ECOLOGICAL SPECIFICITY OF GROWTH PROMOTING BACTERIA FOR INTERIOR SPRUCE (*PICEA GLAUCA X PICEA ENGELMANII*)?

BY

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ABSTRACT

Soil, rhizobacteria and interior spruce seed originating from two disparate ecosystems were used to examine the effect of rhizobacterial inoculation and the role of coexistence between rhizobacteria, seed provenances and soil sources on germination and spruce seedling growth in two experiments.

Statistically significant enhancement of germination due to inoculation with bacteria was rare. Germination of seed inoculated with coexistent bacteria was significantly lower than germination of seed inoculated with non-coexistent bacteria.

Inoculation of seed with bacteria resulted in significant enhancement of seedling growth in both experiments. Maximum shoot and root dry weight increases of 53% and 67%, respectively, were observed. The effect of inoculation on seedling growth varied greatly with seed provenance and soil source.

Coexistent bacteria (i.e. originating from the same location as the target seed or soil) were not more effective growth promoters than non-coexistent bacteria. However, uninoculated seedlings grown in coexistent soil had 27% and 35% heavier shoot and root dry weights, respectively, than uninoculated seedlings grown in non-coexistent soil. The shoot and root biomass stimulation decreased to 17% and 23%, respectively, when coexistent pasteurized soil was used, suggesting that both biotic and abiotic soil factors may have contributed to seed-soil coexistence specificity.

Novel findings in these experiments include the detection of: significant bacterial plant growth promotion of interior spruce; plant growth promotion by a *Staphlococcus* species; and adaptive relationships between seed and soil factors.

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1.0 INTRODUCTION

Poor seedling growth after out-planting on highly stressful or competitive sites is one of the major causes of failed or poorly growing conifer plantations in British Columbia. The use of seedlings which are in optimal physiological condition has been seen as a solution to this problem (Grossnickle *et al.* 1991). Bacterial inoculants may influence a seedling's physiological condition (Sarig *et al.* 1988) and have been shown to enhance shoot and root growth of many agricultural and of some arboreal species in laboratory, greenhouse and field trials. If similar effects were obtained with conifers, bacterial inoculation could predispose planted seedlings to be better competitors for light, nutrients or moisture, and thereby possibly improve their survival and growth.

Bacterial inoculation of seeds has also been shown to influence germination rate and capacity (the percent of seeds which germinate) of some plant species. If such technology was developed for conifer nurseries, it could obviate or minimize expensive over-sowing and subsequent thinning operations, and help to produce a uniformly-sized crop. In addition, bacteria capable of enhancing the rate of germination could help nursery-growers using multiple seedlots to stagger or synchronize maturation as desired, and perhaps permit the use of a wider range of seedlots in regions which have a short growing season.

While several factors may influence plant reactions to rhizosphere bacteria (rhizobacteria), the genotypic match between plants and bacteria has been shown to influence the nature of this response (Chanway *et al.* 1988a,b, 1991b). Genotypic specificity may develop over time between coexistent plants and naturally-occurring rhizosphere bacteria through adaptation of bacterial populations to plant hosts (Holl 1983; Chanway *et al.* 1991b).

Therefore, the ability of rhizobacteria isolated from naturally-regenerating interior spruce [*Picea glauca* (Moench) Voss. x *Picea engelmanii* Parry *ex* Engelm.] seedlings to enhance germination and growth of interior spruce was tested under greenhouse and growth chamber conditions as a

preliminary evaluation of their potential for nursery and field applications. In particular, the role of coexistence in specificity between conifer provenances, soil and growth promoting bacteria in the germination and growth of interior spruce seedlings was examined.

Specifically, the objectives of the experiments were to determine if:

(1) interior spruce seed inoculated with rhizobacteria germinate faster or more completely, and produce larger seedlings than uninoculated seed;

(2) treatments of coexistent factor combinations (bacteria-seed, bacteria-soil, seed-soil, bacteria-seed-soil) result in more rapid or complete germination, or larger seedlings, than treatments of non-coexistent combinations of the same factors.

2.0 LITERATURE REVIEW

2.1 The Rhizosphere

Lorenz Hiltner (1904) was the first to recognize the potential importance of the intense microbial activity on and around root systems to plant growth. He called this area the *rhizosphere*. Bacteria comprise the most common class of rhizosphere micro-organism (Rovira and Davey 1974) and can attain populations of up to 3×10^9 cells per gram of rhizosphere soil (Rouatt and Katznelson 1961). Bacteria isolated from this area have been termed *ectorhizosphere* bacteria; those isolated from within surface-sterilized roots have been termed *endorhizosphere* bacteria (Lalande *et al.* 1989). (The terms *ectorhizobacteria* and *endorhizobacteria* will be used in this paper to describe bacteria isolated from these two root regions, and the general term rhizobacteria to describe all rhizosphere bacteria.)

Rhizosphere bacteria can exhibit considerable influence on plant nutrient availability through the activities of various intra- and extra-cellular enzymes (e.g. lipase, phosphatase and nitrogenase). They may also suppress plant pathogens through competition or antibiotic production, as well as producing active phytohormones. Since all soil-borne nutrients received by the plant must pass through the rhizosphere, it is not surprising that rhizobacteria may affect plant growth.

The organic compounds contained in senescent root tissue and in root exudates and secretions provide substrate for the growth of heterotrophic soil microbes in the rhizosphere. Radio-isotope labelling experiments have indicated that up to 40% of cereal (Whipps and Lynch 1986) and 50% of conifer (Reid and Mexal 1977; Perry *et al.* 1987) net primary production can be exuded into the rhizosphere. The allocation of such an astonishingly large quantity of photosynthates below-ground further reflects the potential importance of rhizosphere microbial ecology to plant growth.

2.2 Plant Growth Promoting Rhizobacteria

Plant growth promoting micro-organisms have been studied intensively because of their potential

impact on agricultural and forest productivity (Gaskins *et al.* 1985; Schroth and Weinhold 1986;

Chanway *et al.* 1991a). The term 'plant growth promoting rhizobacteria' (PGPR) has been used to

describe soil bacteria which, when applied to seed, tubers or roots, are able to colonize roots and

stimulate plant growth (Kloepper and Schroth 1978). Many strains that belong to commonly

occurring genera of soil bacteria, such as *Arthrobacter, Azospirillum, Azotobacter, Bacillus,*

Pseudomonas and *Serratia* have been found in association with plant roots and to promote plant

growth (Brown 1974; Gaskins *et al.* 1985).

Recent success in growth enhancement of agricultural (Kloepper and Schroth 1981; Kapulnik and

Okon 1983; Elad *et al.* 1987; Chanway *et al.* 1988a,b; Reddy and Rahe 1989) and tree species

(Akhromeiko and Shestakova 1958; Gardner *et al.* 1984; Strobel and Nachmias 1985; Pandey *et al.*

1986; Caesar and Burr 1987; Chanway *et al.* 1991b; Chanway and Holl 1991) through inoculation

with PGPR has stimulated a renewed interest in rhizosphere biology. However, variability of the

plant growth response remains a major impediment to the implementation of PGPR technology in

agriculture and forestry (Kloepper *et al.* 1989). When rhizosphere synecology and the mechanisms

by which PGPR stimulate plant growth are better understood, the likelihood of being able to select

and manage more effective PGPR strains will be greatly increased.

2.3 PGPR in Agriculture

The first attempts to improve plant growth by 'bacterization' (coating of seeds with bacteria before

planting) were made in Russia in the 1940's with strains of *Azotobacter* and *Bacillus* (Allison 1947;

Mishustin and Naumova 1962) which were capable of *in vitro* nitrogen fixation and phosphate

solubilization, respectively. It was claimed that inoculation with these strains could result in yield

increases in the order of 10% in fifty to seventy percent of the crops to which they were applied

(Cooper 1959). Unfortunately, lack of statistical analysis and poor reproducibility have precluded

useful interpretation of these studies (Mishustin 1970).

Experimentation with PGPR in the western world was first reported in 1963, when inoculation with

asymbiotic N-fixing *Bacillus* and *Clostridium* rhizobacteria was shown to stimulate growth of tomato (*Lycopersicon esculentum* L.), maize (*Zea mays* L.) and wheat (*Triticum* sp.) (Rovira 1963). Subsequently, representatives of these and other genera of bacteria have been used experimentally as PGPR for agricultural crops. Howie and Echandi (1983) and Kloepper and Schroth (1981) reported significant (all uses of the word 'significant' in this thesis imply *statistically* significant) increases in the weight of potato (*Solanum tuberosum* L.) inoculated with antibiotic producing strains of *Pseudomonas*. Growth, emergence and vigor of canola (*Brassica campestris* L.) were also enhanced by strains of *Pseudomonas, Arthrobacter* and *Serratia* in greenhouse and field trials (Kloepper *et al.* 1988). Growth stimulation of perennial ryegrass (*Lolium perenne* L.), crested wheatgrass (*Agropyron cristatum* L.), white clover (*Trifolium repens* L.) (Chanway *et al.* 1988a; Holl *et al.* 1988) and spring wheat (*Triticum aestivum L.*) (Chanway *et al.* 1988b) were achieved using *Bacillus* inocula. In some cases, seedling emergence was also stimulated (Holl *et al.* 1988; Chanway and Nelson 1990). Other strains have promoted growth of radish (*Raphanus sativus* L.) (Kloepper and Schroth 1978), tomato, pepper (*Capsicum annuum* L.), melon (*Cucumis melon* L.), bean (*Phaseolus vulgaris* L.), tobacco (*Nicotiana tabaccum* L.), cucumber (*Cucumis sativus* L.) (Elad *et al.* 1987) and ornamental plants (Yeun and Schroth 1986).

The nature and magnitude of the growth response vary considerably. Examples of plant growth stimulation relative to uninoculated controls include: grain yield (11%) (Kapulnik *et al.* 1983); height (38%) (Reddy and Rahe 1989); shoot dry weight (56%) (Elad *et al.* 1987); number of roots (42%) (Tien *et al.* 1979); root dry weight (40%) (Hussain and Vancura 1970); root surface area (18%) (Kapulnik and Okon 1983) and root length (29%) (Pandey *et al.* 1986). Variation in the growth response also occurs between experiments, inoculants and target plants, and not all growth responses are positive. For example, the fluorescent *Pseudomonas* strain 599NR inhibited shoot and root growth of sweet orange (*Citrus sinenis* Osbeck) 39% and 41%, respectively, compared with uninoculated controls, but promoted shoot and root growth of lemon (*Citrus jambhiri* Lush.) 38% and 21%, respectively (Gardner *et al.* 1984).

2.4   PGPR in Arboreal Species

Plant growth promotion of several arboreal species following PGPR inoculation has also been observed. Enhanced dry weight of oak (*Quercus* sp.) and ash (*Fraxinus* sp.) seedlings (13 and 26%, respectively) after inoculation with *Azotobacter chroococcum* was the first report of an arboreal PGPR (Akhromeiko and Shestakova 1958). Stimulation of almond (*Prunus* sp.) root stock (Strobel and Nachmias 1985), apple (*Malus* sp.) seedlings and rootstock (Caesar and Burr 1987), rough lemon (*Citrus jambhiri* Lush.) (Gardner *et al.* 1984) and eucalyptus (*Eucalyptus camaldulensis* Dehn.) (Mohammad and Prasad 1988) growth by bacterial inoculants has also been reported.

Growth of coniferous species may also be stimulated by PGPR. Shoot length of Scots Pine germinants (*Pinus sylvestris* L.) was increased after inoculation with Coryneform bacteria or treatment with the supernatant of the Coryneform growth media (Pokojska-Burdziej 1982). Shoot and root dry weight, height, root surface area and root collar diameter of lodgepole pine (*Pinus contorta* Dougl.), and shoot biomass, root collar diameter and root surface area of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings were increased by bacterial inoculation (Chanway and Holl 1991a,b; Chanway *et al.* 1991a). Container-grown Douglas-fir inoculated with a mixed suspension of forest floor bacteria showed significantly greater stem height and diameter than uninoculated controls when grown under nutrient limited conditions (Parker and Dangerfield 1975).

2.5   Mechanisms of Action of PGPR

The mechanism(s) by which PGPR stimulate plant growth have yet to be conclusively determined. However, four mechanisms have been frequently postulated. These include: (1) production of phytohormones; (2) inhibition of deleterious rhizobacteria (DRB) and plant pathogens; (3) increased nutrient availability, and (4) nitrogen fixation. Most PGPR researchers recognize that a single strain may possess more than one plant growth promoting attribute (Curl and Truelove 1986; Holl *et al.* 1988) and that these may interact with other biotic (Bowen and Theodorou 1979; Garbaye and Bowen 1987; McAfee and Fortin 1988) or abiotic (McArthur *et al.* 1988) factors in the soil. As a consequence of the numerous factors influencing the biology of the rhizosphere, Schroth and

Weinhold (1986) termed investigations in this area 'a masochist's delight'.

2.5.1 Production of Phytohormones

Phytohormones that are essential for plant morphogenesis, such as auxins, giberellins and cytokinins, are produced by several genera of rhizosphere bacteria (Brown and Burlingham 1968; Eklund 1970; Hussain and Vancura 1970; Brown 1972; Lynch 1976; and Tien *et al.* 1979). Plant growth promotion by *Azotobacter paspali* (Barea and Brown 1974; Brown 1976), *Azospirillum brasilense* (Tien *et al.* 1979), *Bacillus megaterium* (Katznelson and Cole 1965), *B. polymyxa* (Holl *et al.* 1988), *B. subtilus* (Brown *et al.* 1968) and *Pseudomonas* species (Katznelson and Cole 1965; Eklund 1970; Hussain and Vancura 1970) has been attributed to their ability to produce phytohormones. Venkateswarlu and Rao (1983) correlated root growth stimulation and an increase in the number of lateral roots and root hairs after bacterial inoculation with the magnitude of *in vitro* auxin production by several strains of *Azospirillum brasilense*.

However, most evidence for the involvement of phytohormones in PGPR activity is indirect, and has been derived from experiments in which the effects of bacterial inoculation are mimicked by exogenous application of phytohormones. For example, similar growth effects were observed after inoculation of tomato with either gibberellic acid-producing *Azotobacter* or with synthetic gibberellic acid (Brown *et al.* 1968), and by inoculating wheat with various phytohormone-producing rhizobacteria or with synthetic phytohormones (Brown 1972). In addition, growth of small plants can be increased by adding plant growth substances and live or heat-killed bacteria to the soil (Jackson *et al.* 1964; Gaskins and Hubbell 1979).

Though indirect, these observations suggest that bacterial production of plant growth substances may contribute to PGPR activity. Despite the minute concentration (i.e. nanomolar or picomolar) of growth regulators produced by rhizobacteria, they are absorbed in the region of root-hair development (Rivière 1963; Libbert and Silhengst 1970; Brown 1972). Their production in the rhizosphere in synchrony with the development of new tissue may explain their effectiveness in

altering plant growth (Gaskins *et al.* 1985). Production of phytohormones as a mechanism of plant growth promotion by rhizobacteria continues to receive considerable attention; however, more conclusive evidence in support of this mechanism may await development of techniques by which bacterial phytohormone production *in situ* can be effectively monitered.

2.5.2   Inhibition of Deleterious Rhizobacteria

Plant growth promotion by PGPR may also occur through the inhibition of minor plant pathogens termed 'deleterious rhizobacteria' (DRB). DRB colonize roots and reduce plant growth without causing symptoms of disease (Salt 1979; Suslow and Schroth 1982). For example, Rovira (1972) found that root hair number and length, both considered to be extremely important for plant growth in phosphorus-limited soils, were reduced in the presence of many strains of rhizobacteria. Similarly, Bowen and Rovira (1961) found root-growth inhibiting microorganisms in soils collected from a stand of Monterey pine (*Pinus radiata*) and from three agricultural crops.

Postulated mechanisms by which PGPR inhibit proliferation of DRB include niche exclusion through competition for root binding sites (Burr and Caesar 1984), production of compounds that are toxic to DRB, such as antibiotics or hydrogen cyanide (Weller 1988), and production of siderophores, which chelate soil Fe^{3+}, thereby limiting its availability to, and subsequent growth of, DRB (Kloepper *et al.* 1980; Curl and Truelove 1986).

In some cases, supporting evidence for the DRB-inhibition hypothesis is fairly convincing. Kloepper and Schroth (1981) found that inoculation of potato seed pieces with antibiotic-producing strains of *Pseudomonas* caused significant growth increases (300 - 500%) in total plant weight of potato, but inoculation with non-antibiotic producing strains had no effect. Suslow and Schroth (1982) showed that co-inoculation of sugar beet seed with strains of PGPR and DRB resulted in inhibition of root colonization by DRB and increased plant growth compared to inoculation with DRB alone. Growth promotion did not occur when experiments were conducted in sterile non-soil media or when autoclaved field soils were used, presumably because DRB were not present (Suslow 1982).

However, several studies have shown that plant growth promotion with members of the genus *Bacillus* and other Pseudomonads can occur under both sterile and non-sterile conditions (Lifshitz *et al.* 1987; Holl *et al.* 1988; Chanway and Nelson 1990; Chanway *et al.* 1989; Chanway and Holl 1991a,b). Therefore, PGPR activity does not appear to be strictly related to DRB inhibition.

2.5.3   Increased Nutrient Availability

Aside from enhanced nutrient uptake resulting from the larger root systems associated with inoculated seedlings, most nutrient-related mechanistic hypotheses have focused on phosphorus availability. Phosphorus may become more available to plants through the production of organic acids by rhizobacteria, which reduce the local pH and thereby solubilize otherwise insoluble phosphorus compounds and other soil minerals (Mishustin and Naumova 1962; Bajpai and Sundara Rao 1971). Alternatively, bacterial production of phosphatase can solubilize organic sources of phosphorus directly. Reviews of the effects of bacteria on the mineral uptake by plants (Katznelson 1965; Barber 1978) reveal an accumulation of conflicting evidence (Gaskins *et al.* 1985). However, after reviewing all available evidence, Tinker (1984) concluded that bacterial solubilization of phosphate is probably of minor importance in the growth response of plants inoculated with PGPR, and his contention has not been challenged.

2.5.4   Nitrogen Fixation

Members of various genera of rhizosphere bacteria, including *Azotobacter, Azospirillum, Bacillus, Beijerinckia, Clostridium, Desulfovibrio, Klebsiella* and *Pseudomonas* are capable of fixing atmospheric nitrogen. While symbiotic nitrogen-fixation accounts for most of the combined nitrogen input into forested ecosystems (Kimmins 1987), asymbiotic N-fixation by free-living bacteria may contribute significantly to the long-term productivity of agricultural (Gaskins *et al.* 1985) and forest ecosystems (Dawson 1983; Marschner 1986, p. 189; Kimmins 1987).

Early estimates of asymbiotic nitrogen input of 199 kg N/ha/y for bushlands (Jaiyebo and Moore 1963), 165 kg N/ha/y for lowland forest (Greenland and Nye 1959) and up to 313 kg N/ha/y for some

agricultural crops (Evans and Barber 1977) were erroneously high (Davey and Wollum 1984). Their over-estimation has been attributed to several factors, including faulty assay techniques (Van Berkum and Bohlool 1980), inaccurate extrapolation of fixation rates obtained from short-term assays (Brown 1982), inappropriate sample collection, and a lack of consideration of diurnal and seasonal fluctuations in nitrogen-fixation rates (Sims and Dunigan 1984).

Current estimates of asymbiotic nitrogen input are usually below 30 kg N/ha/y, and most are below 5 kg N/ha/y (Evans and Barber 1977; Davey and Wollum 1984). However, relatively few accurate estimates are available. According to Sprent (1979, p. 114), the paucity of information on the ecological importance of asymbiotic nitrogen fixation "reflects lack of information, rather than lack of importance".

