance, the degree of herbivore damage on trees between years was examined. If plants develop herbivore resistance after receiving damage then there should be an inverse relationship between years, in the degree of herbivore damage on

individual plants. A feeding preference experiment was also set up test the hypothesis that leaves from trees that were severely damaged the previous year are less palatable to sawflies than leaves from undamaged trees. A difference in palatability can indicate the existence of an induced chemical defense (Lowell et al. 1991).

## METHODS AND MATERIALS

The experimental design is outlined in chapter one. Throughout the lower Fraser valley, *A. rubra* was attacked by woolly alder sawfly (*E. ovata* ) in 1993 and 1994. My transplants started showing signs of herbivore damage in early May, 1993 with damage peaking in late August, some trees being totally defoliated. On all but the most severely attacked trees, herbivore damage was confined to leaf tissue in the lower part of the crown. Where herbivore damage was heavy, the leaves in the top of the crown and soft stem tissue near the ends of branches were also eaten. Trees which were defoliated produced new leaves which were generally free of herbivore damage.

On August 17, 1993 and July 29, 1994 I quantified the herbivore damage on each tree in the experiment into the following 6 classes:

0) no damage,

1) 1 - 25 % of leaves damaged,

2) 26 - 50% of leaves damaged,

3) 51 - 75% of leaves damaged,

4) 76 - 100% of leaves damaged,

5) complete defoliation.

A preliminary sampling of wild red alder showed that these classes could consistently be distinguished on experimental trees. Although classes 1 to 4 are based on the proportion of leaves

on a plant that were damaged and classes 0 and 5 on the proportion of leaf tissue eaten, plants with a large proportion of damaged leaves also had a higher percentage of tissue consumed per leaf and per plant (personal observation).

The effect of herbivore damage on the growth of plants was determined by examining both the mass and growth of plants experiencing different levels of herbivore damage. Mass was determined from the relationship between height x diameter$^2$ and the mass of harvested plants and growth was calculated as the RGR of plants on a yearly and monthly basis (Chapter 1). The effect of herbivore damage on plant mass and growth was analyzed using single factor ANOVAs with ln mass and RGR as dependent variables and herbivore damage class as the independent variable for each date mass was estimated. Differences between herbivore damage class were determined using a Ryan's Q test (Day and Quinn (1989). Data from high and low elevation sites were treated separately. The effect of the alder/*Frankia* combinations on the degree of herbivore damage experienced by a plant was examined using the ANOVA model from chapter one (equation 1.2) with herbivore damage class as the dependent variable.

### feeding preferences

A feeding preference experiment was set up to determine if changes in herbivory between years were related to changes in the palatability of previously defoliated trees. The experimental design and statistical analysis follow Peterson and Renaud's (1989) recommendations of using replicated controls for changes in plant mass in the absence of herbivores. On July 22, 1994 leaves and sawflies were collected. Undamaged leaves from previously defoliated and undamaged plants were collected from sites G and G40, the only sites with defoliated trees in 1993. Although 12 trees on these sites had been defoliated in 1993, only 7 of these could provide undamaged leaves at the time of sampling. Sawflies were collected from these sites but were not collected from the trees providing the leaf samples.

In the lab, leaves from a single defoliated tree were randomly matched with leaves from a single

undamaged tree. Three feeding preference dishes were made from each tree pair by placing leaf sections of approximately the same weight (mean 0.335 +/- 0.115 grams) into petri dishes. Two of the dishes were used as replicate feeding preference trials with five sawflies placed in each dish. The remaining dish received no sawflies and was used as a control for changes in leaf weight independent of consumption by sawflies. The dishes were placed under 16 hour day artificial lighting at room temperature and the sawflies were allowed to feed for 16 hours after which all leaf sections were weighed. The difference in the change in weight of the two leaf sections was calculated as the percent of the initial leaf weight for each dish. The mean difference of the replicate dishes was compared to the differences in weight change of leaves in the absence of sawflies using a paired t-test. This method is preferred over simply using weight changes in dishes without herbivores as a constant since it retains the variability in weight change in the absence of herbivores (Peterson and Renaud, 1989).

As part of another experiment, leaf C:N ratios were measured on bulked leaf samples from the trees used in this experiment. The analysis was performed on a mass spectrometer (VG Isotech Prism triple-collecting mass spectrometer, Middlewich, England) in the Department of Oceanography (U.B.C.). The leaves for this analysis were harvested in the first week of August, 1994.

## RESULTS

Herbivore damage was significantly greater ($p = 0.010$) on low elevation sites (3.15+/- 0.64, mean +/- 1 s) than on high elevation sites (0.867+/- 0.69) in 1993 (Table 4.1) according to an ANOVA with site nested within planting elevations (not presented). There were also significant differences between sites within elevations ($p = 0.000$), the highest levels of damage occurring on sites G and G40, with plants having on average greater than 50 % of their leaves damaged. Herbivore damage in 1994 was less, with no significant difference ($p = 0.296$) between low (1.06+/- 0.28) and high ( 0.62+/- 0.42) elevation sites. There were however significant differences ($p = 0.000$) between sites within elevations in 1994. The differences were quite small with no sites having, on average,

plants with greater than 25 % of their leaves damaged. Overall, herbivore damage was most severe on low elevation sites in 1993 only. Effects of herbivore damage on growth will therefore focus on the damage on these sites.

**Table 4.1.** Mean (s) herbivore damage class per site in 1993 and 1994. The herbivore damage class ranged from O (no damage) to 5 (complete defoliation). Common letters indicate no significant difference between sites according to a Ryan's Q test using the MSerror of a nested ANOVA. Data for site H110 was not included in the analysis for 1993 because all values are 0.

| | Planting elevation | | | | | |
|---|---|---|---|---|---|---|
| | Low | | | | High | |
| | G | G40 | K | H30 | H90 | H110 |
| 1993 | 3.27a | 3.73a | 2.45b | 0.88c | 1.42c | 0 |
| | (0.92) | (1.31) | (1.41) | (1.20) | (1.33) | (0) |
| 1994 | 0.80a | 1.55b | 0.84a | 0.55a | 0.62 a | 1.00b |
| | (0.76) | (1.00) | (0.68) | (0.50) | (0.55) | (0.92) |

**effects on size and growth**

Plants with high levels of herbivore damage damage in 1993 were significantly larger than plants with low levels of herbivore damage at the end of the 1993 growing season (Figure 4.1). On low elevation sites by the end of the growing season, plants with no herbivore damage were significantly smaller than plants with 1 to 50% of their leaves damaged, which were significantly smaller than plants with greater than 50% of their leaves damaged. These differences started to occur by May, before any sawflies could be seen on the plants. Although the degree of herbivore damage increased with plant size, the relative growth rate (RGR) varied over the growing season for plants with different levels of herbivore damage (Figure 4.2). On low elevation sites, from April until June, there was a positive relationship between the degree of herbivore damage and RGR. By August however, the relationship was negative with defoliated plants having significantly lower RGRs than all other plants. Plants with 50 to 100% leaf damage had significantly lower RGRs than plants with less than 50% leaf damage. This pattern suggests that sawflies selected the largest, fastest growing plants on which to lay their eggs early in the season which then resulted in reduced growth later in the season. Herbivore damage in 1993 had no significant effect on RGR in 1994 (Figure 4.3). This suggests that any reduction in growth in 1993 due to herbivore damage, did not carry over into the 1994 growing season.