Relatively low rates of nitrogen fixation notwithstanding, inoculation of seeds and plants with nitrogen-fixing bacteria has resulted in significant yield increases of several agricultural species (Smith *et al.* 1976; Rennie and Larson 1979; Kapulnik *et al.* 1981; Schank *et al.* 1981; Chanway *et al.* 1988a,b), and in root growth increases of oak (*Quercus serrata*) (Pandey *et al.* 1986) and lodgepole pine (Chanway *et al.* 1991a) seedlings. However, results of inoculation experiments using nitrogen-fixing PGPR with ^{15}N dilution techniques or with nitrogen rich media (which should suppress nitrogenase activity) suggest that nitrogen fixation is of secondary (Okon *et al.* 1983; Kapulnik *et al.* 1985; Chanway and Holl 1991a) or no importance (O'Hara *et al.* 1981; Brown 1982) in the plant growth response, and increases in plant growth by diazotrophic PGPR are often attributed to bacterial production of phytohormones (Barea and Brown 1974; Holl *et al.* 1988). In retrospect, this conclusion is not surprising if Barber and Lynch (1977) were correct in asserting that "if all the carbon released by the roots were available only to known nitrogen-fixing [rhizobacteria], and if all the nitrogenases of the bacteria functioned at their maximum rates, then only 15% of the N content of temperate cereals could be provided in this way". Therefore, asymbiotic root-associated nitrogen fixation has all but been dismissed as a primary mechanism by which diazotrophic PGPR operate.

2.6   Bacteria-Host Specificity

Qualitative and quantitative differences in root exudates exist between plant species (Rovira and Davey 1974; Curl and Truelove 1986) and between cultivars and genotypes of the same species (Baldani and Dobereiner 1980). These differences, coupled with the reliance of rhizosphere microbes on root exudates for organic nutrients, may result in the proliferation of microbial populations that are specific to plant species or to genotypes within species (Neal *et al.* 1973; Burr and Caesar 1984; Chanway *et al.* 1991b).

Chanway *et al.* (1991b) proposed that specificity between plants and growth promoting micro-organisms can occur at either of two stages of these associations: during infection of the root system to form root nodules or mycorrhizas (i.e. infection specificity), or during subsequent growth of the infected plant host (i.e. growth response specificity). Where the relationship is not symbiotic, but microbial association with the host is required (e.g. PGPR), specific colonization of the rhizosphere may occur.

Infection (or colonization) specificity may be determined by a cell wall recognition mechanism, in which plant lectins (specific plant glycoproteins which adhere to unique carbohydrates on the cell wall of bacteria) operate in a manner similar to that of antigens in immunological reactions (Sumner 1990; Chanway *et al.* 1991b). The possible involvement of lectins in the specificity observed in *Rhizobium*-legume associations has been recognized for nearly two decades (Bohlool and Schmidt 1974) and was recently postulated in the adsorption-recognition process between plants and PGPR by Okon and Kapulnik (1986). More recently, infection specificity in *Rhizobium*-legume associations was also shown to involve biochemical signals secreted by plant roots which activate nodulation genes in specific *Rhizobium* strains (Long 1989). *Growth* specificity may result from bacterial production of compounds of the type, or in an amount that specifically affects growth of individual or related groups (i.e. ecotypes) of plants.

Chanway *et al.* (1991b) also suggest that the development of plant-specific rhizosphere microflora may arise in either of two ways. Pre-existing genetic variability among resident soil bacteria may predispose particular bacterial strains to experience a competitive advantage over other strains in the rhizosphere of a particular plant genotype or species. Consequently, the populations of those strains would increase and possibly dominate in the rhizosphere due to superior fitness when in association with that plant.

An alternative and perhaps less likely mechanism would involve the adaptation of particular bacterial strains to the host plant. This could occur as a result of the generation of genetic variation in the rhizosphere bacteria population through point mutations and/or various forms of genetic recombination (conjugation, transduction or transformation), with subsequent selection of superior bacterial genotypes in the rhizosphere. Genotypic specificity between plants and microbes may therefore result from small genetic differences between host plants that affect root exudation and consequently the size and nature of the bacterial population that proliferates in the rhizosphere.

Some strains of PGPR are capable of promoting the growth of a number of plant species (Elad *et al.* 1987; Holl *et al.* 1988; Bashan *et al.* 1989). However, PGPR are not universally effective and differences in growth promotion between PGPR-plant combinations are well documented (Rovira 1963; Gardner *et al.* 1984,1985; Chanway *et al.* 1989). The basis of these differences is not understood, but host-plant genetics (Burr and Caesar 1984; Chanway *et al.* 1989) and the history of coexistence between bacterial and plant genotypes (Chanway *et al.* 1988a,b) are important.

The occurrence of specific relationships between strains of associative nitrogen-fixing bacteria and plant genotypes is well known (Baldani and Dobereiner 1980; Holl 1983). Chanway *et al.* (1988a) tested the hypothesis that genotype specific plant growth promotion by PGPR may develop between *coexistent* plant genotypes and associative PGPR (or rhizobacterial populations). Using physically contacting (i.e. *coexistent*) pairs of white clover and perennial ryegrass plants, and strains of *Bacillus* isolated from the roots of the white clover, they tested the growth-promoting ability of the bacteria

using clones of the coexistent and non-coexistent 'parental plants'. They discovered that as the experimental environment became more 'familiar' by growing the clover predominantly with (1) non-coexistent *Bacillus* and ryegrass, then with (2) coexistent *Bacillus* but not ryegrass, and finally with (3) coexistent *Bacillus* and ryegrass, the yield of the legume component of the species mixture increased from condition (1) to condition (3). Furthermore, no inoculation reponse was detected when plants were inoculated with non-coexistent *Bacillus* strains.

Perhaps the most pointed display of a specific PGPR-plant relationship emerged from the experiments by Rennie and Larson (1979) involving the inoculation of disomic chromosome substitution lines of wheat with a diazotrophic *Bacillus* isolated from a parental wheat cultivar. (Disomic chromosome substitution lines contain 20 pairs of indigenous chromosomes plus one pair from a donor line, allowing for the study of the effects of the 'donated' pair in an otherwise constant genetic background.) Using this system they were able to attribute significant plant growth increases and nitrogen accumulation following bacterial inoculation to the presence of a single chromosome in the wheat genome. Their results emphasize how relatively small changes in plant genotype can have substantial effects on growth promotion by rhizosphere bacteria.

These findings prompted Chanway *et al.* (1988b) to argue that if beneficial microbes are positively selected over time in the rhizosphere, then the probability of finding a positive effect on plant performance due to inoculation with coexistent bacterial strains should be greater than if plants are inoculated with strains to which they have not been previously exposed. To test this hypothesis, Chanway *et al.* (1988b) isolated *Bacillus* strains from the rhizosphere of spring wheat cultivar 'Katepwa' which was growing in a field that had been cropped continuously with this cultivar for the preceeding five years and to other wheat cultivars for the preceeding 22 years. When inoculated onto cv. 'Katepwa', a related cultivar to which the field had also been cropped, and an unrelated Mexican cultivar, six of seven *Bacillus* isolates promoted growth of cv. 'Katepwa', but none promoted growth of the other two cultivars. These results indicated that cultivar-specific adaptation of rhizosphere bacteria (or the bacterial population) to wheat can occur within a period of five years.

Chanway *et al.* (1989) suggested that some of the variability observed in plant growth promotion by PGPR could be explained by genotypic specificity between plants and inoculant microbes. They proposed, therefore, that the probability of securing consistent and effective PGPR could be increased by using strains which were isolated from (i.e. had coexisted with) the target crop.

Though beyond the scope of this review, it is interesting to note that varying degrees of plant-microbe specificity have also been documented involving plants and root nodule bacteria (Mytton *et al.* 1977; Holl 1983; Florence and Cook 1984) and mycorrhizae (Molina and Trappe 1982; Cline and Reid 1982; Kendrick and Berch 1984), and more than three decades ago, Moser (1958) recommended using the same provenance of trees and fungi to stimulate optimal mycorrhizal formation and tree growth.

It was with these ideas in mind that I undertook a search for interior spruce PGPR. The term 'coexistent' has been used to indicate a common origin of organisms (rhizosphere bacteria, interior spruce seedlings and seed) and soil (i.e. collected from the same micro-site as were the seed, seedlings and bacteria). The term 'coexistence specificity' will refer to the seedling growth response that results from testing coexistent organisms and soil compared with non-coexistent (i.e. collected from an alternative site) organisms and soil.

The identification of bacteria-plant or bacteria-soil coexistence specificity in bacterial promotion of germination or growth (i.e. increased germination or growth when the bacterial inoculant and seed, or bacterial inoculant and soil, have the same geographic origin), would make the isolation of a general PGPR unlikely, and would potentially limit the effective range of seedlots or soils that could be used with a particular PGPR. Nevertheless, consideration of coexistence specificity, if important, could facilitate the isolation of consistent germination or growth promoters, albeit with a restricted range of seedlots or sites.

3.0 MATERIALS AND METHODS

3.1   Soil and Seedling Collection

Naturally-established interior spruce (*Picea glauca* x *englemanii*) seedlings (1-5 years old) and soil were collected during the summer of 1989 from the understory of two mature forest sites in central British Columbia. The first of these ecologically disparate sites was located 10 km south of Mackenzie in the Sub-Boreal Spruce biogeoclimatic zone (Krajina *et al.* 1982), within 1 km of a British Columbia Ministry of Forest's (MoF) interior spruce parent-tree stand at Buth Creek (latitude 55^0 11', longitude 122^0 58', elevation 780 m). The main vegetation in this valley-bottom site, consisted of interior spruce, black cottonwood (*Populus trichocarpa* Torr. and Gray *ex* Hook), coltsfoot (*Petasites palmatus* [Ait.] Gray) and *Pleurozium schreberi* ((Brit.) Mitt.).

The second site was located 30 km north-west of Salmon Arm in the Engelmann Spruce Sub-alpine Fir biogeoclimatic zone, within 50 m of the MoF's interior spruce 'plus tree' #3010 (latitude 51^0 04', longitude 119^0 26', elevation 1250 m). The main vegetation on this mountain-top site included interior spruce, sub-alpine fir [*Abies lasiocarpa* (Hook.) Nutt.], Canada thistle (*Cirsium arvensis*), red raspberry (*Rubus ideus* L.), huckleberry (*Vaccinium membranaceum* Dougl. *ex* Hook.) and western mountain-ash (*Sorbus scopulina* Greene).

Interior spruce seedlings and their intact root mass contained in forest soil were collected to a depth of 20 cm. Seedlings and soil were placed separately in plastic bags and transferred to the Forest Biotechnology Centre laboratory at the British Columbia Research Corporation in Vancouver where they were stored at 4^0 C. All bacterial isolations took place within seven days of seedling collection. Soil samples within each location were pooled and soil nutrient analysis was conducted using standard methodology according to Black (1965).

3.2 Bacterial Isolation

A total of 25 seedlings from each location were used for bacterial isolations. Seedlings were divided

into five groups of five in order to maximize the diversity of bacterial strains isolated from the roots.

Root masses of each group of seedlings were shaken vigorously to dislodge loosely adherent soil, and

were then cut aseptically into 3-5 cm segments. To isolate the ectorhizobacteria, approximately 2.0 g

of root segments (0.4 g/plant) from each group of five seedlings were placed in a 250 mL flask

containing 20-30 glass beads and 150 mL of 10 mM sterile phosphate buffer (SPB - 1.21 g K_2HPO_4,

0.34 g KH_2PO_4, 1.0 L distilled water pH 7.0). Flasks were agitated gently on a rotary shaker (100

rev/min) for 20 minutes.

To obtain the endorhizobacteria, roots treated as described above were removed from the flask, and

were surface sterilized by soaking for five minutes in 70% ethanol and then for ten minutes in 3%

HClO (50% Chlorox bleach). Roots were then rinsed three times in 200 mL sterile distilled water,

and blended at high speed for 60 s in a sterile Waring blender containing 20 mL of 10 mM SPB.

The root-wash suspensions from unsterilized roots and from the surface-sterilized, blended roots

were diluted serially from 10^{-1} to 10^{-5} in 10 mM SPB, and 0.1 mL aliquots of the dilutions were

plated onto duplicate petri-plates. In order to further increase the diversity of strains recovered

from the dilutions, three culture methods were employed: (1) aerobic growth on diazotroph-enriching

combined carbon media (CCM) (Rennie 1981); (2) anaerobic growth on CCM in anaerobic jars

(Baltimore Biological Laboratory, Inc.); and (3) aerobic growth on Pseudomonad-enriching King's B

media (King et al. 1954).

All media were supplemented with 100 mg/L cyclohexamide and 30 mg/L benomyl (Benlate, W.P.

Dupont Inc.) to inhibit the growth of fungi. Plates were held at 28°C for 72 h for aerobic incubation,

or for three weeks for anaerobic incubation. After incubation, bacterial colonies of distinct

morphology were isolated by streaking onto new plates of the same media from which the bacteria

were originally cultured. Isolated strains were grown aerobically for 1-4 days, then purified by re-

streaking a single isolated colony onto tryptic soy agar (TSA - 20 g Difco tryptic soy agar, 10.0 g agar, 1.0 L distilled water). Of the strains which grew on King's B medium, only those which fluoresced under UV light (300 nm) were considered to be siderophore producers and were purified.

3.3   Bacterial Storage

Purified strains were stored at -80°C in order to minimize the potential for genetic mutation which may occur with serial re-culturing. This was achieved by culturing strains in tryptic soy broth (TSB - Difco) until turbid and adding 0.5 mL of each culture to 2 mL plastic cryovials containing 0.5 mL TSB in 40% (v/v) glycerol. The resulting suspensions were stirred to facilitate immersion of bacteria into the glycerol. Suspensions were then held stationary for two hours at room temperature to allow for glycerol uptake into the cells before storing at -80°C.

3.4   Acetylene Reduction Assay

To test for acetylene-reducing capability, isolates were inoculated from frozen cultures into 3 mL of liquid CCM contained in sterile 5 mL glass vials which were fitted with a rubber seal. Vials were incubated for 72 h at 28°C on a rotary shaker (120 rev/min). Acetylene was then injected into the vials to a final concentration of 10% (v/v). One mL of gas was withdrawn from each vial 24 h later and analyzed for C_2H_4 by flame-ionization gas chromatography, following separation in a stainless steel column (0.3 x 180 cm) containing Porapak N (80-100 mesh) at 55°C with N_2 carrier gas at a flow rate of 40 mL/min. Strains registering rates of acetylene reduction ten times greater than the 'background' were considered to possess nitrogenase activity.

3.5   Strain Selection and Inocula Preparation

Twenty strains from each collection site were selected based on three criteria: the ability to reduce acetylene (an indication of their ability to fix nitrogen); the medium on which the primary isolation was made (to secure a representative sample of isolates from each type of isolation medium); and the population size of the strain in the rhizosphere of the naturally regenerating seedling, based on the dilution plate from which it was selected (i.e. those present in the largest numbers were selected).

The selected bacterial strains were inoculated onto TSA plates from frozen culture and grown for 48 h at 28°C. Plate cultures were used to inoculate 150-300 mL of TSB in flasks, which were then incubated at 28°C for 24-48 h on a rotary shaker (150 rev/min). Bacterial cells were centrifuged (3000 x g for 20 min) and washed by re-suspending the pellet with SPB to the original volume, re-centrifuging and resuspending again. Washed cells were adjusted with SPB to an optical density (OD) (600 nm) intended to give 10^7 colony forming units (cfu) per mL according to previously established OD/cfu concentration functions for each strain.

3.6   Experiment 1

3.6.1   Seed Sowing and Inoculation

Seed from the Mackenzie parent-tree stand (seedlot 29144) and the Salmon Arm 'plus tree' were provided by the MoF and were stratified by immersion in 250 mL of distilled water for 24 h, followed by surface drying, and storage at 4°C for 30 days. During the first week of April 1990, the two seedlots were sown, three seed per cell, into plastic cones (Super Cell 160 cm^3, 4.0 x 21.0 cm, Ray Leach 'conetainer' Nursery, Canby, Oregon) filled with a 50:50 mixture of Turface (montmorillonite clay - Applied Industrial Materials Corporation, Deerfield, Illinios) and their corresponding coexistent soil (i.e. Mackenzie or Salmon Arm). Soil was sifted through a 1 cm mesh before use. Seed were then drenched with 3 mL of 10 mM SPB which contained 10^7 cfu/mL of one of the twenty coexistent bacterial strains (i.e. Mackenzie seed were sown onto Mackenzie soil and inoculated with Mackenzie bacterial strains and Salmon Arm seed were sown onto Salmon Arm soil and inoculated with Salmon Arm bacterial strains). Control seed was drenched with 3 mL of 10 mM SPB. Seeds were then covered with 5 mL of 'Forestry Sand' (Target Products Ltd., Vancouver) and watered lightly.

3.6.2   Experimental Design and Seedling Culture

Twenty-one treatments (twenty bacterial strains plus the uninoculated control) were tested on each spruce seedlot (n=20). The 420 'conetainers' of each seedlot (21 treatments x 20 'conetainers'/treatment) were arranged in a completely randomized design, and the seedlings were

grown in the University of British Columbia Plant Science greenhouse. Daily maximum temperature ranged from approximately 20-28°C and occasionally reached 35°C. Seedlings were watered to saturation on alternate days, and daily on the hotter days. Due to extremely slow growth during the first month after germination, Mackenzie seedlings each received approximately 5 mL of soluble fertilizer (650 mg/L 20-8-20 (Plant Products) supplemented with 150 mg/L $Fe_2(SO_4)_3$) once a week for four weeks, commencing seven weeks after sowing. An extended photoperiod of 18 h was achieved with the use of fluorescent lights. Seedlings were thinned to the single largest germinant five weeks after sowing.

3.6.3   Germination Survey, Harvests and Statistical Analysis

The number of germinants/cell was counted on nine occasions during the active germination period, with the final count taking place 30-45 days after sowing. The germination capacity (GC - final percent germination) and germination value (GV), a measure of the speed and completeness of germination (Czabator 1962), were calculated for each treatment and compared with the control using a two-tailed Least Significant Difference (LSD) at $p < 0.05$. The germination value (which is currently being incorporated into the tree seed registry system of the B.C. Ministry of Forests) gives an overall estimate of a seedlot's germinative quality. To obtain the GV, the germination rate (% germination/days since sowing) was calculated for each cell on each of the nine observation days. The maximum germination rate was then determined, and multiplied by the final germination rate to give the GV.

Seedlings were harvested 13 weeks after sowing. Shoot height and root collar diameter were measured. Roots were separated from shoots and washed to remove adherent soil. Projected root surface area of fresh roots was obtained with the use of a LiCor 3000 surface area meter. The mean of three measurements/root was calculated and multiplied by π to estimate actual root surface area. Shoots and roots were oven-dried for three days at 70°C before shoot, root and total dry weights were measured. LSDs were calculated as they were for the germination parameters and used to compare treatment means with that of the control.

3.7 Experiment 2

Following analysis of seedling growth in the first experiment, the three bacterial strains from each

provenance which elicited the greatest stimulation of shoot dry weight were selected for further

study as putative PGPR in a second inoculation experiment. These six putative PGPR were

identified to the species level at Auburn University, Georgia, by gas chromatographic analysis of

bacterial fatty acids (as methyl esters) using the MIDI (Microbial ID, Inc.) Microbial Identification

System which has been described by Mertz and Yao (1990).

The influence of conifer ecotype, soil source and PGPR on germination and seedling growth were

evaluated in a complete factorial experiment (see Appendix 1). The experimental design permitted

evaluation of the influence of bacteria-seed, bacteria-soil, seed-soil and bacteria-seed-soil specificity

on germination and seedling growth through the use of contrasts involving control (i.e. uninoculated)

treatments, and treatments of coexistent and non-coexistent factor combinations.