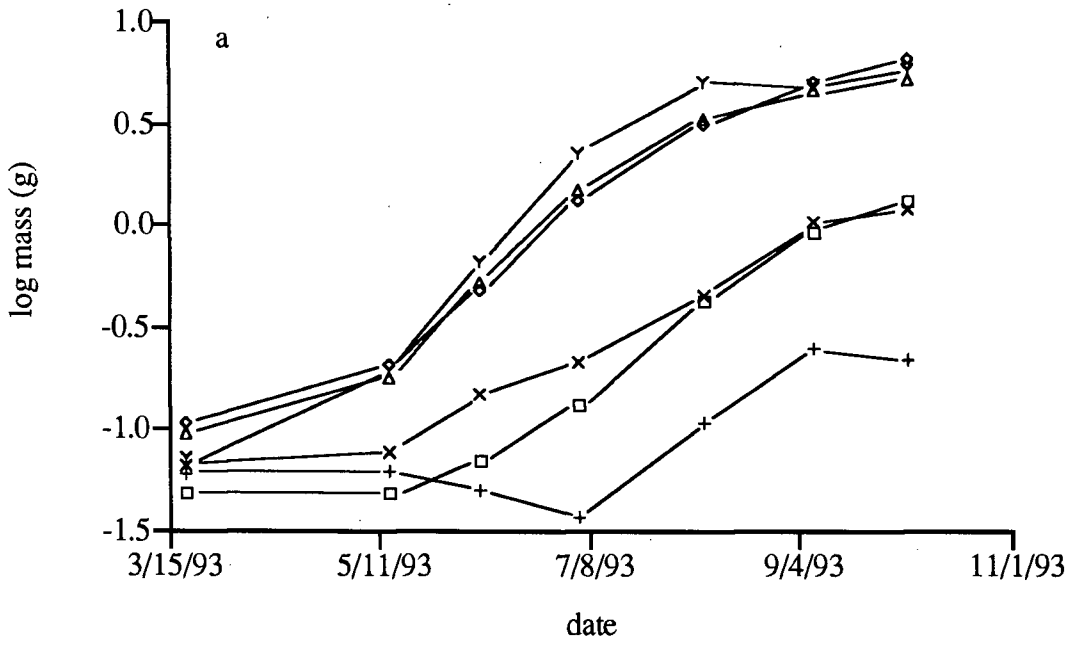


**Figure 4.1.** Log mass (in grams) of plants on low elevation planting sites over the 1993 growing season with different levels of herbivore damage. Symbols are as follows: + no damage; x 1 - 25% leaves damaged; □ 26 - 50 % leaves damaged; ◇ 51 - 75% leaves damaged; Δ 76 - 100% leaves damaged; Y complete defoliation.

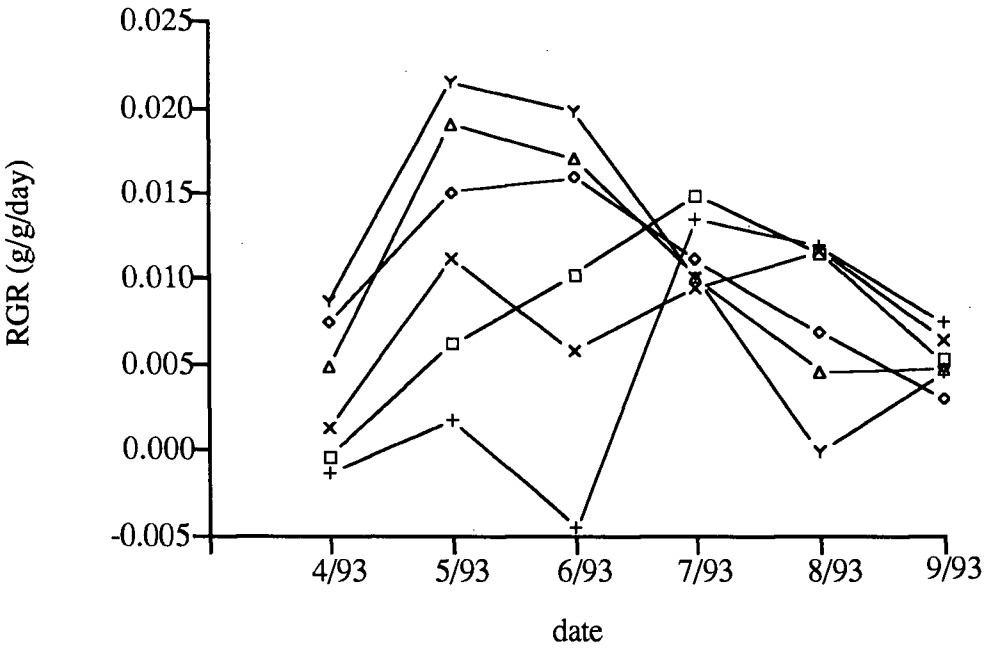


**Figure 4.2.** Relative growth rate (RGR) in grams/gram/day over the 1993 growing season for plants on low elevation planting sites with different levels of herbivore damage in 1993. Symbols are as follows: + no damage; x 1 - 25% leaves damaged; □ 26 - 50 % leaves damaged; ◇ 51 - 75% leaves damaged; Δ 76 - 100% leaves damaged; Y complete defoliation.

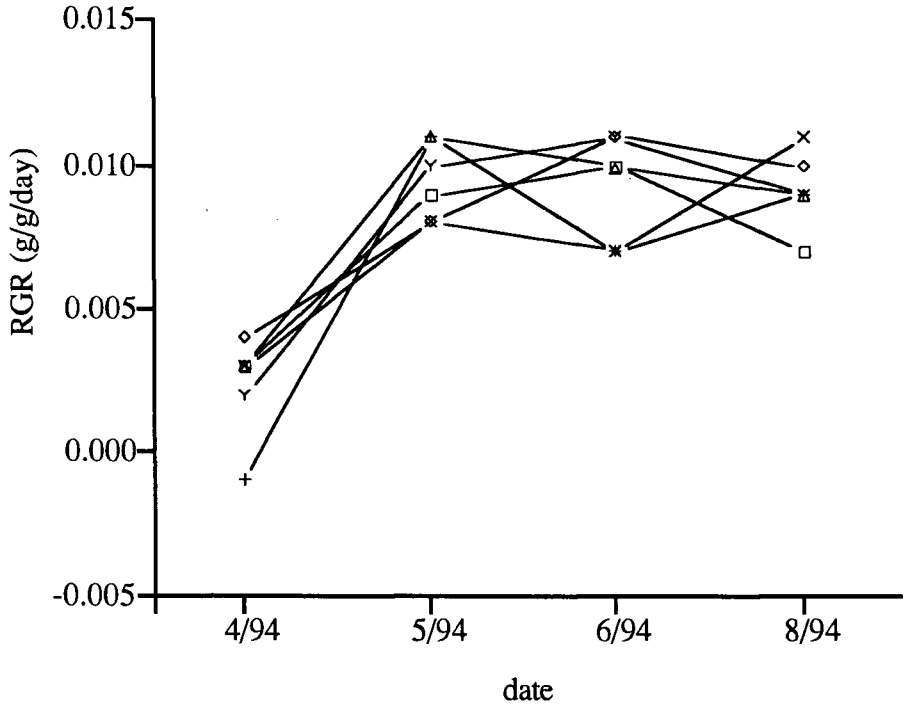


**Figure 4.3.** Relationship between RGR of plants in 1994 and herbivore damage level in 1993 on low elevation planting sites. Symbols are as follows: + no damage; x 1 - 25% leaves damaged; □ 26 - 50 % leaves damaged; ◇ 51 - 75% leaves damaged; Δ 76 - 100% leaves damaged; Y complete defoliation.

A mixed model ANOVA was run to determine if any of the experimental treatments were associated with variation in herbivore damage in 1993 (Table 4.2). Most of the variation was accounted for by planting elevation and site, with low elevation sites having significantly more damage than high elevation sites. Plants with neighbours had significantly higher herbivore damage ( 2.22 +/- 1.69) than plants without neighbours (1.84 +/- 1.74) on both high and low elevation sites. Except for the effect of neighbour presence increasing herbivore damage, treatments which resulted in the largest and/or fastest growing plants had the highest level of herbivore damage. A number of linear regressions between the level of herbivore damage and plant size or growth per treatment throughout the season (Table 4.3, Figure 4.4) accounted for up to 75.5% of the variation in the level of herbivore damage when all the data are grouped. When the data were split by neighbour presence or absence up to 92.0% of the variation is accounted for.

A regression between the level of herbivore damage on a plant in 1993 and 1994 for plants on low elevation sites showed no relationship in herbivore damage between years. However, there was a positive relationship between herbivore damage in 1993 and 1994 for plants experiencing high herbivore damage (Table 4.4). Of the 10 plants which had greater than 50% damaged leaves in 1994, 9 of these had greater than 50% damaged leaves in 1993. So, although the overall reduction in herbivory in 1994 may indicate that the trees have a defensive reaction against future herbivore attacks, individual trees with high levels of herbivore damage, did not seem particularly able to reduce future herbivore damage.

**Table 4.2.** ANOVA for the effect of planting elevation, parent elevation, inoculation source, neighbor presence, site (nested within planting elevation) and parent watershed on herbivore damage class. Site and watershed are random factors, all other factors are fixed. The error mean square is the denominator of the F ratio for all effects, except elevation. Site H110 has not been included in the analysis since all values are zero.

| Source | MS | DF | F | P |
|---|---|---|---|---|
| elevation | 290.366 | 1 | 19.6519* | 0.0108 |
| parent | 0.01198 | 1 | 0.0090 | 0.9247 |
| elevation x parent | 0.78268 | 1 | 0.5855 | 0.4450 |
| inoculation | 0.31163 | 2 | 0.2331 | 0.7923 |
| elevation x inoculation | 0.62955 | 2 | 0.4709 | 0.6251 |
| parent x inoculation | 0.95889 | 2 | 0.7173 | 0.4893 |
| elevation x parent x inoculation | 7.30641 | 2 | 5.4656 | 0.0049 |
| neighbours | 13.2236 | 1 | 9.8920 | 0.0019 |
| Site | 16.6047 | 4 | 12.4213 | 0.0000 |
| watershed | 0.17631 | 2 | 0.1319 | 0.8765 |
| Error | 1.337 | 211 | | |

* Synthetic denominator = $0.880MS_{site} + 0.120MS_{error}$
For a balanced design the denominator is the mean $square_{site}$.