By utilizing pasteurized and non-pasteurized growing media, the role of biotic and abiotic soil factors

in seed germination and seedling growth were also assessed. One half of the soil-Turface mixtures

were pasteurized by heating 5 kg soil aliquots twice to $100^{\circ}C$ for 30 min (24 h between heat

treatments) before the 'conetainers' were filled. Fifty-six factorial treatment combinations (n=20)

resulted from the use of two seed provenances (Mackenzie and Salmon Arm), two soil sources

(Mackenzie and Salmon Arm), two soil types (pasteurized and non-pasteurized) and seven

inoculation treatments (three Mackenzie and three Salmon Arm bacterial strains, and a SPB

control).

Seed were sown and inoculated and seedlings were grown as described for Experiment 1, with the

following exceptions: seed were re-inoculated three days after the initial inoculation at sowing;

seedlings were grown in two growth chambers (day/night temperatures $24/16^{\circ}C$); a 19 h photoperiod

was used (photosynthetically active radiation was 400-700 umol/m^2/s), and the quantity of fertilizer

applied to the seedlings growing in Mackenzie soil was increased to 13 mL/seedling. Trays of

'conetainers' were rotated within and between growth chambers every two days in an attempt to distribute evenly positional effects among trays. Germination and growth parameters were measured as described in Experiment 1.

Multi-way analysis of variance was performed on all parameters. After ANOVA, untransformed residuals were well distributed and no improvement of the homogeneity of the treatment variances was observed when data were log, inverse, or square root transformed. Therefore, ANOVA was conducted using untransformed data. The Least Significant Difference test (two-tailed) at $p < 0.05$ based on the experiment mean square error was used to identify treatments which differed significantly from their control.

Four sets of contrasts were used to test the general hypothesis that germination and/or growth of interior spruce seedlings were greater when coexistent factor combinations (bacteria, seed and soil) were present. The specific sets of contrasts were:

C1 - coexistent bacteria, seed and soil vs. non-coexistent bacteria, seed and soil

C1 - coexistent bacteria, seed and soil vs. control

C1 - non-coexistent bacteria, seed and soil vs. control

C2 - coexistent bacteria and seed vs. non-coexistent bacteria and seed

C2 - coexistent bacteria and seed vs. control

C2 - non-coexistent bacteria and seed vs. control

C3 - coexistent bacteria and soil vs. non-coexistent bacteria and soil

C3 - coexistent bacteria and soil vs. control

C3 - non-coexistent bacteria and soil vs. control

C4 - coexistent seed and soil vs. non-coexistent seed and soil

These contrasts were also performed on seedlings grown in pasteurized soil to estimate the contribution of biotic versus abiotic factors to any observed specificity. The overall comparison error rate was controlled at $\alpha < 0.10$ with the use of the Bonferroni procedure (Wilkinson 1988 p. 490). The treatments comprising each contrast are outlined in Table 6 and the treatment number designations are illustrated in Appendix 1.

4.0 RESULTS

4.1 Soil Analysis and Bacterial Isolation

The pooled soil sample from Salmon Arm was richer in all micro- and macro-nutrients and in organic matter, than that from Mackenzie (Table 1). The only exception was calcium, which was present at a level of 2300 ppm in Mackenzie, but at only 900 ppm in Salmon Arm soil. The two soils also differed substantially in pH (i.e. Salmon Arm soil 4.8; Mackenzie 6.4).

Approximately 150 bacterial strains were recovered from the roots of seedlings from each site. Of these, 80% originated from the ectorhizosphere, and each of the isolation techniques recovered approximately equal numbers of strains. Three of the Mackenzie and six of the Salmon Arm strains were capable of reducing acetylene (Tables 2a and 2b).

4.2 Experiment 1

4.2.1 Germination Responses

Statistically significant increases in GC or GV due to inoculation were not observed in either seedlot, although strains M4 and M13 both stimulated GC 13% over the control treatment (i.e. GC treatment - GC control) (Fig. 1a) (Absolute germination values are presented in Table 3). Strain M13 also elicited a 23% increase in GV over the control. Inhibition of GC and GV in the Mackenzie seedlot was frequent and substantial, with GV inhibition greater than 20% occurring with the use of six of the strains (Fig. 1a). Maximum GV inhibition occurred from inoculation with strain M15 (38%).

The magnitude of the effects on germination in the Salmon Arm seedlot (Fig. 1b) was much smaller than those in the Mackenzie seedlot. Significant inhibition of GV resulted with the use of strains S15 (21%) and S20 (21%), and GV inhibition exceeded 10% when strains S8 and S16 were tested. The maximum enhancement of GC and GV was 5% and 8%, and both were achieved with the use of strain S2.

Table 1. Characteristics of pasteurized and unpasteurized Mackenzie and Salmon Arm soils.

			Soil Characteristics					Available Nutrients (ppm)					
	pH	organic matter %	total N %	P	K	Ca	Mg	Cu	Zn	Fe	Mn	B	S
Mackenzie unpasteurized	6.4	2.8	0.05	25	290	2300	180	2.4	3.0	130	108	1.3	106
Mackenzie pasteurized	6.8	1.7	0.06	14	375	2000	170	2.6	2.8	150	122	1.3	94
Salmon Arm unpasteurized	4.8	2.9	0.09	39	510	900	205	2.4	5.6	180	110	1.5	163
Salmon Arm pasteurized	4.6	5.6	0.09	43	495	950	255	2.0	3.6	190	290	1.7	163

Table 2a. List of Mackenzie bacterial strains used in Experiment 1.
Isolation method: (1) CCM, aerobic; (2) CCM, anaerobic; (3)
King's B media, aerobic. Habitat: endo - strain isolated from
endorhizosphere; ecto - strain isolated from ectorhizosphere.
Ethylene reduction: + - ethylene reducing strain.

Mackenzie

Strain designation	Isolation method	Habitat	Ethylene reduction	Rank in Expt. 1
M1	3	endo		
M2	2	endo		
M3	1	ecto	+	
M4	3	endo		
M5	2	ecto		
M6	1	endo		
M7	3	endo		
M8	1	endo		
M9	1	endo		
M10	1	endo		
M11	2	ecto		
M12	3	ecto		
M13	1	ecto		
M14	3	ecto		1
M15	2	endo		
M16	3	ecto		
M17	1	ecto		
M18	2	ecto	+	2
M19	1	ecto	+	3
M20	1	ecto		

Strain identification: M14 - *Pseudomonas putida*; M18 and M19 -
Hydrogenophaga pseudoflava. The top 3 ranking strains from
Experiment 1 with respect to seedling shoot dry weight promotion
were used in Experiment 2.

Table 2b. List of Salmon Arm bacterial strains used in Experiment 1.
Isolation method: (1) CCM, aerobic; (2) CCM, anaerobic; (3)
King's B media, aerobic. Habitat: endo - strain isolated from
endorhizosphere; ecto - strain isolated from ectorhizosphere.
Ethylene reduction: + - ethylene reducing strain.

Salmon Arm

Strain designation	Isolation method	Habitat	Ethylene reduction	Rank in Expt. 1
S1	2	endo		3
S2	1	endo	+	
S3	1	endo	+	
S4	1	ecto		
S5	1	endo		
S6	2	endo	+	
S7	1	endo		
S8	3	endo		
S9	1	ecto		
S10	1	endo		2
S11	3	endo		
S12	1	endo		
S13	3	endo		
S14	2	endo		
S15	2	endo		
S16	2	ecto	+	
S17	1	ecto		
S18	2	ecto	+	
S19	2	ecto	+	
S20	1	ecto		1

Strain identification: S1 - *Pseudomonas putida*; S10 - *Staphlococcus hominis*; S20 - *Bacillus polymyxa*. The top 3 ranking strains from Experiment 1 with respect to seedling shoot dry weight promotion were used in Experiment 2.

Fig. 1. Effect of Mackenzie and Salmon Arm bacterial isolates on the germination
capacity and germination value of their respective seedlots. Seed was
grown in soil collected from the same location as was the seed. * -
indicates values which differ significantly from the control at p < 0.05.

a   Mackenzie

GC treatment - GC control   % change from control (GV)

strain

■ germination capacity   ▱ germination value

b   Salmon Arm

GC treatment - GC control   % change from control GV

strains

■ germination capacity   ▱ germination value

Table 3. Effect of bacterial inoculation with Mackenzie and
Salmon Arm isolates on germination capacity (GC) and
germination value (GV) of Mackenzie and Salmon Arm
seedlots in Experiment 1. Ctrl - SPB control; MSE -
mean square error; * - indicates treatments which
differ significantly from the control at p < 0.05.

	Mackenzie				Salmon Arm		
Strain	GC (%)	GV		Strain	GC (%)	GV	
M1	72.5	10.8		S1	90.0	23.9	
M2	53.7	6.9		S2	99.5	27.2	
M3	68.7	9.3		S3	91.6	25.3	
M4	77.5	11.6		S4	95.0	27.1	
M5	63.7	9.5		S5	95.0	26.0	
M6	51.2	7.3		S6	96.6	25.6	
M7	57.5	7.2		S7	95.0	24.1	
M8	52.5	7.5		S8	90.0	22.4	
M9	57.5	8.5		S9	90.0	23.1	
M10	58.7	8.8		S10	95.0	25.1	
M11	65.0	10.1		S11	93.3	23.6	
M12	67.5	11.0		S12	91.6	23.4	
M13	77.5	13.0		S13	96.6	25.9	
M14	72.5	11.9		S14	96.6	26.8	
M15	55.0	6.4	*	S15	86.6	20.0	*
M16	68.7	10.4		S16	88.3	21.3	
M17	62.5	9.0		S17	91.6	23.6	
M18	63.7	8.5		S18	96.6	25.4	
M19	65.0	9.5		S19	93.3	25.5	
M20	61.2	8.4		S20	88.3	19.8	*
Ctrl	**63.8**	**10.6**		**Ctrl**	**95.0**	**25.3**	
LSD	**16.80**	**3.78**			**10.80**	**4.70**	
MSE	734.26	37.28			303.68	57.71	

4.2.2 Growth Responses

While Mackenzie strains inhibited Mackenzie seedling growth (Figs. 2a and 3), most of the Salmon Arm rhizobacteria promoted the growth of Salmon Arm seedlings (Figs. 2b and 4). However, effects were rarely significant. (See Tables 4a and 4b for treatment means of both seedlots.)

Although inhibition of growth was widespread and often substantial among Mackenzie inoculants, the only significant decreases were in height with strains M7 and M9 (20% and 21% respectively) and in root dry weight with strain M7 (24%) (Figs. 3a and 3c). Considerable increases occurred in shoot dry weight with strains M14 (19%), M18 (12%) and M19 (16%) (Fig. 2a), and in root dry weight, also with M14 (20%) (Fig. 3c). The only significant growth promotion due to inoculation with Mackenzie bacteria was of stem diameter, by strain M18 (10%) (Table 4a).

Height growth of Salmon Arm seedlings was greater in all bacterial treatments, relative to the control, and was significantly promoted by strains S1 (12%), S5 (13%), S11 (11%) and S20 (18%) (Fig. 4a). Strain S20, the most effective height growth promoter, also produced significant shoot dry weight (27%), root surface area (20%), and total dry weight (21%) increases (Figs. 2b, 4b and 4d). Six other strains produced non-significant increases in shoot dry weight ranging from 11-18%.

Root/shoot dry weight ratio was lower (relative to the control) in all but two of the Salmon Arm treatments (range 3 to -20%) (Table 4b), and was increased in 13 of 20 Mackenzie treatments (range -15 to 42%) (Table 4a). The increase in root/shoot dry weight ratio, however, generally resulted from inhibition of SDW, and not from promotion of root dry weight. Inoculation with strain M15, for example, resulted in a 42% increase in root/shoot dry weight ratio, which was due to a slight decrease in root dry weight and a moderate decrease in SDW (Figs. 2a and 3c). The only significant effects on root/shoot dry weight ratio occurred with strains M15 (42% greater than controls) and S11 (20% below controls).

Fig. 2. Effect of inoculation with (a) Mackenzie and (b) Salmon Arm bacterial strains on shoot dry weight of their respective seedlots. Seed was grown in soil collected from the same location as was the seed. * - indicates treatments which differ significantly from the control at p < 0.05.
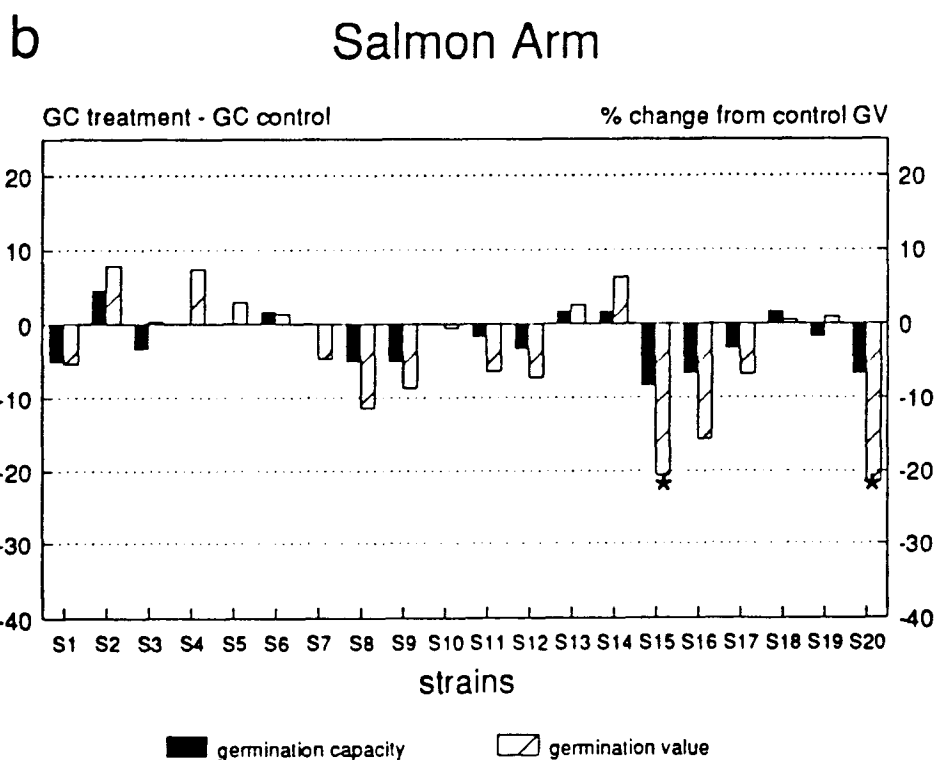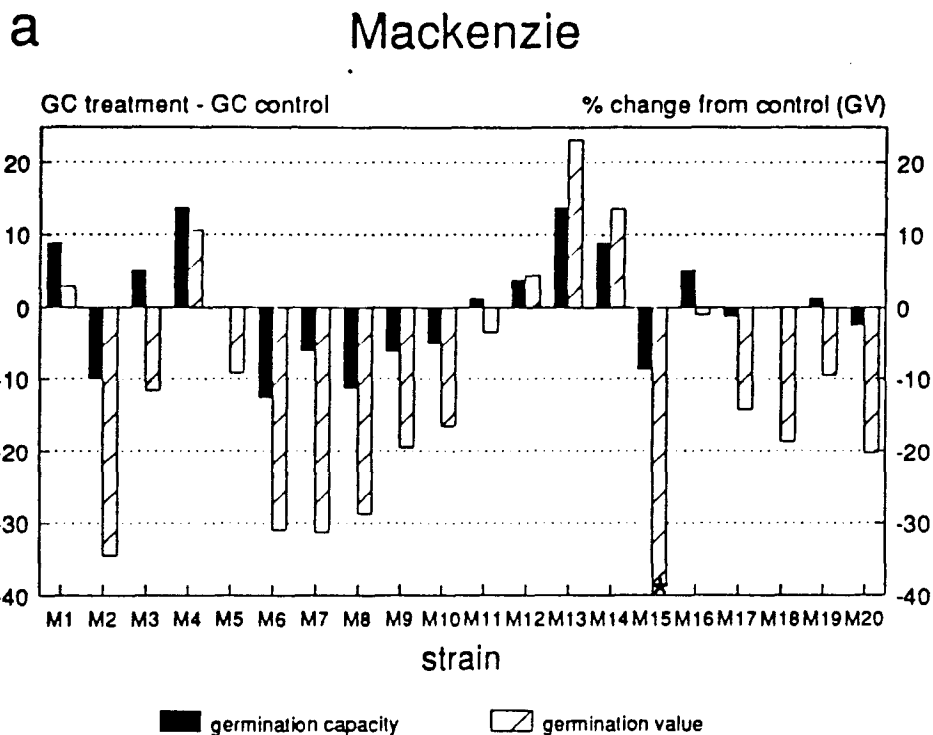