**Table 4.3.** Coefficients of determination for the mean herbivore damage per treatment combination vs plant size and growth for all treatments with and without neighbours. RGR 1993 is measured as g/g/year and monthly RGRs are measured on a g/g/day basis. RGR 4/93 was calculated using the time when 50% of the buds flushed for each tree as the initial time (see Chapter one).

| | all treatments | neighbours present | neighbours absent |
|---|---|---|---|
| log mass 1992 | 0.532 | 0.611 | 0.217 |
| log mass 5/93 | 0.696 | 0.820 | 0.651 |
| log mass 6/93 | 0.755 | 0.920 | 0.693 |
| log mass 10/93 | 0.379 | 0.831 | 0.693 |
| RGR 1993 | 0.488 | 0.599 | 0.660 |
| RGR 4/93 | 0.569 | 0.526 | 0.902 |
| RGR 5/93 | 0.331 | 0.674 | 0.308 |
| RGR 6/93 | 0.520 | 0.634 | 0.701 |

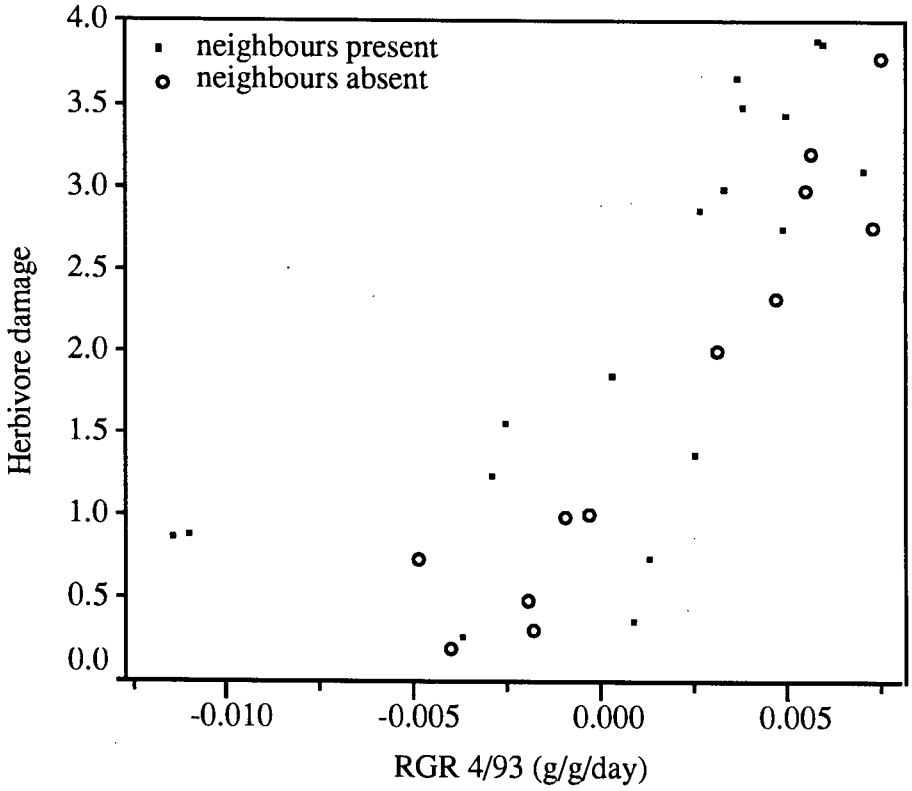


**Figure 4.4.** Mean herbivore damage class vs. mean relative growth rate in April (RGR 4/93) for each treatment combination (planting elevation, parent source, inoculum and neighbour type) outlined in Table 2.

**Table 4.4.** Frequency of plants in each herbivore damage classes for plants on low elevation sites in 1993 and 1994.

| | | Damage class in 1993 | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| Damage class in 1994 | 0 | 2 | 1 | 3 | 10 | 9 | 1 |
| | 1 | 2 | 5 | 4 | 23 | 31 | 4 |
| | 2 | 0 | 3 | 0 | 2 | 1 | 4 |
| | 3 | 1 | 0 | 0 | 0 | 4 | 2 |
| | 4 | 0 | 0 | 0 | 1 | 1 | 1 |
| | 5 | 0 | 0 | 0 | 0 | 0 | 0 |

**feeding preferences**

After being exposed to the sawflies for 16 hours most leaves from both damaged and undamaged trees showed visual signs of being eaten. Leaf sections from undamaged trees lost approximately two times more mass in the presence of sawflies than did leaf sections from damaged trees, while there was no difference in the absence of sawflies (Table 4.5). Mass loss from leaves of defoliated and undamaged trees ranged from -6.96 to 46.8% and 1.73 to 77.6 % respectively. In one case of very high mass loss, the only tissue remaining was the mid rib and veins which the sawflies did not seem to feed on. A paired t-test on the differences in mass loss between the two leaf types in containers with and without sawflies showed that there was a significantly greater difference in mass loss when sawflies were present ($p = 0.020$) indicating that the sawflies showed a preference for leaves from previously undamaged plants.

Leaves harvested from previously defoliated trees had a significantly ($p = 0.024$) lower C:N ratio (19.7 +/- 2.8) than leaves harvested from undamaged trees (23.7 +/- 2.8) indicating sawflies preferred leaves with a lower nitrogen content.

**Table 4.5.** Summary of feeding preference experiment. Mean percent mass loss and standard deviation of leaves from defoliated and undamaged plants in the presence and absence of sawflies. Differences in percent mass loss (Δ) were calculated as the mean of the mean of the replicate dishes, of the difference in percent mass loss of leaves from undamaged trees minus defoliated trees.

| | sawflies present | | | sawflies absent | | |
|---|---|---|---|---|---|---|
| | defoliated | undamaged | Δ | defoliated | undamaged | Δ |
| mean | 22.28 | 40.95 | 18.50 | 12.50 | 10.52 | -1.97 |
| s | 15.01 | 9.10 | 13.75 | 14.46 | 15.31 | 14.75 |

## DISCUSSION

Although herbivory did result in the reduction in growth of plants late in the growing season, the overall reduction in plant yield was difficult to quantify. If sawflies had attacked trees at random then the loss in growth could be quantified by calculating yield based on the difference in RGR between plants with and without herbivore damage. However, herbivore damage was not randomly distributed but was concentrated on plants which had a higher RGR earlier in the growing season. The selection of fast growing plants by the sawflies makes an estimation of the RGR of these plants, in the absence of herbivory, later in the growing season impossible. If it is assumed that the peak RGR in the spring would be maintained in the absence of herbivory, then losses due to herbivory can be estimated by calculating plant yield using the maximum spring RGR, an estimate of plant mass at the start of the growing season and the length of the growing season. When this was done, differences in expected mass in the absence of herbivory and estimated mass when sawflies were present were up to 20 times different, with losses being greater in the absence of neighbours and increasing with the degree of herbivore damage (Table 4.6). This assumption is tenuous however since red alder, like other Betulaceae, tends to lose leaves in mid summer (Kikuzawa 1982 and Chapter 1) and summer drought conditions in the region tend to retard growth (Harrington et al. 1994). A higher incidence of herbivory on faster growing individuals has been predicted since they make less of an investment in defensive structures (Loehle 1987, Coley et al. 1985) but is not a universal trend. For example, increased herbivore attack by *Fenusa dohrnii* on more productive *Alnus glutinosa* stands has also been observed, but there was no relation between or within other alder species tested (Hendrickson et al. 1991).