Mackenzie
shoot dry weight
a

Salmon Arm
shoot dry weight
b

Fig. 3. Effect of inoculation with Mackenzie bacterial strains on: (a) height; (b) root surface area; (c) root dry weight; (d) total dry weight of Mackenzie seedlings grown in Mackenzie soil in Experiment 1. * - indicates treatments which differ significantly from the control at p < 0.05.
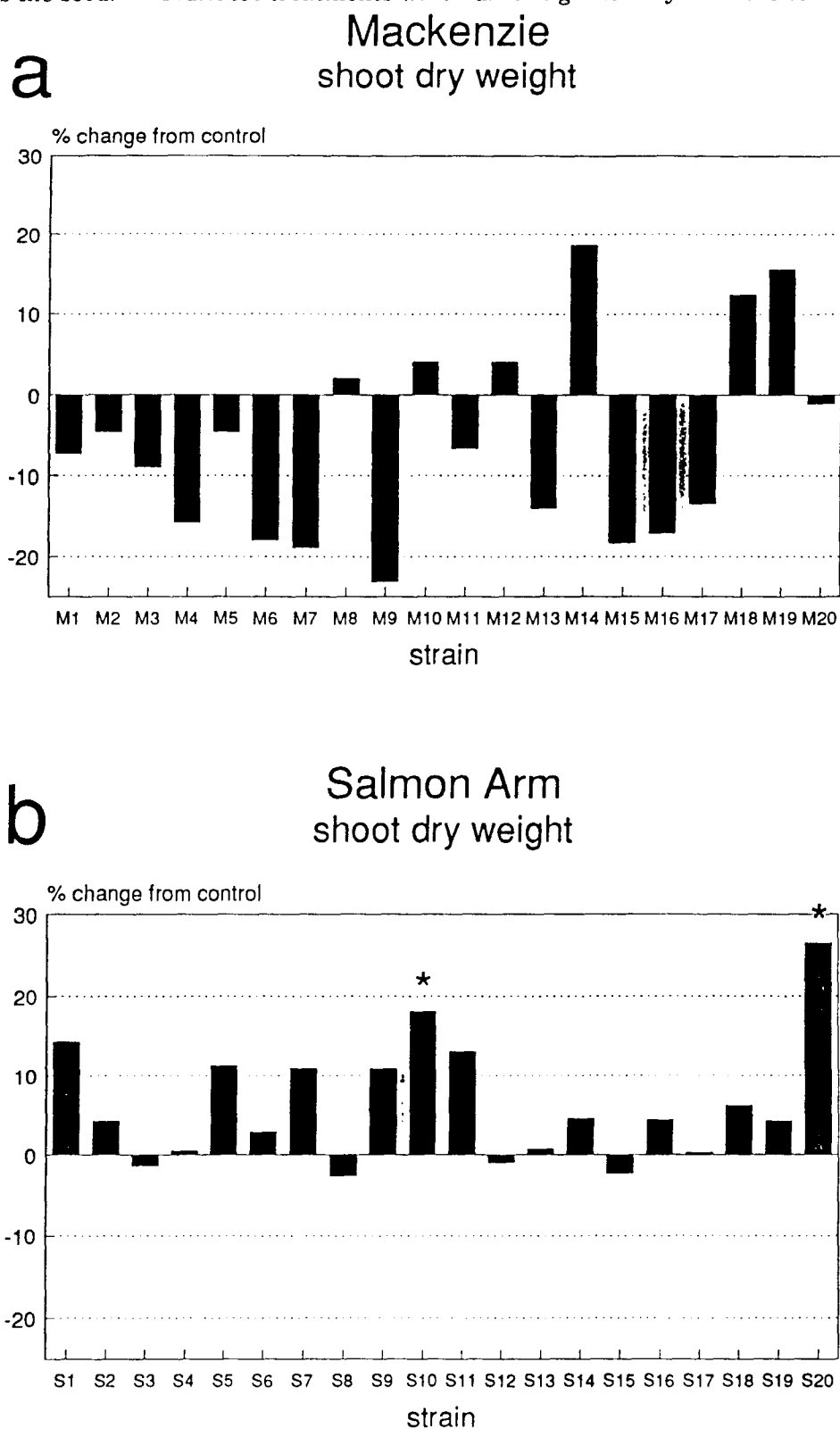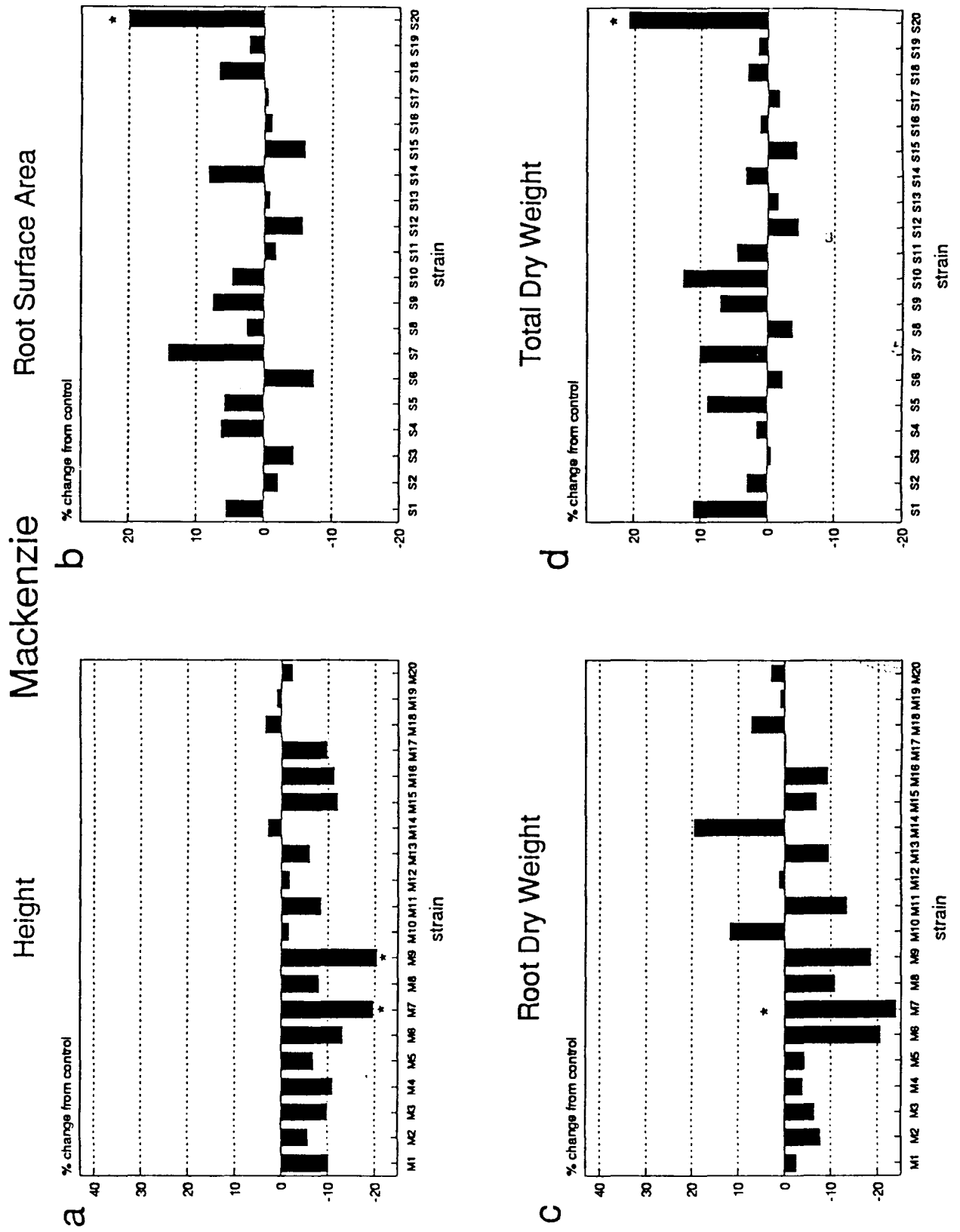
Fig. 4. Effect of inoculation with Salmon Arm bacterial strains on: (a) height; (b) root surface area; (c) root dry weight; (d) total dry weight of Salmon Arm seedlings grown in Salmon Arm soil in Experiment 1. * - indicates treatments which differ significantly from the control at p < 0.05.
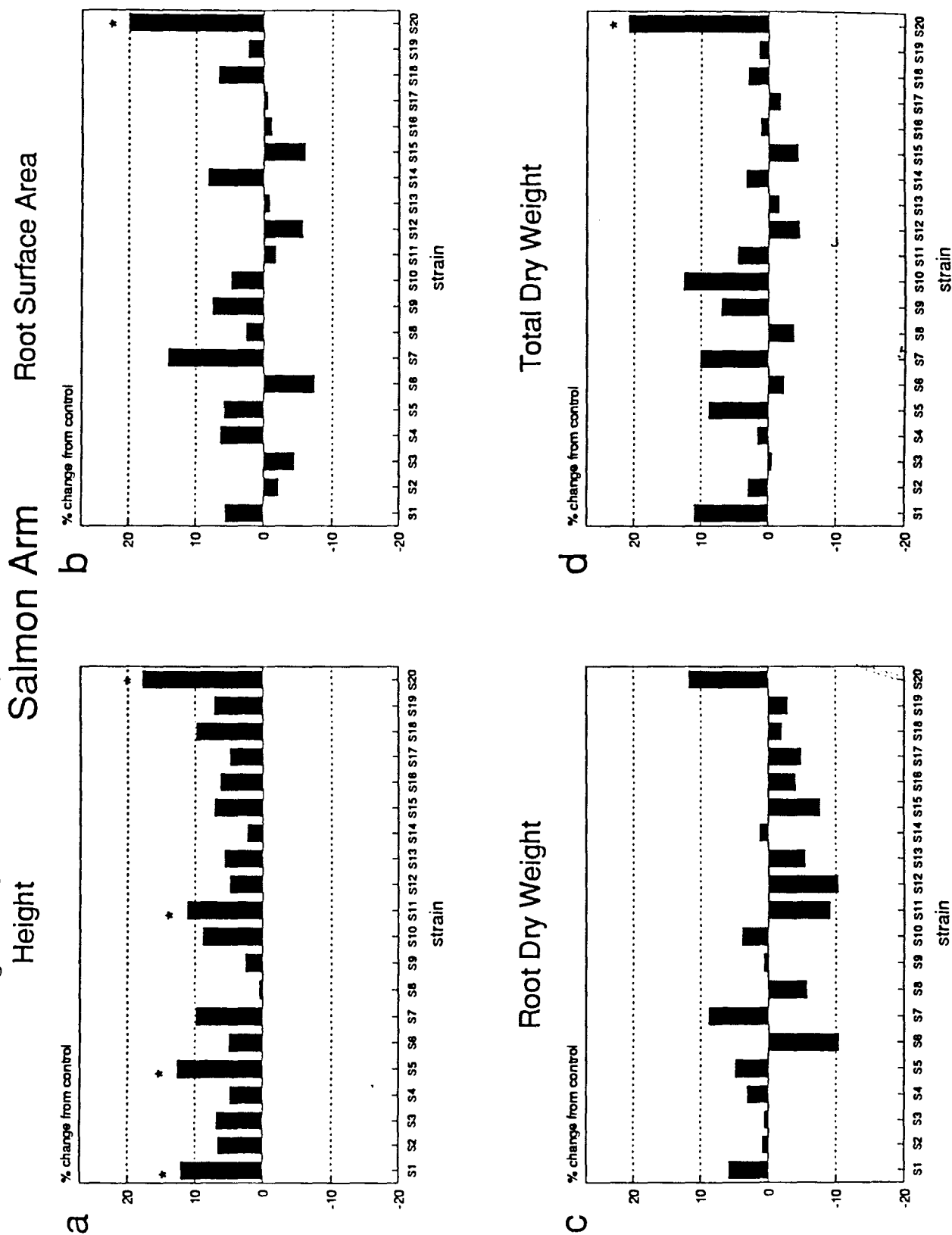
Table 4a. Experiment 1 treatment means for Mackenzie seedlings. TRT - strain used for inoculation; SDW -shoot dry weight; HT - height; DIAM - stem diameter; RSA - root surface area; RDW - root dry weight; R/S - root/shoot dry weight ratio; TOTDW - total seedling dry weight; LSD - least significant difference; MSE - mean square error. * - indicates values which differ significantly from control at p < 0.05.

			Mackenzie Provenance				
TRT	SDW (mg)	HT (mm)	DIAM (mm)	RSA (cm^2)	RDW (mg)	R/S	TOTDW (mg)
M1	22.4	24.1	0.514	6.67	15.1	0.76	37.5
M2	23.1	25.2	0.531	6.95	14.2	0.67	37.3
M3	22.0	24.1	0.535	6.69	14.4	0.76	36.5
M4	20.4	23.8	0.528	6.98	14.9	0.87	35.3
M5	23.1	24.9	0.542	6.42	14.8	0.87	37.9
M6	19.8	23.2	0.506	5.56	12.2	0.66	32.1
M7	19.6	21.5 *	0.536	5.55	11.7 *	0.65	31.4
M8	24.7	24.6	0.527	6.66	13.8	0.62	38.5
M9	18.6	21.3 *	0.504	5.71	12.6	0.80	31.2
M10	25.2	26.4	0.524	7.36	17.3	0.77	42.5
M11	22.6	24.5	0.547	6.40	13.4	0.61	36.0
M12	25.2	26.3	0.565	7.48	15.7	0.73	40.9
M13	20.8	25.2	0.525	6.90	14.0	0.70	34.8
M14	28.7	27.6	0.573	8.31	18.6	0.75	47.4
M15	19.8	23.6	0.500	6.34	14.4	0.97 *	34.1
M16	20.1	23.8	0.515	6.67	14.0	0.87	34.1
M17	20.9	24.2	0.527	6.69	15.5	0.85	36.4
M18	27.2	27.7	0.589 *	7.52	16.6	0.66	43.8
M19	28.0	27.1	0.532	7.16	15.6	0.58	43.6
M20	23.9	26.2	0.541	7.53	15.9	0.76	39.9
control	24.2	26.8	0.534	7.16	15.6	0.69	39.8
LSD	7.30	4.05	0.0542	1.672	3.69	0.237	10.3
MSE	138.78	42.90	0.0076	7.275	35.35	0.146	276.9

Table 4b. Experiment 1 treatment means for Salmon Arm seedlings. TRT - strain used for inoculation; SDW -shoot dry weight; HT - height; DIAM - stem diameter; RSA - root surface area; RDW - root dry weight; R/S - root/shoot dry weight ratio; TOTDW - total seedling dry weight; LSD - least significant difference; MSE - mean square error. * - indicates values which differ significantly from control at p < 0.05.

				Salmon Arm Provenance			
TRT	SDW (mg)	HT (mm)	DIAM (mm)	RSA (cm^2)	RDW (mg)	R/S	TOTDW (mg)
S1	64.4	33.8 *	0.842	16.81	37.5	0.61	101.9
S2	58.7	32.2	0.835	15.00	35.8	0.67	94.5
S3	55.5	32.2	0.845	14.66	35.6	0.67	91.1
S4	56.6	31.6	0.812	16.30	36.5	0.66	93.2
S5	62.7	34.0 *	0.853	16.21	37.2	0.60	99.9
S6	57.9	31.7	0.826	14.21	31.7	0.63	89.7
S7	62.5	33.1	0.864	17.50	38.5	0.64	101.0
S8	54.9	30.3	0.810	15.71	33.4	0.65	88.3
S9	62.4	30.9	0.860	16.47	35.6	0.61	98.1
S10	66.5 *	32.8	0.866	16.06	36.8	0.57	103.3
S11	63.7	33.5 *	0.827	15.08	32.2	0.52	95.9
S12	55.8	31.6	0.804	14.46	31.8	0.60	87.6
S13	56.7	31.9	0.818	15.21	33.5	0.64	90.3
S14	58.8	30.9	0.831	16.57	35.9	0.66	94.7
S15	55.0	32.3	0.844	14.40	32.7	0.64	87.8
S16	58.7	32.0	0.836	15.16	34.0	0.60	92.8
S17	56.5	31.6	0.818	15.25	33.7	0.71	90.2
S18	59.8	33.1	0.841	16.34	34.7	0.60	94.5
S19	58.7	32.3	0.824	15.67	34.4	0.62	93.1
S20	71.3 *	35.5 *	0.862	18.38 *	39.6	0.56	110.9 *
control	56.3	30.2	0.805	15.32	35.5	0.67	91.8
LSD	7.40	3.28	0.073	2.661	5.46	0.127	15.26
MSE	142.54	28.01	0.014	18.429	77.49	0.042	606.19

4.3 Experiment 2

4.3.1 Germination Responses

The three most effective SDW promoting strains from each location in the first experiment were selected for re-testing in the second experiment. The most effective SDW promoter from Mackenzie was strain M14 and was identified as *Pseudomonas putida*. The second and third best strains were M18 and M19, and both were identified as *Hydrogenophaga pseudoflava*. Both strains M18 and M19 were capable of reducing acetylene. The most effective SDW promoting strains from Salmon Arm tested in the first experiment were (in decreasing order of effectiveness) S20, S10 and S1. These were identified as *Bacillus polymyxa, Staphlococcus hominis, and Pseudomonas putida,* respectively.

Soil source and pasteurization had a significant effect on germination capacity, which was 3.6% greater in Mackenzie than in Salmon Arm soil, and 6.4% greater in pasteurized than in unpasteurized soil (Fig. 5a). (See Table 5 for absolute germination values and Appendix 2 for ANOVA.) A significant seed x bacteria interaction for GC was also detected, although no strain produced a GC response significantly different from its corresponding control (Fig. 5c).

Germination value also varied significantly between seed provenances and soil types (Fig. 5b). GV was higher in the Salmon Arm (8.3) than in the Mackenzie (6.3) provenance, but lower in the Salmon Arm (7.0) than in the Mackenzie (7.7) soil. Although the bacteria x seed provenance interaction was technically non-significant (p = 0.107), the effect of most of the inoculants on GV depended greatly on the seedlot used. For example, strains M14, S10 and S20 all promoted GV by greater than 15%, but had an inhibitory or negligible effect on the Salmon Arm seedlot (Fig. 5d).

4.3.1.1 Coexistence Specificities

In most of the factor combinations which were tested, coexistence had little effect on germination. The germination capacity of treatments comprised of coexistent bacteria and unpasteurized soil was slightly, but significantly lower (5.8%) than those comprised of non-coexistent factor combinations (Table 6 C3$_a$). Coexistence of seed and pasteurized soil also resulted in a significantly lower

Fig. 5. Effect of: (a) seed provenance, soil source and pasteurization on germination capacity; (b) seed provenance and soil source on germination value; seed provenance and bacterial strain on (c) germination capacity and (d) germination value in Experiment 2. Only statistically significant (p < 0.05) effects are shown for graphs a and b. No statistically significant effects were observed in graphs c and d.
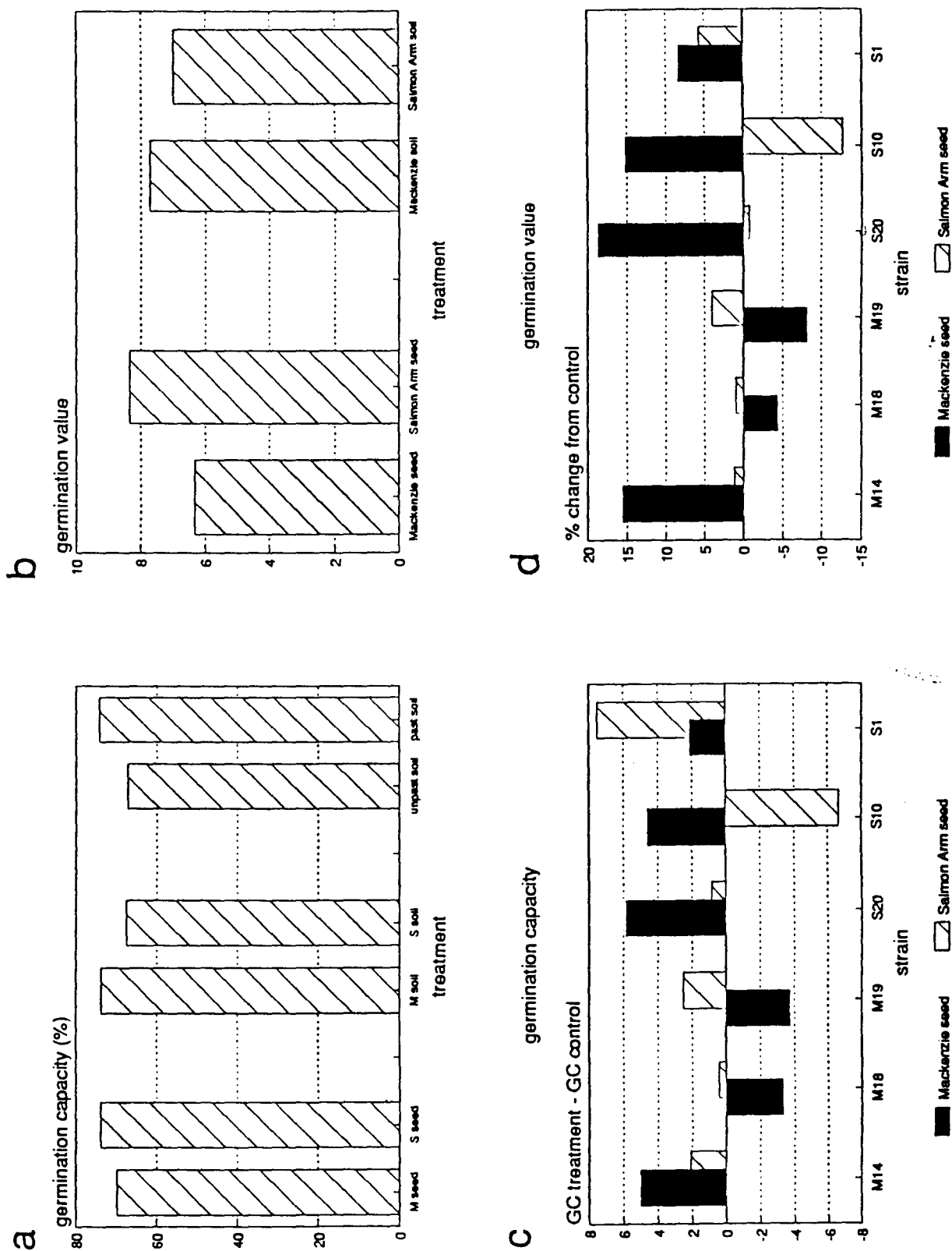
Table 5. Mean germination capacity (GC) and germination value (GV) of 56 treatments in Experiment 2. SEED - seed provenance; SOIL - soil source; PAST - pasteurization treatment; BACT - putative PGPR strain used; M - Mackenzie; S - Salmon Arm; unpast - unpasteurized; past - pasteurized; MSE - mean square error; LSD - Least Significant Difference. * - indicates values which differ significantly from control at p < 0.05. Horizontal lines group treatments with their appropriate control. Shaded regions contain treatments in unpasteurized soil.