**Table 4.6.** Effect of herbivore damage on plant mass at the end of the growing season in 1993 for plants in the presence and absence of neighbours. The final mass of plants in the presence of herbivores, $M_{fH+}$, is the mass estimated from the height and diameter of the plants in Oct.,1993. The mass of plants in the absence of herbivory, $M_{fH-}$, is estimated using the equation

$$M_{fH-} = M_i \times 10^{(t \times RGR_{max})}$$

where $M_i$ is the estimated mass (in grams) at the start of the growing season, t is the length of the growing season (117 days, i.e., the mean date of budflush until the October 1993 plant census) and $RGR_{max}$ is the maximum mean RGR (in g/g/day) for plants in each herbivore damage class. Only herbivore damage grater than 50% is presented.

| Neighbours | Herbivore damage | $RGR_{max}$ | Mi | $M_{fH+}$ | $M_{fH-}$ | $M_{fH-}/M_{fH+}$ |
|---|---|---|---|---|---|---|
| absent | 51 - 75% | 0.0185 | 0.750 | 38.74 | 109.8 | 2.84 |
| | 76 - 100% | 0.0207 | 0.692 | 32.10 | 182.7 | 5.69 |
| | defoliated | 0.0245 | 0.180 | 6.89 | 13.9 | 19.80 |
| present | 51 - 75% | 0.0151 | 0.551 | 9.76 | 32.0 | 3.28 |
| | 76 - 100% | 0.0184 | 0.411 | 8.83 | 33.09 | 3.74 |
| | defoliated | 0.0180 | 0.321 | 5.16 | 40.9 | 7.90 |

Since herbivores tended to attack larger plants, and therefore plants from treatments resulting in larger plants, herbivore damage reduced potential differences between treatments. Therefore differences found between different alder/*Frankia* combinations (Chapter one) may be conservative estimates of potential differences. This is not true in the case of neighbour presence which resulted in smaller plants but a greater degree of herbivore damage. Reduction in growth, due to neighbour presence is therefore partially due to an increased incidence of herbivory and can be considered apparent competition (Connell 1990, Reader 1992). Since no controls for herbivory were established, the proportion of reduced growth due to herbivory and resource competition cannot be separated. However, since the reduction in growth was just as large on sites with low as well as a high degree of herbivore damage (Chapter 3), it seems that the proportion of apparent competition is quite small. This also suggests that apparent competition due to herbivory reduces resource competition and, in the absence of herbivory, resource competition would equal resource competition plus apparent competition in the presence of herbivory. This hypothesis has not been addressed in discussions of apparent competition.

The overall pattern of herbivore damage fits well with the assumption of plant apparency theory (Feeney 1976), that plants which are more visible (apparent) in time and space are more likely to be attacked by herbivores. Apparency will increase as the size of an individual increases and as the density of conspecifics increases. Since red alder is more abundant at low elevation sites in this region (Harrington et al. 1994), sawflies are more likely to be abundant and find the low elevation planting sites, explaining the higher herbivore damage on my low elevation sites. Within sites, sawflies are more likely to encounter larger trees either through actively searching for a host or through random dispersal. Plants with neighbours, although smaller, will also be more conspicuous since, in this experiment, the neighbours were other red alder trees. Since *E. ovata* prefers a shaded habitat (Mackay and Wellington 1977) the presence of neighbours may also increase herbivore presence by providing a more shaded habitat on the target plant.

The general, reduction in herbivory in 1994 suggests that red alder has an induced resistance to

herbivore damage. Induced chemical resistance has been found in a number of woody species (Rhoades 1985) and is often assumed to be a defensive response by the plant (Myers and Karban 1989). The feeding preference experiment also supports the hypothesis of an induced resistance since the sawflies showed a preference for leaves from undamaged trees. The higher C:N ratio in undamaged leaves does not support the assertion that intense herbivore damage results in lower quality food in red alder (Myers and Williams 1987) but moderate damage improves food quality (Myers and Williams 1984). The damaged trees used in the feeding preference experiment had been completely defoliated yet had a higher nitrogen content although they were not preferred by the sawflies. The C:N data indicate that the sawflies prefer leaves with less nitrogen (leaves from undamaged trees). The C:N ratio differences do not however indicate differences in the quality of nitrogen in the leaves. Since the sawflies preferred leaves with a lower nitrogen content, it would seem that either the quality of the nitrogen was lower in leaves from undamaged trees or that there was some form of chemical defense altering the leaves palatability. The higher nitrogen content in the leaves from defoliated trees does agree with Matson's (1980) review which found defoliation to consistently increase total protein in new plant tissue. It is also consistent with the finding of faster growing plants receiving a higher degree of herbivore damage. Fast growing plants will tend to have higher protein content and invest less in defensive compounds (Coley 1988, Loehle 1987, Coley et al. 1985).