TRT	SEED*SOIL*		PAST*	BACT	GC (%)	GV
1	M	M	unpast	M14	60.0	5.4
2	M	M	unpast	M18	61.7	6.1
3	M	M	unpast	M19	55.0	4.6
4	M	M	unpast	CONTROL	70.0	6.2
5	M	M	unpast	S20	75.0	7.5
6	M	M	unpast	S10	78.3	8.3
7	M	M	unpast	S1	65.0	5.9
8	M	M	past	M14	80.0	8.1
9	M	M	past	M18	73.3	7.1
10	M	M	past	M19	75.0	6.7
11	M	M	past	CONTROL	66.7	5.4
12	M	M	past	S20	81.7	8.0
13	M	M	past	S10	80.0	7.4
14	M	M	past	S1	83.3	8.5 *
15	M	S	unpast	M14	76.7 *	8.1 *
16	M	S	unpast	M18	66.7	5.9
17	M	S	unpast	M19	63.3	5.6
18	M	S	unpast	CONTROL	56.7	4.5
19	M	S	unpast	S20	65.0	6.1
20	M	S	unpast	S10	61.7	5.5
21	M	S	unpast	S1	63.3	5.1
22	M	S	past	M14	76.7	6.0
23	M	S	past	M18	58.3 *	3.7 *
24	M	S	past	M19	65.0	4.9
25	M	S	past	CONTROL	80.0	7.7
26	M	S	past	S20	75.0	6.7
27	M	S	past	S10	71.7	6.1
28	M	S	past	S1	70.0	6.3
29	S	M	unpast	M14	76.7	9.3
30	S	M	unpast	M18	68.3	7.8
31	S	M	unpast	M19	66.7	7.4
32	S	M	unpast	CONTROL	75.0	9.0
33	S	M	unpast	S20	81.7	10.2
34	S	M	unpast	S10	65.0	7.9
35	S	M	unpast	S1	81.7	9.2
36	S	M	past	M14	71.7	8.2
37	S	M	past	M18	78.3	9.0
38	S	M	past	M19	85.0	10.2
39	S	M	past	CONTROL	83.3	9.5
40	S	M	past	S20	76.7	8.1
41	S	M	past	S10	66.7	6.8
42	S	M	past	S1	80.0	8.6
43	S	S	unpast	M14	71.7	7.5
44	S	S	unpast	M18	66.7	7.9
45	S	S	unpast	M19	70.0	8.4
46	S	S	unpast	CONTROL	66.7	7.7
47	S	S	unpast	S20	70.0	7.9
48	S	S	unpast	S10	63.3	8.2
49	S	S	unpast	S1	80.0	9.5
50	S	S	past	M14	80.0	8.8
51	S	S	past	M18	80.0	9.1
52	S	S	past	M19	80.0	8.7
53	S	S	past	CONTROL	66.7	7.3
54	S	S	past	S20	66.7	7.0
55	S	S	past	S10	69.7	6.3
56	S	S	past	S1	80.0	8.0

LSD   17.03   2.84
MSE   755.38   21.06

Table 6. Coexistent and non-coexistent factor combination treatment means for germination capacity (GC) and germination value (GV) in Experiment 2. Treatment number designations are explained in Appendix 1. Means within a group of comparisons which are not followed by a letter or are followed by the same letter do not differ significantly at p < 0.10.

CONTRASTS IN UNPASTEURIZED SOIL

			TREATMENTS	GC	GV
C1	bact-seed-soil	coexistent	1,2,3,47,48,49	65.0	6.95
		non-coexistent	5,6,7,15,16,17,19,20,21,29,30,31,33,34,35,43,44,45	70.2	7.43
		non-coexistent control	4,18,32,46	67.1	6.83
		coexistent control	4,46	68.3	6.93
C2	bact-seed	coexistent	1,2,3,15,16,17,33,34,35,47,48,49	68.7	7.39
		non-coexistent	5,6,7,19,20,21,29,30,31,43,44,45	69.0	7.23
		control	4,18,32,46	67.1	6.83
C3	bact-soil	coexistent	1,2,3,19,20,21,29,30,31,47,48,49	66.0 a	6.91
		non-coexistent	5,6,7,15,16,17,33,34,35,43,44,45	71.8 b	7.71
		control	4,18,32,46	67.1 ab	6.83
C4	seed-soil	coexistent	4,46	68.3	6.93
		non-coexistent	18,32	65.8	6.73

CONTRASTS IN PASTEURIZED SOIL

			TREATMENTS	GC	GV
C1	bact-seed-soil	coexistent	8,9,10,54,55,56	74.1	7.21
		non-coexistent	12,13,14,22,23,24,26,27,28,36,37,38,40,41,42,50,51,52	75.6	7.51
		coexistent control	11,53	66.7	6.36
		non-coexistent control	11,25,39,53	74.2	7.48
C2	bact-seed	coexistent	8,9,10,22,23,24,40,41,42,54,55,56	72.3 a	6.78 a
		non-coexistent	12,13,14,26,27,28,36,37,38,50,51,52	78.1 b	8.09 b
		control	11,25,39,53	74.2 ab	7.48 ab
C3	bact-soil	coexistent	8,9,10,26,27,28,36,37,38,54,55,56	74.7	7.48
		non-coexistent	12,13,14,22,23,24,40,41,42,50,51,52	75.7	7.39
		control	11,25,39,53	74.2	7.48
C4	seed-soil	coexistent	11,53	66.7 a	6.36 a
		non-coexistent	25,39	81.7 b	8.60 b

germination capacity and germination value, compared with non-coexistent seed and pasteurized

soil factor combinations (Table 6 C4$_b$).

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4.3.2   Growth Responses

The results and discussion of growth parameters in Experiment 2 will concentrate on SDW due to

the high correlation between this parameter and the others (height - 0.72; diameter - 0.80; root

surface area - 0.73; root dry weight - 0.79; total dry weight - 0.95), and the importance of SDW in

seedling growth and survival.

Due to the strong 4-way treatment interaction (bacterial strain x seed provenance x soil source x

pasteurization treatment) (Appendix 3), the effect of each of the six bacterial strains on SDW is

presented separately for the eight seed x soil x pasteurization combinations (Fig. 6). (Effects on the

other growth variables are illustrated in Appendices 4-9, and means of the 56 treatments are

presented in Table 7.) Uninoculated control means are illustrated separately (Fig. 7), in order to

emphasize the effects of the 3 other main effects (seed provenance, soil source and soil pasteurization

type), and to best distinguish their effects from the effects of the bacteria on seedling growth.

Four of the six selected bacterial strains caused statistically significant growth promotion (Fig. 6),

however, the growth response depended on seed and soil source, and on soil pasteurization. A strain

which was effective with a particular factor combination often inhibited SDW in other factor

combinations. For example, strains S1 and S10 increased SDW of Mackenzie seedlings by 32% and

53%, respectively, (over uninoculated controls) in pasteurized Salmon Arm soil (Fig. 6b); but these

two strains inhibited SDW of the same seed provenance by 12% and 19%, respectively, when grown

in pasteurized Mackenzie soil (Fig. 6a). Similarly, strain M19 promoted SDW of Salmon Arm

seedlings by 37% when grown in pasteurized Mackenzie soil (Fig. 6c), but growth of Mackenzie

seedlings when seed was inoculated with the same bacteria and grown in the same media was

inhibited by 12% (Fig. 6a).

Fig. 6. Effect of inoculation with putative PGPR on seedling shoot dry weight in all seed, soil and pasteurization treatment combinations in Experiment 2. Data represent the percent change in shoot dry weight due to inoculation (relative to uninoculated control treatments). * . indicates treatments which differ significantly from control at p < 0.05.
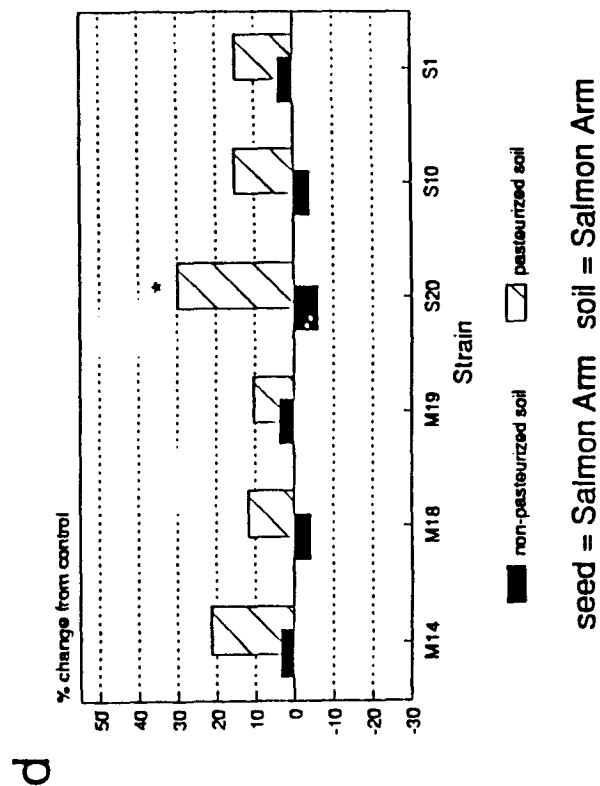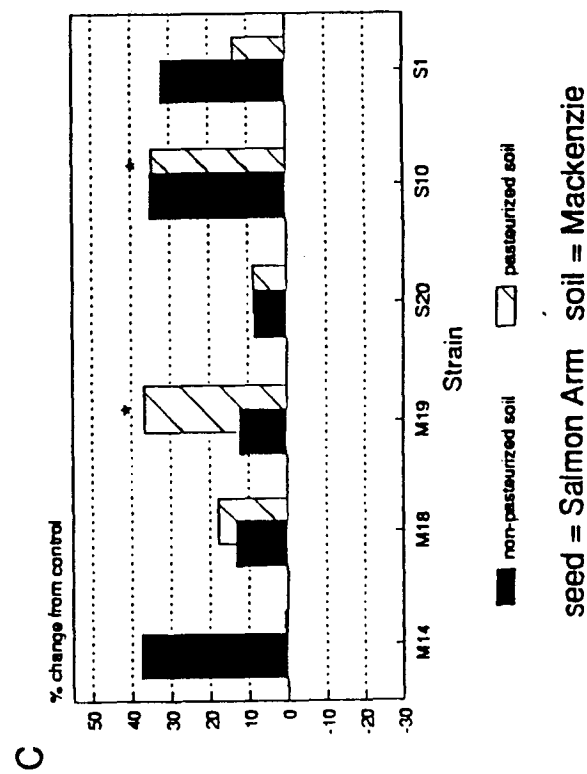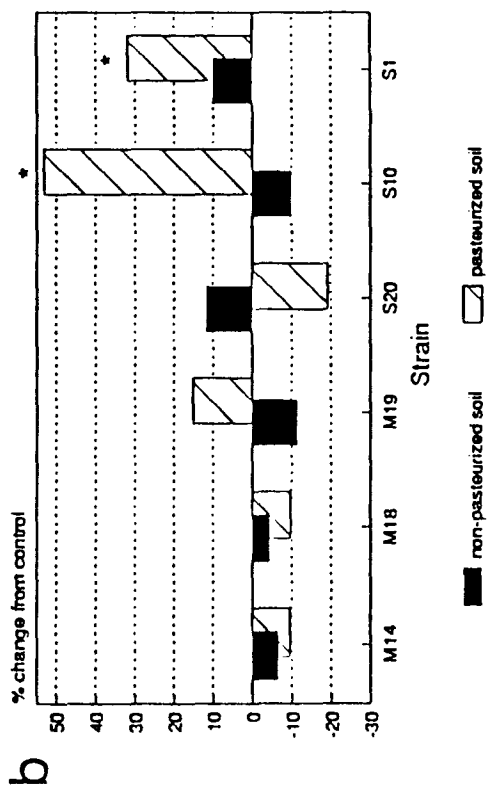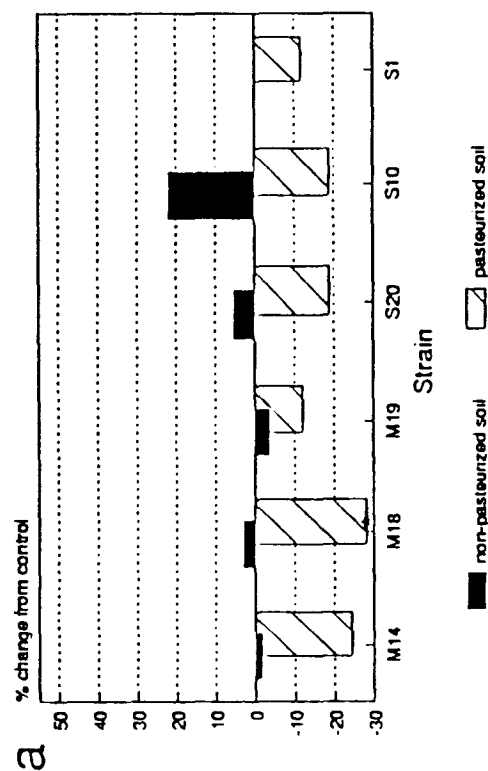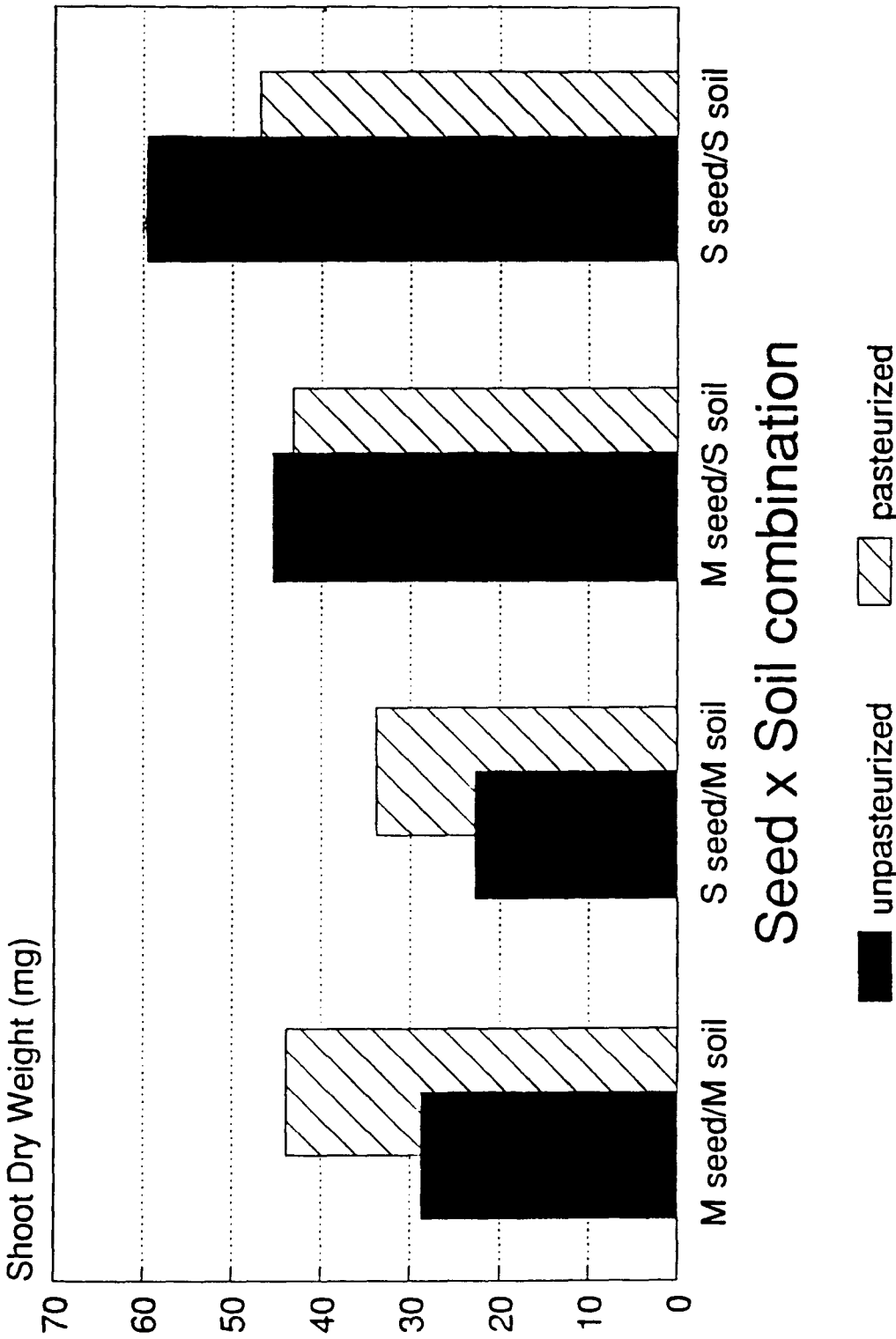
Shoot Dry Weight

Table 7. Growth parameter means of 56 treatments in Experiment 2. TRT - treatment number designation; SEED - seed provenance; SOIL - soil source; PAST - pasteurization treatment; BACT - putative PGPR strain used; SDW - shoot dry weight; HT - height; DIAM - stem diameter; RSA - root surface area; RDW - root dry weight; R/S - root/shoot dry weight ratio; TOTDW - seedling total dry weight. past - pasteurized; unpast - unpasteurized; LSD - Least Significant Difference; MSE - mean squares error. * - indicates values which differ significantly from control at p < 0.05. Horizontal lines group treatments with their appropriate control. Shaded regions contain treatments in unpasteurized soil.

TRT	SEED	SOIL	PAST	BACT	SDW (mg)	HT (mm)	DIAM (mm)	RSA (cm^2)	RDW (mg)	R/S	TOTWT (mg)
1	M	M	unpast	M14	28.3	25.00	0.551	6.4	16.9	0.779	45.2
2	M	M	unpast	M18	27.7	27.94	0.489	6.7	18.3	0.679	46.0
3	M	M	unpast	M19	29.6	28.80	0.577	7.3	19.3	0.701	48.9
4	M	M	unpast	CONTROL	28.9	28.05	0.551	7.0	18.8	0.711	47.7
5	M	M	unpast	S20	30.3	28.25	0.548	7.1	18.2	0.647	48.6
6	M	M	unpast	S10	35.1	28.85	0.580	8.0	20.7	0.613	55.9
7	M	M	unpast	S1	28.8	27.68	0.559	7.1	19.4	0.666	48.2
8	M	M	past	M14	33.2	26.70	0.551 *	8.0	22.1	0.693	55.4
9	M	M	past	M18	31.5 *	23.70	0.557 *	8.3	22.2	0.732	53.7
10	M	M	past	M19	38.6	21.75 *	0.574	9.9	25.4	0.675	64.1
11	M	M	past	CONTROL	43.9	25.65	0.648	11.6	28.1	0.664	72.1
12	M	M	past	S20	35.6	21.20 *	0.599	9.8	25.4	0.793	61.0
13	M	M	past	S10	35.6	22.85	0.554 *	9.2	21.4	0.613	57.1
14	M	M	past	S1	38.7	23.55	0.608	10.2	24.0	0.647	62.7
15	M	S	unpast	M14	42.5	25.40	0.633	11.7	32.7	0.795	75.3
16	M	S	unpast	M18	43.5	30.36	0.653	11.9	30.1	0.701	73.7
17	M	S	unpast	M19	40.3	26.78	0.638	10.8	27.4	0.748	67.7
18	M	S	unpast	CONTROL	45.5	28.63	0.639	11.2	31.0	0.669	76.6
19	M	S	unpast	S20	50.8	29.05	0.692	12.0	32.4	0.669	83.3
20	M	S	unpast	S10	40.9	27.45	0.652	10.5	28.9	0.730	69.9
21	M	S	unpast	S1	50.0	30.50	0.704	14.2	35.0	0.706	85.2
22	M	S	past	M14	39.1	24.80	0.655	18.4	39.3	1.007	78.5
23	M	S	past	M18	39.0	25.70	0.635	18.7	39.4	0.973	78.5
24	M	S	past	M19	49.7	26.45	0.718	22.2	47.9	1.021	97.7
25	M	S	past	CONTROL	43.2	26.75	0.699	19.5	38.9	0.934	82.2
26	M	S	past	S20	34.9	25.45	0.652	17.5	38.3	1.107 *	73.3
27	M	S	past	S10	66.1 *	33.75 *	0.828 *	30.1 *	64.7 *	1.034	130.9 *
28	M	S	past	S1	57.0 *	32.42 *	0.732	26.8 *	60.1 *	1.144 *	117.2 *
29	S	M	unpast	M14	31.4	24.80	0.644	7.8	19.2	0.658 *	50.6
30	S	M	unpast	M18	25.8	23.33	0.618	8.3	20.1	0.804	46.1
31	S	M	unpast	M19	25.6	23.35	0.592	9.9	20.1	0.766 *	47.0
32	S	M	unpast	CONTROL	22.8	22.84	0.570	8.7	19.3	0.976	42.2
33	S	M	unpast	S20	24.7	22.78	0.629	8.2	19.3	0.809	44.1
34	S	M	unpast	S10	30.8	25.38	0.625	8.7	22.1	0.841	53.7
35	S	M	unpast	S1	30.1	23.75	0.617	8.9	21.1	0.768 *	51.6
36	S	M	past	M14	34.0	20.90	0.681	12.8	28.1	0.811	61.4
37	S	M	past	M18	39.8	23.70	0.679	14.0	33.2	0.863	73.2
38	S	M	past	M19	46.2 *	23.84	0.699	15.1	35.7	0.906	82.1 *
39	S	M	past	CONTROL	33.8	20.65	0.685	12.4	27.0	0.874	60.8
40	S	M	past	S20	36.7	20.90	0.680	12.9	28.8	0.838	65.7
41	S	M	past	S10	45.5 *	23.90	0.750	15.4	33.2	0.772	78.7
42	S	M	past	S1	38.4	23.05	0.717	13.7	30.6	0.893	69.1
43	S	S	unpast	M14	61.5	32.40	0.793	19.6	50.6	0.877	112.2
44	S	S	unpast	M18	56.9	29.72	0.776	17.6	47.3	0.830	105.3
45	S	S	unpast	M19	61.7	33.10	0.778	18.5	50.5	0.834	112.4
46	S	S	unpast	CONTROL	59.5	29.94	0.770	17.0	50.9	0.852	110.5
47	S	S	unpast	S20	55.8	31.95	0.766	17.2	46.1	0.829	102.0
48	S	S	unpast	S10	57.0	30.29	0.788	16.3	46.2	0.825	103.3
49	S	S	unpast	S1	61.7	32.55	0.813	18.2	47.9	0.805	109.7
50	S	S	past	M14	56.9	32.21	0.814	30.6	64.9	1.179	121.9 *
51	S	S	past	M18	52.4	29.84	0.788	26.2	54.8	1.135	107.3
52	S	S	past	M19	51.8	28.89	0.748	26.9	59.4	1.154	111.3
53	S	S	past	CONTROL	46.9	27.27	0.774	25.4	54.6	1.169	101.6
54	S	S	past	S20	60.9	30.66	0.840	29.2	62.9	1.082	123.8 *
55	S	S	past	S10	54.0	29.60	0.810	25.2	62.2	1.202	116.3
56	S	S	past	S1	53.9	29.60	0.771	24.2	57.6	1.127	111.6

LSD (2-tailed, p < 0.05)					11.19	3.768	0.0810	4.28	9.36	0.1739	19.26
MSE					325.88	36.963	0.0171	47.63	228.15	0.0787	965.66

Fig. 7. Shoot dry weight of uninoculated control seedlings in all seed, soil and pasteurization treatments.

Shoot Dry Weight (mg)

Seed x Soil combination

M seed/M soil   S seed/M soil   M seed/S soil   S seed/S soil

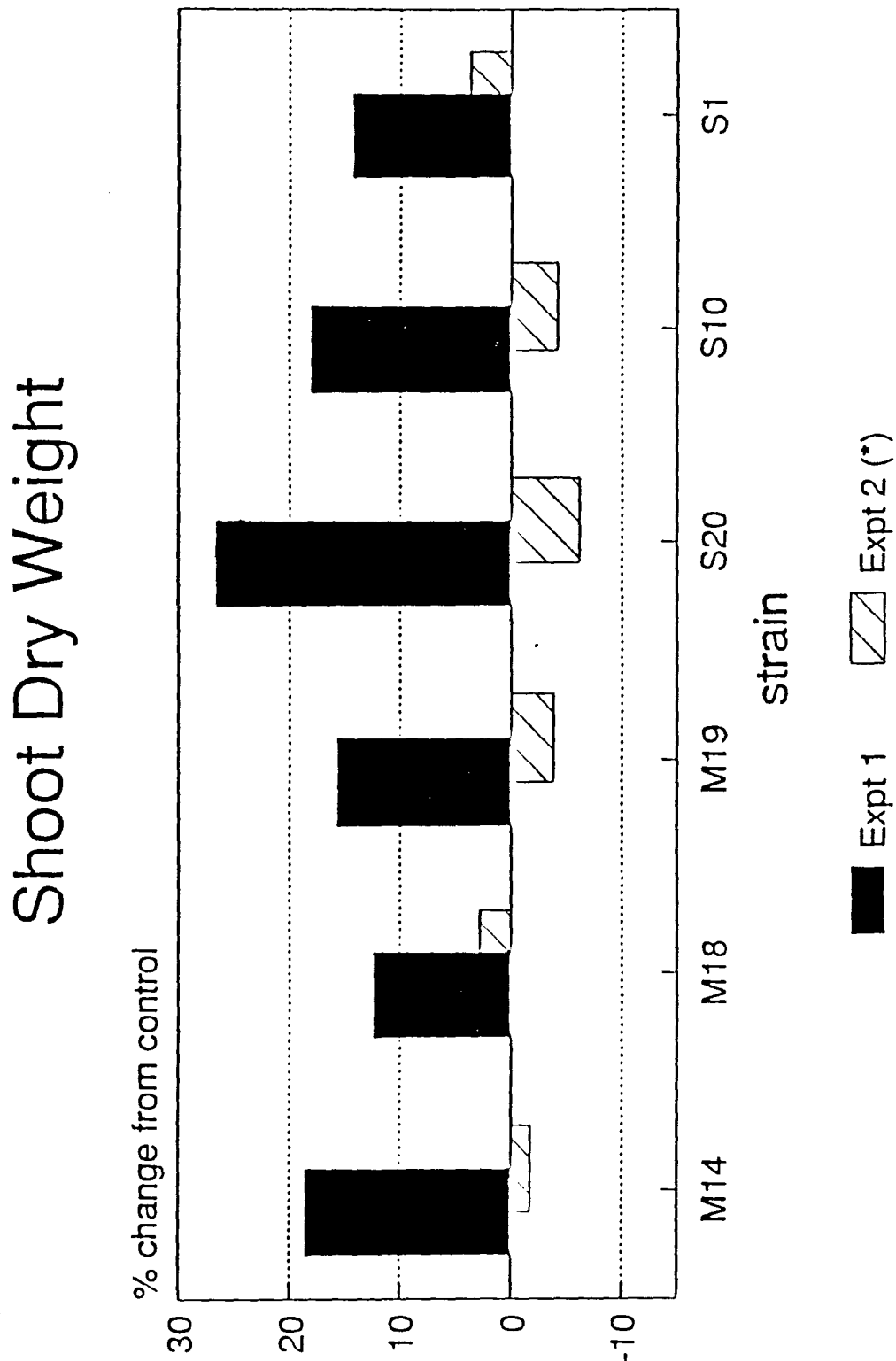■ unpasteurized   ▨ pasteurized

M - Mackenzie   S - Salmon Arm

Given the preceding examples of the extreme dependance of the PGPR effect on particular seed provenance, soil source and soil pasteurization combinations, strain S10 nonetheless stimulated SDW (and other growth parameters) in 4 of the 8 seed x soil x pasteurization treatment combinations and strain S1 caused promotion of SDW in five of the eight combinations (Fig. 6). However, the promotion of SDW in Experiment 1 due to inoculation by the selected strains was not repeated in the same coexistent bacteria-seed-soil treatments of Experiment 2 (Fig. 8), although height was generally promoted in both experiments.

Despite the variation in PGPR response between the different seed x soil x pasteurization treatment combinations, most of the bacteria responded somewhat similarly within each factor combination. Inoculation of Salmon Arm seed in either soil source generally resulted in SDW promotion regardless of pasteurization, with five of the strains promoting SDW greater than 30% above uninoculated controls in at least one of the four soil source x pasteurization type combinations (Figs. 6c and 6d).

Mackenzie seed on the other hand, varied considerably in its response to inoculation. All strains depressed SDW of the Mackenzie provenance when grown in pasteurized Mackenzie soil, and, with the exception of S10, had a negligible effect (<5%) in unpasteurized Mackenzie soil (Fig. 6a). The single exception to the generalization that strains within a seed x soil x pasteurization combination responded similarly, occurred with the inoculation of Mackenzie seed in pasteurized Salmon Arm soil: responses ranged from 53% promotion (S10) to 19% inhibition (S20) (Fig. 6b). There was no significant effect of inoculation of the same seed x soil combination in unpasteurized soil.

Strong interactions were detected involving the three other main effects (seed provenance, soil source and soil pasteurization) (see control means Fig. 7 and ANOVA - Appendix 3). Mackenzie seedlings were 22% larger than Salmon Arm seedlings when grown in Mackenzie soil, and Salmon Arm seedlings were 31% larger than Mackenzie seedlings when grown in Salmon Arm soil. The same pattern of growth occurred in pasteurized soil. In general, the SDW of seedlings grown in Salmon Arm soil exceeded that of seedlings grown in Mackenzie soil regardless of seed x

Fig. 8. Shoot dry weight promotion due to bacterial inoculation in coexistent bacteria-seed-soil (unpasteurized) treatments in Experiments 1 and 2.

Shoot Dry Weight

* only treatments containing coexistent
seed, soil (unpasteurized), and bacteria
considered.

pasteurization combination. The only exception was Mackenzie seed sown in pasteurized Mackenzie soil; resultant seedling SDW exceeded very slightly (2%) the SDW of the same seed in pasteurized Salmon Arm soil. Pasteurization resulted in profoundly larger seedlings when Mackenzie soil was tested (57% for Mackenzie seed and 49% for Salmon Arm seed), however, it had the opposite effect on seedling growth in Salmon Arm soil (3% inhibition of Mackenzie seedling SDW and 25% inhibition of Salmon Arm seedling SDW) (Fig. 7).

4.3.2.1 Coexistence Specificities

The SDW of seedlings in treatments comprised of coexistent three-factor combinations (i.e. Mackenzie seed inoculated with Mackenzie bacteria and grown in Mackenzie soil, and Salmon Arm seed inoculated with Salmon Arm bacteria and grown in Salmon Arm soil) was significantly greater than the SDW of uninoculated control seedlings (coexistent and non-coexistent) and seedlings in treatments which were not comprised of coexistent seed, soil and bacteria combinations (i.e. the mean of all other inoculated treatments) (Table 8 $C1_a$ and Fig. 9a). Seedlings in treatments comprised of coexistent bacteria-seed-pasteurized soil, bacteria-seed and bacteria-soil did not differ in SDW from seedlings with the same non-coexistent factor combinations, nor from the control seedlings. (Inoculated seedlings grown in unpasteurized soil were contrasted only with controls grown in unpasteurized soil, and inoculated seedlings grown in pasteurized soil were contrasted only with controls grown in pasteurized soil.)

However, treatments comprised of coexistent combinations of seed and soil (Mackenzie seed grown in Mackenzie soil and Salmon Arm seed grown in Salmon Arm soil) resulted in seedlings which had a significantly and substantially larger SDW (27%) in comparison with treatments comprised of non-coexistent combinations of the same factors (Fig. 9a and Table 8 $C4_a$). The same contrast (coexistent seed-soil versus non-coexistent seed-soil) of seedlings grown in pasteurized soil did not indicate a significant influence of pasteurization, although the SDW advantage due to the presence of coexistent combinations was reduced to 17% (compared with non-coexistent factor combinations) (Fig. 9b).

Table 8. Coexistent and non-coexistent factor combination treatment means for growth parameters in Experiment 2. Treatment number designations are illustrated in Appendix 1. SDW - shoot dry weight; HT - height; DIAM - stem diameter; RSA - root surface area; RDW - root dry weight; R/S - root/shoot dry weight ratio; TOTDW - seedling total dry weight. Means within a group of comparisons which are not followed by a letter or are followed by the same letter do not differ significantly at p < 0.10.

CONTRASTS IN UNPASTEURIZED SOIL

			SDW	HT	DIAM	RSA	RDW	R/S	TOTDW	TREATMENTS
C1	bact-seed-soil	coexistent	44.66 a	29.5 a	0.671 a	12.47	33.56 a	0.773	78.89 a	1,2,3,47,48,49
		non-coexistent	39.62 b	27.4 b	0.652ab	11.22	28.71 b	0.746	68.54 b	5,6,7,15,16,17,19,20,21,29,30,31,33,34,35,43,44,45
		non-coexistent control	38.77 b	27.1 b	0.630 b	10.89	29.60ab	0.800	68.37 b	4,18,32,46
		coexistent control	43.37ab	28.9 ab	0.654ab	11.77	33.99ab	0.777	77.36 ab	4,46
C2	bact-seed	coexistent	39.82	27.6	0.651	11.26	29.46	0.775	69.71	1,2,3,15,16,17,33,34,35,47,48,49
		non-coexistent	41.71	28.2	0.661	11.76	30.22	0.731	72.11	5,6,7,19,20,21,29,30,31,43,44,45
		control	38.77	27.1	0.630	10.89	29.60	0.800	68.37	4,18,32,46
C3	bact-soil	coexistent	41.04	27.9	0.661	11.46	29.72	0.745	71.18	1,2,3,19,20,21,29,30,31,47,48,49
		non-coexistent	40.56	27.9	0.652	11.57	29.99	0.759	70.72	5,6,7,15,16,17,33,34,35,43,44,45
		control	38.77	27.1	0.630	10.89	29.60	0.800	68.37	4,18,32,46
C4	seed-soil	coexistent	43.37 a	28.9 a	0.654	11.77	33.99 a	0.777	77.36 a	4,46
		non-coexistent	34.17 b	25.7 b	0.605	10.01	25.21 b	0.823	59.37 b	18,32

CONTRASTS IN PASTEURIZED SOIL

			SDW	HT	DIAM	RSA	RDW	R/S	TOTDW	TREATMENTS
C1	bact-seed-soil	coexistent	45.12	27.0 a	0.681	17.31	41.77 a	0.916	86.89	8,9,10,54,55,56
		non-coexistent	44.27	25.7 a	0.696	18.30	40.31 a	0.935	84.55	12,13,14,22,23,24,26,27,28,36,37,38,40,41,42,50,51,52
		non-coexistent control	42.02	25.0 b	0.700	17.12	36.90 b	0.904	78.93	11,25,39,53
		coexistent control	45.39	26.4 ab	0.708	18.20	40.68ab	0.904	86.08	11,53
C2	bact-seed	coexistent	43.31	25.5	0.687	17.10	39.15 a	0.916	82.47 ab	8,9,10,22,23,24,40,41,42,54,55,56,
		non-coexistent	45.64	26.5	0.697	19.00	42.21 b	0.945	87.84 a	12,13,14,26,27,28,36,37,38,50,51,52
		control	42.02	25.0	0.700	17.12	36.90 a	0.904	78.93 b	11,25,39,53
C3	bact-soil	coexistent	45.71	26.8	0.696	18.32	42.48 a	0.946	88.18 a	8,9,10,26,27,28,36,37,38,54,55,56
		non-coexistent	43.24	25.2	0.688	17.77	38.87ab	0.915	82.10 ab	12,13,14,22,23,24,40,41,42,50,51,52
		control	42.02	25.0	0.700	17.12	36.90 b	0.904	78.93 b	11,25,39,53
C4	seed-soil	coexistent	45.39	26.4	0.708	18.20	40.68	0.904	86.08	11,53
		non-coexistent	38.65	23.7	0.692	16.05	33.12 b	0.905	71.77	25,39

Fig. 9. Shoot dry weight of treatments comprised of coexistent and non-coexistent factor combinations and control treatments in (a) unpasteurized and (b) pasteurized soil in Experiment 2. Blank control bar represents non-coexistent control for bacteria-seed-soil contrasts. Slashed control bar represents coexistent control for bacteria-seed-soil contrasts. Within a set of contrasts, means having no letter or the same letter do not differ significantly at p < 0.10.
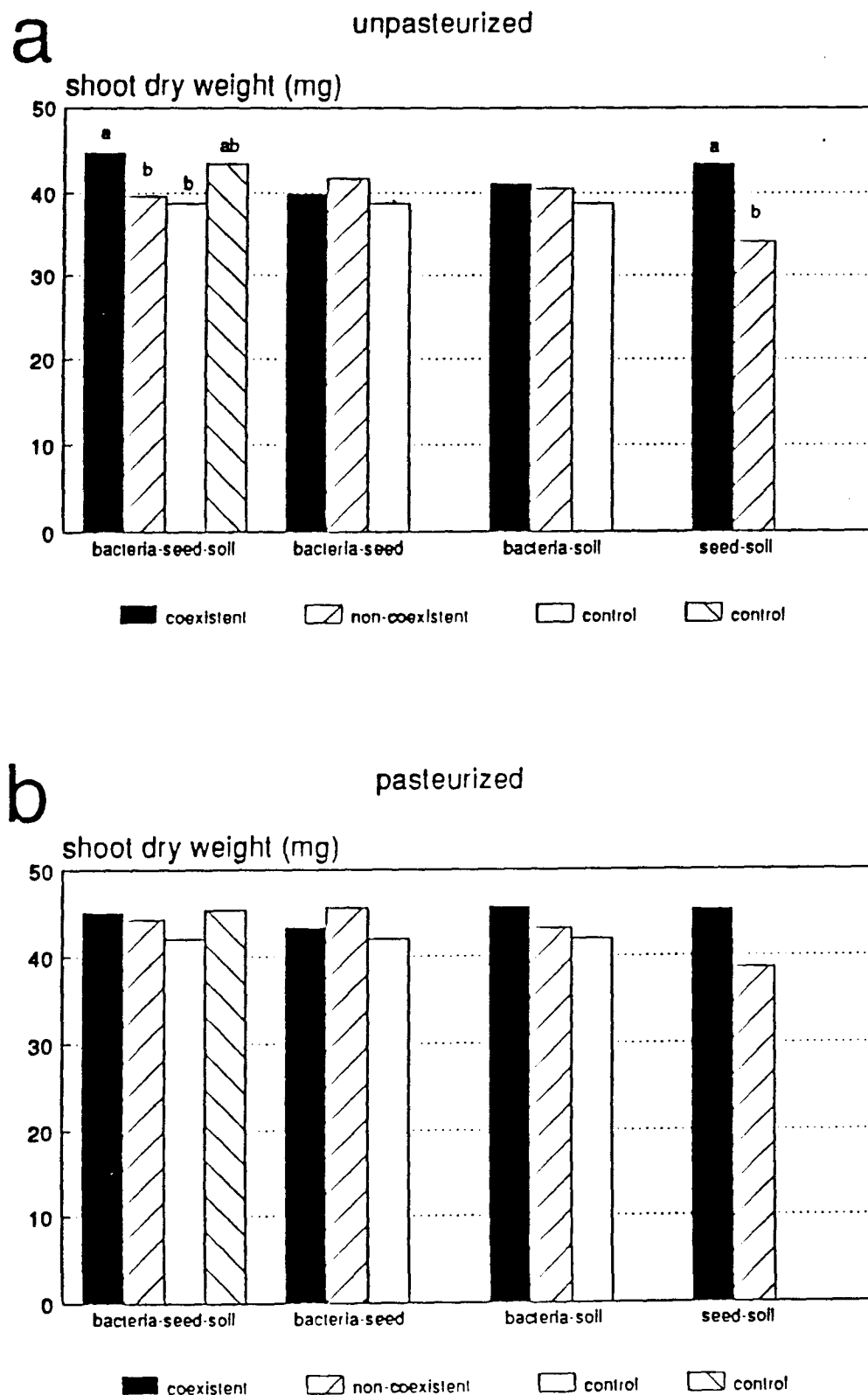
5.0 DISCUSSION

5.1   Germination

When applied to seed in pure culture, some bacterial strains have promoted the germination of several important agricultural species (Hussain and Vancura 1970; Holl *et al.* 1988; Kloepper *et al.* 1988; Chanway *et al.* 1989; Chanway and Nelson 1990). Chanway *et al.* (1991a) recently demonstrated that the germination rate of white spruce [*Picea glauca* (Moench) Voss.], and the germination capacity of lodgepole pine can be increased in response to inoculation with *Bacillus polymyxa* in a controlled environment. Germination capacity of white spruce was also stimulated in an outdoor nursery experiment with the same bacterial inoculum (O'Neill *et al.* 1991).