The fact that plants with greater than 50% of their leaves damaged in 1994 were likely to have greater than 50% of their leaves damaged in 1993 would seem to reject the induced defense hypothesis. This may result from the overwintering behavior of the sawflies. In the fall, sawflies drop to the ground and overwinter under the host tree. They may not pupate (Rose and Lindquist 1982) in which case the emerging larvae would then feed on the same host in the spring and adults would likely encounter and lay eggs on the same host plant, even though more palatable individuals may exist. Myers and Williams (1987) also found that, while the quality of leaves from trees repeatedly attacked by western tent caterpillar decreased, in terms of their effect on larval development, the caterpillars continued to feed on these trees.

# CONCLUSIONS

To date, cross inoculation studies with actinorhizal plants have tended to concentrate on differences between actinorhizal species and families, while employing a small number of *Frankia* strains as inocula. This is the first study to look in depth at interactions between different populations of *Frankia* and its hosts within a single host species. The results revealed complex interactions between *Frankia* and red alder populations which are dependent on environmental conditions. The patterns found were consistent with the prediction that there will be selection for a cheating strategy in a mutualism, as the likelihood that the relationship will break down increases, the most probable situation for red alder given its early successional status. *Frankia* strains inoculated on seedlings from their host population acted more like parasites than mutualists when grown on the low elevation sites, resulting in lower growth and proportion of fixed nitrogen in the plants. The induction of the response may have been triggered by higher levels of soil nitrogen on the low elevation sites. The differences between the different alder/ *Frankia* combinations were not affected by the presence of neighbours, indicating that alder/*Frankia* combinations which produced the largest plants produced plants with the highest fitness.

It was assumed that the interactions found between alder and *Frankia* ecotypes were the result of coevolution. However, since this study provides no information on changes in gene frequencies and selection pressure over time, it should be considered only weak evidence for coevolution (Endler 1986, Janzen 1980). Much more work is required to determine long term selection trends between host and endophyte genotypes. These are however, most intriguing results and suggest there is still much to be learned about the dynamics of actinorhizal plant/*Frankia* interactions. More information on the free living nature of *Frankia* and patterns of regional strain diversity are also needed to answer a number of questions raised by this study. There has been a tendency for some researchers to disregard variation in the interactions between *Frankia* genotypes and their hosts, leading to a view that *Frankia* can essentially be considered an organelle of the plant which

produces fixed nitrogen in response to plant needs (see for example Ager 1987). The results presented here clearly show that *Frankia* genotypes can have a strong effect on the growth of different alder populations, regardless of the interpretation of the patterns of variation.

The planting sites in this study represent a broad range of productivity as measured by total mass of all transplants. Since no competition could be detected on the lowest productivity site (and plants were in fact larger in the presence of neighbours) these data do not support Tilman's prediction of no change in competitive intensity across a gradient of site productivity. However, the effects of neighbours were quite similar across the remaining sites, so changes in competitive intensity may only be important at the extremes of a productivity gradient. The problems associated with the measurement of competition, as discussed here, are likely to prevent any consensus about the effect of competition under different environmental conditions.

While herbivory was not considered in the design of this study, the degree of herbivore damage observed suggested that its effect on the growth of the plants needed to be addressed. Although it would have been preferable to systematically control the sawflies, this was not practically possible. The data presented here do show that while herbivore damage did affect plant growth, with the exception of neighbour presence, the herbivore damage had the effect of reducing differences between treatments. Such an effect could therefore result in a reduction in differences in fitness between the different alder/*Frankia* combinations. This would then represent another level of complexity in the interactions between red alder and its abiotic and biotic environment.

## management implications

These findings have a number of ecological and management implications. The evolution of less effective *Frankia* strains is likely to drive selection for variation in hosts and increase genetic variation within populations just as other antagonistic relationships are thought to (Barrett 1983, Van Valen 1973). In terms of management, these complex interactions between host, endophyte and the environment make selection of superior strains (in terms of plant growth or nitrogen

fixation) impossible. The best recommendation at this point would be to inoculate plants with strains they are not normally associated with, although the effects will depend on environmental conditions. It may be that in young stands, in which some thinning has occurred, selection against less effective symbionts has taken place and selection for the evolution of cheating in *Frankia* is not present. These stands would therefore be a better source of inoculum than mature stands. These results do however highlight the potential gain in plant growth through proper strain selection, with early inoculation effects lasting for at least three growing seasons. This indicates that inoculation of seedlings at the time of planting can have long term effects on individual plant yield. Given the low cost of performing inoculation, it could become a practical tool in the management of red alder.

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