Most of the bacterial strains from both locations inhibited germination when inoculated onto the seed (Fig. 1). Although these effects were generally not statistically significant, the magnitude was was substantial in several cases. Inhibition of germination of maize following inoculation with bacterial supernatant was reported by Hussain and Vancura (1970) for one of the strains they tested. However, germination was subsequently promoted by a lower concentration of the same supernatant. This observation and the large quantity of several phytohormones in the supernatant provide anecdotal evidence for the involvement of phytohormones in this process. By examining phytohormone production of the strains tested in the present experiment and the effect of inoculation with supernatant dilutions of these strains on germination, the influence of bacterial phytohormones on spruce germination could be assessed.

Numerous factors (mainly abiotic) regulate germination to ensure that the environment in which germination occurs is optimal (Kramer and Kozlowski 1979). The inhibition of germination observed here suggests that rhizosphere bacteria may be included within the repertoire of methods which plants use to maximize their fitness.

Despite the dirth of statistically significant positive germination effects, inoculation with several of the Mackenzie and Salmon Arm strains substantially altered both the germination capacity and the germination value, particularly in the Mackenzie seedlot. In Experiment 1, GC of Mackenzie seed was stimulated 14% compared with uninoculated controls after inoculation with strains M4 and M13, and GV increased by 23%, also in response to strain M13 (Fig. 1a). In Experiment 2, strain S20 increased the GC and GV of Mackenzie seed an average of 5.8% and 18%, respectively, over uninoculated controls in the four soil x pasteurization combinations (Figs. 5c and 5d).

The mechanisms by which such promotive effects occur have yet to be elucidated, but germination enhancement by soil bacteria has been postulated to involve solubilization of organic phosphate (Holl *et al.* 1988) and production of plant growth regulators (Hussain and Vancura 1970). The latter authors observed enhanced germination capacity of maize (*Zea mays* L.) by up to 40% after inoculation with either growth regulator-producing bacteria or their supernatants. Kramer and Kozlowski (1979) suggested that the ratio of growth inhibitors (mainly abscisic acid) to growth promoters (gibberellins and cytokinins) in seeds is largely responsible for regulating seed dormancy. Alternatively, Elad *et al.* (1987) related bacterial promotion of tobacco, radish and cucumber seed germination to the ability of inoculum bacteria to suppress *Fusarium*, a common fungal pathogen of agricultural and conifer species. However, some fungi have been found to enhance germination, possibly through inactivation of germination inhibitors within the seed coat (Jones and Waid 1963).

Storage temperature, seed moisture content and seed age are also known to influence germination (Kramer and Kozlowski 1979). Mackenzie and Salmon Arm seedlots were stored at different locations and were different ages, therefore, it was not surprising that significant differences were detected in the GC and the GV between seedlots (Fig. 5b and Appendix 2). In addition, variation in germination between populations of interior spruce (Khalil 1986) and differences in the percent of filled seed of the two seedlots may also have contributed to the observed differences between the two provenances.

However, causes of the observed differences in germination parameters associated with soil source and soil pasteurization are not so obvious (Fig. 5a and Appendix 2). Allelochemicals, such as organic cyanides, terpenes or phenolic acids, which are leached from living and dead leaves, or are exuded from plant roots of many species can inhibit germination and growth (Rice 1974). Their presence and activity may have differentially influenced germination in the two soils because different plant species were found associated with each of the soil collection sites.

The significant enhancement of germination capacity after soil pasteurization suggests that microbial inhibitors of germination may exist within these soils. This concurs with the work of Jones and Waid (1963), in which greater germination capacity and germination rate were observed in sterile vermiculite than in unsterile soil. The preponderance of germination-inhibiting over germination-promoting bacteria in Experiment 1 implies that rhizobacteria are at least partially responsible for this phenomenon.

The utility of a bacterial germination promoter in a conifer nursery would require that the strain be able to elicit consistently a positive response under a variety of environmental conditions. Despite the relatively similar environmental conditions of Experiments 1 and 2, the reproducibility of the effects of individual strains on germination between experiments was poor: of the six strains tested in both experiments, the strongest promoter of GC in Experiment 1 (M14 - 9% greater than control) was the second strongest GC inhibitor in Experiment 2 (-5%). Similarly, the second strongest promoter in Experiment 1 (2%) was the strongest GC inhibitor in Experiment 2 (M19 - -15%). It appears therefore, that the factors influencing the expression of germination promotion by rhizobacterial inoculation must be more thoroughly understood before this technology can be successfully implemented in conifer nurseries.

Analysis of the effects of phosphorus fertilization on germination, seedling performance assays related to organic phosphate metabolism and growth regulator production of Mackenzie and Salmon Arm strains, and the use of bacterial mutants that are deficient in phosphatase or hormone

production could help to elucidate the mechanisms by which germination is enhanced by these rhizobacteria. Inactivation of germination inhibitors by rhizobacteria, as has been reported for fungi, should also be explored.

5.1.1 Coexistence Specificities

The GC of coexistent bacteria-soil treatments was significantly lower (6%) than that of non-coexistent treatments (Table 6 C3$_a$). While the biological significance of this difference is questionable, it may suggest that the expression of germination regulation by rhizobacteria may have evolved through a period of coexistence between the rhizobacteria and soil.

In general, the presence of coexistent factor combinations (seed provenance, soil source and bacteria) did not substantially affect seedling germination (Table 6). However, the presence of coexistent combinations of seed and pasteurized soil resulted in a substantial inhibition of GC and GV relative to non-coexistent combinations (Table 6 C4$_b$). The same specificity was not observed in unpasteurized soil (Table 6 C4$_a$). The influence of pasteurization on seed germination is difficult to explain and effects may have resulted from altered soil nutrient availability (e.g. the nearly three-fold increase in manganese in Salmon Arm soil), synthesis of inhibitory compounds, or simply from the removal of beneficial micro-organisms that buffer seed from the abiotic environment.

The lack of a stimulatory effect on germination when coexistent factor combinations were tested against uninoculated controls and treatments comprised of non-coexistent factor combinations does not mean that germination-promoting bacteria are non-specific with regard to seed and soil (i.e. that germination promoters are effective on a range of seed genotypes). On the contrary, a significant bacteria x seed interaction for GC was detected: strain S10 promoted the GV of Mackenzie seed 15%, but inhibited that of Salmon Arm seed 12%, while strain M19 had the opposite effect (Mackenzie seed was inhibited 8%, but Salmon Arm seed was promoted 4%) (Fig. 5c). This suggests that bacteria-seed specificity may influence germination, but that such specificity is unrelated to coexistence.

Nonetheless, bacteria-seed, bacteria-soil or seed-soil specificities resulting from coexistence may indeed exist, but may have been undetected in the present experiments because of the absence of the necessary genetic differences between the rhizobacteria, seed or soil biota of the two sites. In other words, we may have wrongly assumed that the geographically disparate sites which were utilized would provide rhizobacteria, seed or soil biota possessing the appropriate qualitative or quantitative genetic differences required to detect coexistence specificities.

Similarly, coexistence specificities may have been missed if their expression is dependent upon some environmental factor which differed between experimental and natural conditions. If growth regulating substances produced by soil bacteria in nature are assumed to cause enhanced germination, production of these substances may be altered substantially under the experimental conditions that were used. This, in turn, may have affected seedling emergence in a way that does not reflect the natural course of events.

5.2   Seedling Growth

5.2.1   Effects of Bacterial Inoculation

Bacterial inoculation had a substantial effect on seedling growth in some treatments. Shoot dry weight increases of up to 27% in Experiment 1 and 53% in Experiment 2, as well as equivalent or larger increases in root surface area, root dry weight and total dry weight relative to uninoculated controls were observed. These findings represent the first report of bacterial growth promotion of spruce and indicate that bacteria capable of stimulating early spruce seedling growth, in controlled conditions at least, can be secured by isolating rhizobacteria from naturally-regenerating conifer seedlings.

However, bacterial inoculation also resulted in the inhibition of shoot dry weight accumulation (maximum inhibition was 28% relative to uninoculated controls) and the effect of a particular strain varied greatly between seed x soil x pasteurization treatments. The successful acquisition of PGPR in these experiments and the variation between treatments may be related to several factors,

including: (1) host specificity; (2) coexistence specificity; (3) the use of endorhizosphere bacteria; (4) the use of bacteria isolated from young plants, and (5) the natural abundance of selected bacteria in the rhizosphere.

5.2.1.1 Host Specificity

Variability of host plant growth responses to inoculation with growth promoting rhizosphere bacteria is not uncommon and its cause remains elusive (Kloepper et al. 1989). Although not always the case (Kloepper et al. 1988; Bashan et al. 1989), plant cultivar or genotype specificity (i.e. differential growth responses of differing cultivars or genotypes due to inoculation with a particular microbe) may comprise a substantial component of the variation in plant growth responses to PGPR (Burr and Caesar 1984; Chanway et al. 1988b,1989). Differential growth responses of the Mackenzie and Salmon Arm seed provenances to the putative PGPR (as indicated by the significant seed x bacteria interaction (Appendix 3)) demonstrate the occurrence of provenance specificity in these spruce-rhizobacteria associations.

Host specificity may also exist at the species level (i.e. species specificity) (Baldani and Dobereiner 1980; Gardner et al. 1984). *Azospirillum brasilense* is most frequently isolated from the roots of wheat, while *Azospirillum lipoferum* is most frequently isolated from the roots of sorghum and maize. Both bacterial species are often cited as PGPR of the plant species from which they were isolated, but rarely do they promote the growth of other species (Sumner 1990).

Inoculation of Douglas-fir, lodgepole pine and interior spruce with *Bacillus* strains isolated from, and capable of promoting the growth of, perennial ryegrass and white clover, resulted in little or no growth promotion of spruce and Douglas-fir (Chanway et al. 1991a; O'Neill et al. 1991), while the stimulatory effects of inoculation on lodgepole pine were short-lasting unless the seedlings were re-inoculated (Chanway et al. 1991a). Therefore, the successful acquisition of interior spruce PGPR in the present experiments may have been related to the use of bacterial inoculants isolated from the target species, interior spruce.

5.2.1.2 Coexistence Specificities

In contrast to germination capacity and value, the presence of coexistent bacteria, seed and soil factor combinations resulted in seedlings with significantly greater SDW than seedlings in non-coexistent factor combinations (Fig. 9 and Table 8 $C1_a$). However, analysis of the three 2-factor combinations (bacteria-seed, bacteria-soil, seed-soil) comprising the 3-factor combination indicated that the seed-soil-bacteria coexistence specificity was almost entirely due to the use of coexistent seed and soil (Table 8 $C3_a$, $C4_a$, $C5_a$).

The SDW of seedlings grown in coexistent soil was significantly larger (27%) than that of seedlings grown in non-coexistent soil. However, the difference in SDW between coexistent and non-coexistent treatments decreased to 17% when the soils were pasteurized, suggesting that both biotic and abiotic elements may have been involved in the manifestation of coexistence specificity.

This trend was also reflected when seedling height, root dry weight and seedling total dry weight were analyzed (Table 8 $C4_a$ and $C4_b$). For example, the root dry weight of seedlings associated with coexistent seed and soil factor combinations was 35% greater than those in non-coexistent combinations, and this advantage decreased to 23% after pasteurization.

In addition to bacteria and mycorrhizae, other microflora (fungi, actinomycetes and algae) and microfauna (protozoa, nematodes, mites and insects) inhabit the rhizosphere, and can influence plant growth (Curl and Truelove 1986). In theory, these could also affect coexistence specificities. However, the less specific nutritional demands and the greater ability of the microfauna to adapt to differing environments are likely to make them less dependent than the microflora on specific hosts. Hence, their involvement in seed-soil coexistence specificity is less probable than that of the more abundant microflora.

Abiotic soil factors which could potentially facilitate the expression of coexistence specificity between seed and soil include soil physical properties, the inorganic micro- or macro-nutrients, and organic

compounds, such as hormones, vitamins, amino acids or enzymes. In these experiments notable differences in the levels of many of the inorganic elements between the two soils were detected (Fig. 1) and may have contributed to the abiotic component of the observed seed-soil coexistence specificity.

Although most of the seed-soil coexistence specificity effect appears to have been due to abiotic factors (inferred from the decrease in the relative shoot dry weight difference between coexistent and non-coexistent seed-soil combinations from 27% to 17% upon soil pasteurization), biotic elements are often directly responsible for the quality and quantity of many abiotic factors, such as organic and inorganic compounds, and soil physical properties, particularly in the rhizosphere. For example, siderophores produced by bacteria are known to influence available ferric iron levels in the rhizosphere (Powell *et al.* 1982) and many species of rhizobacteria secrete various phytohormones (Whightman *et al.* 1980). Therefore, the overall influence of biotic factors in seed-soil coexistence specificity, and consequently, in the potential benefit to plant growth through manipulation of the rhizosphere with coexistent soil micro-organisms, may be under-estimated from the degree with which soil pasteurization affected this specificity.

It is tempting to ascribe the decrease in seedling growth associated with seed-soil coexistence specificity following soil pasteurization solely to the removal of the indigenous microflora, but alterations in soil nutrients and other soil properties following soil pasteurization may also have contributed. Mulder (1979) notes that soil pasteurization can result in manganese and ammonium toxicity in some agricultural species. Consequently, if these nutrients or other soil properties which were altered by soil pasteurization were responsible for the manifestation of the abiotic component of seed-soil specificity, then they too may have contributed to its reduction following pasteurization. No differences were observed in the physical characteristics of the soil, and most of the soil nutrient levels were altered only slightly following soil pasteurization. However, the level of available manganese almost tripled after pasteurization of Salmon Arm soil (Table 1), and seedlings grown in pasteurized Salmon Arm soil had potentially toxic levels of manganese in their tissue (data not

shown) (Brady 1974 p. 486). Therefore, soil pasteurization effects may be confounded with manganese toxicity and these should be interpreted with caution.

By contributing to competitive ability, enhanced plant growth rates can increase the likelihood of dominance and reproductive success within a plant population, and thereby influence the structure of plant communities (Sarukhan *et al.* 1984). The significant seedling growth advantage associated with coexistent seed-soil combinations compared with non-coexistent combinations suggests that seed-soil adaptation could be an important determinant of plant population distribution by increasing community resistance to invasion by non-coexistent genotypes within a species.

Chanway *et al.* (1988a,b) have shown that a history of coexistence between plants and microbes can influence the plant response to bacterial inoculation. However, bacteria-seed coexistence specificity not was detected in the present experiments. Coexistence specificity between these bacterial strains and the spruce provenances may not have been detected due to the heterozygous nature of interior spruce which results from its tendency (and the tendency of most forest tree species) to outcross (Zobel and Talbert 1984 p. 52). Only when vegetative clones have been used has microbe-plant coexistence specificity been observed (Chanway *et al.* 1988a,1990). Therefore, clonal experimentation should be conducted with spruce to confirm these results.

Notwithstanding the lack of bacteria-seed coexistence specificity, significant adaptive relationships were observed between spruce provenances and soil sources. The manifestation of adaptive relationships in the complex environment of soil (as opposed to in less complex non-soil media) attests to the influence these adaptive relationships may have on plant growth. Buffering this observation, however, is the fact that the seed x soil interaction accounted for only 2% of the total experimental variation (Appendix 3); clearly, factors other than seed-soil specificity had considerable influence on plant growth.

In support of this argument is the general observation from provenance tests of many forest tree species that local provenances are often not among the best performing provenances at a given site. This would imply that seed adaptation to climate is much stronger than adaptation to soil. By re-testing the effect of seed-soil coexistence on plant growth using a larger number of seed/soil sources the strength of the inference of this relationship to other seed and soil sources would be increased. Additionally, by growing each provenance in each of the soils and at each location, the contribution of climatic and soil effects to seed-soil specificity could be partitioned.

The contribution of biotic elements to seed-soil coexistence specificity could also have significant silvicultural implications. The benefit of inoculating seedlings at planting with soil possessing the 'proper' microbes was demonstrated by Amaranthus and Perry (1987). They increased Douglas-fir seedling survival by 50% on an unsuccessfully reforested clear-cut by inoculating seedlings at planting with a small amount of soil from a young Douglas-fir plantation. Incorporation of the concept of seed-soil coexistence specificity into silvicultural practice could result in a simple method of improving the efficacy of soil inoculants.

Bacteria-soil coexistence also had a negligible effect on SDW relative to uninoculated control seedlings and to seedlings associated with non-coexistent factor combinations (Fig. 9 and Table 8 $C3_a$). These results may indicate that plant-microbe and soil-microbe coexistence specificity is not important in these ecosystems, and that no advantage would accrue through the use of 'adapted' PGPR for interior spruce.

Alternatively, coexistence specificity between these factors may exist, but may not have been detected due to the over-riding influence of soil factors or the heterozygosity of spruce seed (discussed above). The failure to impose realistic environmental conditions on the experimental system (Harley and Smith 1983), or the alteration of soil microbial populations as a result of storage of the soil for six months may also have obscured the detection of these specificities.

5.2.1.3 Age of the Host Plant

Growth promotion of spruce seedlings in the present study may in part also have been related to the use of young seedlings for the isolation of bacterial inoculants. The variation in the quality and quantity of root exudates between plant species and genotypes described above also exists between plants of differing ages and stages of development (Rovira 1959; Alexander 1977 p. 428). Vancura and Hanzlikova (1972) found that general exudate components (sugars, amino acids, organic acids, lipids, enzymes, etc.), and the specific compounds comprising these broad groupings, varied between seed and seedling exudates. Similar differences between seedlings and mature plants have also been observed (Vancura and Hovadik 1965). For example, Smith (1970) reported that carbohydrates in the root exudate of 3-week-old sugar maple (*Acer saccharum* March.) seedlings were more diverse and abundant than those of 55-year-old trees.

Given the dependence of rhizosphere microbes on root exudates for their supply of organic nutrients (Rovira 1969) and the variation in nutritional requirements between bacterial species (Lochhead and Chase 1943; Boyd 1984), it is not surprising that the kinds and numbers of rhizosphere organisms change with plant growth and development (Rivière 1960; Parkinson *et al.* 1963; Burr and Caesar 1984). Consequently, bacterial inoculants isolated from, and adapted to, young seedlings may show increased survival, proliferation, and efficacy when used as PGPR.

This hypothesis is supported by the soil inoculation experiments of Amaranthus and Perry (1987). In their work, Douglas-fir seedlings which were inoculated at planting with soil from a young conifer plantation exhibited dramatic increases in survival (50%) and basal area (200%) compared with controls, while survival decreased slightly when inoculated with soil from a mature forest. Therefore, growth promotion of spruce seedlings in the present study may have been related to the use of bacterial inoculants that were isolated from seedlings less than 5 years of age.

5.2.1.4 Endorhizosphere Bacteria

Endorhizobacteria accounted for five of six of the most effective Salmon Arm SDW promoters in

Experiment 1 (Table 2b and Fig. 2b), and for the two SDW promoters which were most consistent in

Experiment 2 (Fig. 6). This sugests that bacteria originating *within* the root may be more likely to

promote plant growth than bacteria originating *on* the root, and would appear to contradict the

general assumption that the lack of plant growth promotion by PGPR can be attributed to the failure

of inoculum to thrive in the ectorhizosphere (Gaskins *et al.* 1984). However, Reddy and Rahe (1989)

recently reported that growth promotion of onion by *Bacillus subtilus* was not correlated with

inoculum survival in the ectorhizosphere. They observed the greatest stimulation of shoot and root

dry weight due inoculation during the final week of their study when the population of the marked

inoculant was lowest (95 cfu's/plant) and suggested that growth promotion may have resulted from

PGPR-related manipulation of the indigenous rhizosphere microflora (i.e. DRB and/or pathogens

were suppressed). Holl and Chanway (unpublished data) were also unable to correlate growth

stimulation of pine after inoculation with *Bacillus polymyxa* and rhizosphere colonization by the

inoculant when measured at the same time, but colonization four weeks after inoculation was

correlated with the seedling growth response eight weeks after inoculation.

Alternatively, some species of bacteria may promote plant growth from within the endorhizosphere;

such a strategy may help to explain the apparent incongruencies regarding plant growth promotion

and PGPR colonization of the ectorhizosphere. For example, members of the genus *Azospirillum* are

also frequently cited as being PGPR (Baldani and Dobereiner 1980; Patriquin *et al.* 1983; Umalia-

Garcia *et al.* 1980), and may be more abundant within cereal roots than in the ectorhizosphere

(Baldani and Dobereiner 1980). In addition to Azospirilla, plant growth promoting Bacilli have also

been found within the root (Larson and Neal 1978), and their populations have been demonstrated to

exceed those in the ectorhizosphere (Lalande *et al.* 1989).

Electron microscopy has revealed that endorhizosphere colonization occurs between live root cortical

cells and within dead cortical cells (Umalia-Garcia *et al.* 1980; Bashan and Levanony 1988). Due to

root respiration, oxygen tension within root tissues is lower than ambient (personal communication, Dr. H. Weger, University of Regina). This could facilitate the growth of bacteria which are micro-aerophilic (i.e. *Arthrobacter* and *Azospirillum*) or facultatively anaerobic (i.e. *Bacillus*, *Klebsiella*, *Serratia* and *Staphlococcus*) and reduce the number of aerobic bacteria within the root. Endorhizosphere localization of bacteria may also facilitate an efficient exchange of materials between the two organisms (Sumner 1990).

In addition, it has been suggested that colonization of the endorhizosphere would be conducive to bacterial nitrogen fixation because nitrogenase, the enzyme responsible for nitrogen fixation, is oxygen-labile (Sumner 1990). For example, Pohlman and McColl (1982) found that inoculation of barley with an unidentified nitrogen-fixing bacterium isolated from the rhizoplane of the same crop enhanced nitrogenase activity of excised roots 10-fold. This activity persisted despite washing and sterilizing the roots, suggesting that the diazotroph had colonized the root interior.

Yield increases of cereal crops due to inoculation with rhizobacteria isolated from surface-sterilized roots have been observed by several authors (Lalande *et al.* 1989; Lethbridge and Davidson 1983; O'Hara *et al.* 1981). Baldani *et al.* (1983) provided convincing evidence for the involvement of endorhizosphere colonization by PGPR using growth promoting *Azospirillum* strains isolated from surface sterilized roots of wheat. The number of *Azospirillum* cells detected within wheat roots was strongly correlated (r = 0.92) with total nitrogen accumulation, but no relationship was observed between the number of ectorhizosphere *Azospirillum* cells and total nitrogen accumulation.

Staphlococcus hominis was the most effective and consistent growth promoter in Experiment 2, and has not previously been reported as a PGPR. Its absence from the literature may be due to the infrequency with which endorhizosphere and conifer PGPR are characterized. Staphloccoci are closely related to *Bacillus* (Kloos and Jorgensen 1985) and are found occasionally in the soil (Alexander 1977 p. 26). The anaerobic nature of some Staphloccoci strains may facilitate their colonization in the endorhizosphere.

Bacillus polymyxa, the most effective Salmon Arm SDW promoter in Experiment 1, was isolated from the ectorhizosphere, although endorhizosphere-inhabiting Bacilli have also been detected (Larson and Neal 1978). In a rare enumeration study of endo- and ectorhizosphere bacteria, 88% of the strains within the endorhizosphere of maize were identified as Bacilli, some of which significantly promoted maize growth (Lalande *et al.* 1989). Rennie and Larson (1979) also obtained significant growth promotion of wheat with the use of a *Bacillus* isolated from the endorhizosphere of wheat.

Members of the genus *Bacillus*, particularly *Bacillus polymyxa*, have been shown to promote a variety of yield parameters of several crop and forest species, including white clover, crested wheatgrass (Holl *et al.* 1988), maize (Lalande *et al.* 1989), sorghum (Broadbent *et al.* 1977), potato (Burr *et al.* 1978), onion (*Allium fistulosum* L.) (Reddy and Rahe 1989), spring wheat (Chanway *et al.* 1988b), lodgepole pine and Douglas-fir (Chanway *et al.* 1991a). While no attempt was made in these experiments to specifically isolate endorhizobacteria, cutting the roots into segments, sometimes as short as 5 mm, may have resulted in their exudation from the root interior into the root wash media during bacterial isolation.

Of the six most effective seedling growth promoting strains selected from Experiment 1, two were identified as *Pseudomonas putida* and two were *Hydrogenophaga pseudoflava*, formerly *Pseudomonas pseudoflava* (Willems *et al.* 1989) (Tables 2a and 2b). The ability of pseudomonads to enhance plant growth has been widely attributed to their effectiveness in ectorhizosphere colonization (Burr and Caesar 1984). However, they also appear to be aggressive endorhizosphere colonizers, as members of this genus were the second most abundant within the maize roots examined by Lalande *et al.* (1989), and Lynch (1980) encountered pseudomonads in the intercellular spaces of maize root cortex. Furthermore, the most abundant group of bacteria isolated by Gardner *et al.* (1982) from the xylem of rough lemon roots were pseudomonads, including *Pseudomonas putida*. Numerous other genera, including *Bacillus*, were also isolated from the xylem. The prevalence of these two genera in the endorhizosphere, and their incidence as PGPR in this and

other studies, suggests that the ability to colonize the endorhizosphere may also be related to the ability to stimulate plant growth.

5.2.1.5 Abundance of the Inoculant on the Host Plant

Certain rhizobacteria, particularly those that contribute to the fitness of their host, may have a selective advantage in the rhizosphere over those which do not benefit the plant, and with time, may comprise a significant component of the rhizobacterial population (Chanway *et al.* 1991b). If plant growth promoting ability of bacteria is related to rhizosphere colonization ability (Suslow and Schroth 1982; Bashan 1986), and if the most abundant strains of bacteria in the rhizosphere are the most successful colonizers, then selection of the most abundant rhizobacteria may have assisted in the acquisition of growth promoting strains in the present experiments.

This proposition, however, has not been critically assessed (Kloepper *et al.* 1989) and supporting evidence is lacking (Chanway *et al.* 1991b). Reddy and Rahe (1989) were unable to relate growth promotion of onion in the field by a *Bacillus* PGPR to survival of the inoculum in the rhizosphere, and recent work with maize PGPR confirmed that the rhizosphere population of growth promoting *Azospirillum* is small relative to the total maize rhizobacterial population (Mubyana 1990). In addition, Lalande *et al.* (1989) obtained the best growth promotion of maize with *Serratia liquefaciens*, a species which represented only 2% of the ectorhizobacteria which were isolated. Correlation of the abilities of bacteria to colonize roots and to promote plant growth may therefore depend on the mechanism by which plant growth is stimulated, and hence, on the particular bacterial strain in question.

5.2.2   Effects of Seed Provenance, Soil Source and Soil Pasteurization

Although the primary purpose of these investigations was to explore the effects of bacterial inoculants and coexistent factor combinations on seedling growth, some inferences regarding the effects of seed provenance, soil source and soil pasteurization on seedling growth can be made from the performance of uninoculated control seedlings (Fig. 7). First, that seedlings from Salmon Arm

attained a SDW 5% greater than Mackenzie seedlings is not surprising. Much of the genetic variation within forest tree species for growth traits resides between provenances (Zobel and Talbert 1984 p. 62), and in white spruce, provenances, as well as trees within provenances, are significant sources of genetic variation (Khalil 1986).

Second, seedlings in Salmon Arm soil generally grew much better than seedlings in Mackenzie soil (Fig. 7) regardless of pasteurization or seed provenance. This was also predictable because the Salmon Arm soil had a greater cation exchange capacity, organic matter concentration, total nitrogen and available nutrient content (Fig. 1). The only exception to this generalization was calcium, but Salmon Arm seedlings did not indicate calcium deficiency (data not shown) according to Ballard and Carter 1985. Furthermore, seedlings in Salmon Arm soil did not display twisted and deformed leaves, nor dead or dying meristematic tissues characteristic of calcium deficiency (Salisbury and Ross 1985).

Finally, and most interestingly, pasteurization of the Mackenzie soil resulted in a dramatic increase in the SDW of both Mackenzie (57%) and Salmon Arm (49%) seedlings, while pasteurization of Salmon Arm soil inhibited the SDW of seedlings from Mackenzie (3%) and Salmon Arm (25%). This would suggest that the Mackenzie soil harboured a larger number of deleterious soil micro-organisms than the Salmon Arm soil, or that the effects of the deleterious micro-organisms in the Mackenzie soil were more profound than those of the beneficial ones. Although effects of soil pasteurization on seedling growth are usually attributed to changes in populations of soil microbes, changes in the physical properties and the observed changes in the nutrient concentrations of the soils could also have affected seedling growth. In particular, tripling of the manganese concentration in the Salmon Arm soil following pasteurization (Table 1), and potentially toxic levels of manganese (Ballard and Carter 1985) in Salmon Arm seedlings grown in Salmon Arm soil, could have been responsible for the growth inhibition of these seedlings. Wider soil sampling (e.g. more than one sample/site) and assessment of soil bacteria populations in pasteurized and unpasteurized soils could have shed more light on this observation.

5.2.3 Mechanisms of Action

Although the mechanism(s) of action of the inoculants was not investigated in these experiments, some speculation can be made on the basis of the bacterial species involved and the experimental design utilized. Strains M14 and S1 were identified as *Pseudomonas putida*, and strains M18 and M19 as *Hydrogenophaga pseudoflava* (previously *Pseudomonas pseudoflava*) (Tables 2a and 2b). Pseudomonad PGPR activity is often related to their ability to inhibit deleterious rhizobacteria through the production of Fe^{3+}-chelating siderophores and other antibiotics (Powell *et al.* 1982; Kloepper *et al.* 1980).

If this mechanism had operated in these experiments, then growth promotion should have been observed only in the unpasteurized soil treatments, where DRB would have reduced the growth of control seedlings. In pasteurized soil where DRB would have been absent, PGPR inoculation should have had no effect because the growth of control seedlings would not have been reduced. However, in only two of the 16 seed x soil x inoculant treatments involving these four strains was growth promotion greater in unpasteurized than in pasteurized treatments (excluding two treatments in which promotion was negligible) (Fig. 6). Greater growth promotion in unpasteurized versus pasteurized soil was also rare in treatments which received the *Bacillus polymyxa* or the *Staphlococcus hominis* inocula and suggests that antibiosis did not contribute to the observed growth promotion by any of the strains. However, microbes were probably re-introduced into pasteurized media from the atmosphere and tap water. Therefore, this interpretation should be made with caution.

Bacterial solubilization of phosphate was not examined in these experiments, although foliar nutrient concentrations of the 56 seed x soil x pasteurization x bacteria treatments showed little evidence of enhanced phosphorus uptake among inoculated seedlings relative to uninoculated controls (data not shown). The largest increase in foliar phosphorous content was due to bacterial inoculation with strain M14, but was only 5.6% greater than controls.

The SDW of seedlings inoculated with N-fixing and non-N-fixing strains was contrasted and results indicated that the diazotrophic inoculants as a group were not more effective in promoting shoot dry weight gain than non-diazotrophs (data not shown). When examined individually, the six Salmon Arm diazotrophs in Experiment 1 ranked among the poorest growth promoters. However, in the nitrogen depauperite Mackenzie soil, the three Mackenzie diazotrophs tested in Experiment 1 ranked as the second and third most effective growth promoters. Low levels of available nitrogen were cited by Brown (1982) as a requirement for asymbiotic bacterial nitrogen fixation to contribute significantly to the nitrogen capital of ecosystems. Consequently, the poor nitrogen status of the Mackenzie soil may have facilitated nitrogen fixation by these two strains. However, the contribution of N-fixation by PGPR to plant growth is questionable, and most recent estimates of the quantity of nitrogen fixed in the rhizosphere by PGPR are too low to account for the observed plant growth promotion (Kapulnik *et al.* 1985; Sumner 1990). The failure of nitrogen fixation by associative rhizobacteria to contribute significantly to plant growth was also demonstrated with conifers. Chanway and Holl (1991) determined that asymbiotic nitrogen fixation by a diazotrophic *Bacillus* PGPR inoculated onto lodgepole pine contributed only 4% of the seedling's foliar nitrogen. The significance of bacterial phytohormone production to the observed growth promotion can not be determined from these experiments.

5.2.4   Reproducibility of Effects

As has been previously documented (Kloepper *et al.* 1989), substantial growth response variability between experiments was detected in this work. All putative PGPR selected from Experiment 1, with the exception of strains M18 and S1, inhibited seedling growth in Experiment 2 when similar seed and soil sources were used (Fig. 8). Strains M18 and S1 promoted SDW in both experiments, although promotion in Experiment 2 was less than 5%. Storage of the soil for six months between experiments may have altered the microbial population of the soil, and thereby modified the plant response to bacterial inoculation (in addition to affecting bacteria-seed and bacteria-soil coexistence specificities discussed above).

Adsorption of different strains of *Azospirillum brazilense* to several crops was strongly affected by the growth phase of the inoculum (Kapulnik *et al.* 1985). Consequently, inadequate detail to the precise growth phase of the strains in the two experiments may also have contributed to the different responses to inocula between experiments. Finally, environmental conditions, soil nutrient status and inoculum density can influence the success of bacterial inoculation (Sumner 1990). Therefore, minor differences in inoculation and fertilization, and dissimilar environmental conditions in the greenhouse used in Experiment 1 and in the growth chamber used in Experiment 2, may also have imparted different inoculation responses in the two experiments.

6.0 CONCLUSIONS

Interior spruce PGPR were isolated in these experiments using a relatively cheap and simple 'natural plant enrichment technique'. Attempts to secure PGPR by selecting rhizobacteria possessing *in vitro* attributes presumed to be beneficial to plant growth are expensive and time-consuming, and may not be more effective than the methods used in the present experiments (e.g. Kloepper *et al* 1988).

The isolation of a plant growth promoting strain of *Staphlococcus hominis* is the first report of a PGPR within this genus. The infrequency with which members of this genus are found in the soil may support the use of a 'natural plant enrichment technique', and may signify the importance of 'minor' bacterial species in bacterial plant growth promotion. Additionally, the disproportionate success of endorhizosphere bacteria over ectorhizosphere bacteria in promoting plant growth in these experiments suggests that more attention be paid to endorhizosphere-colonizing PGPR.

Evidence for coexistence specificity involving bacteria and seed or bacteria and soil was not detected. Specific responses unrelated to coexistence were observed between these factors. Consequently, methods of isolating PGPR based coexistence between seed or soil and the bacteria may not prove beneficial.

Evolved specificities between organisms have been detected in the relatively simple environment of non-soil media by others. However, the detection of significant coexistent specificity between seed and soil amidst the numerous interactions of biotic and abiotic soil elements, attests to the importance this phenomenon may have in nature. Seed-soil coexistence specificity could be an important determinant of plant growth and competitive ability, and, therefore, of plant distribution.

7.0 SUMMARY

1. PGPR for interior spruce can be isolated by using a natural plant enrichment technique.

2. The six most effective PGPR strains were identified as *Pseudomonas putida* (2x), *Hydrogenophaga pseudoflava* (2x), *Bacillus polymyxa*, and *Staphlococcus hominis*.

3. PGPR activity depended on specific seed, soil, and pasteurization treatments.

4. The growth response to PGPR inoculation varied between the two experiments.

5. A disproportionately larger number of PGPR strains were isolated from the endorhizosphere than from the ectorhizosphere.

6. Seedling growth was significantly greater when coexistent seed, soil and bacteria were used, compared with seedlings associated with non-coexistent seed, soil and bacteria.

7. Most of the growth advantage when coexistent seed, soil and bacteria were present could be attributed to the seed x soil specificity.

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Appendix 1. Numeric designations of the fifty-six factorial treatment combinations comprised of two seed provenances, two soil sources, two pasteurization treatments and seven inoculation treatments tested in Experiment 2. M - Mackenzie; S - Salmon Arm; unpast - unpasteurized soil; past - pasteurized soil.

bacterial strain	M seed				S seed			
	M soil		S soil		M soil		S soil	
	unpast	past	unpast	past	unpast	past	unpast	past
M14	1	8	15	22	29	36	43	50
M18	2	9	16	23	30	37	44	51
M19	3	10	17	24	31	38	45	52
control	4	11	18	25	32	39	46	53
S20	5	12	19	26	33	40	47	54
S10	6	13	20	27	34	41	48	55
S1	7	14	21	28	35	42	49	56

Appendix 2. Analysis of variance of germination capacity (GC) and
germination value (GV) for Experiment 2. SEED - seed provenance;
SOIL - soil source; PAST - pasteurization treatment; BACT -
putative PGPR strain used.

SOURCE	DF	SUM-OF-SQUARES		PROBABILITY	
		GC	GV	GC	GV
SEED	1	4582	1122	0.014 *	0.000 *
SOIL	1	3589	167	0.029 *	0.005 *
PAST	1	11570	12	0.000 *	0.456
BACT	6	6387	88	0.208	0.651
SEED*SOIL	1	35	6	0.830	0.579
SEED*PAST	1	1155	33	0.216	0.208
SEED*BACT	6	9682	220	0.047 *	0.107
SOIL*PAST	1	197	23	0.609	0.295
SOIL*BACT	6	4702	51	0.399	0.874
PAST*BACT	6	2364	107	0.792	0.535
SEED*SOIL*PAST	1	567	10	0.386	0.498
SEED*SOIL*BACT	6	4311	134	0.457	0.385
SEED*PAST*BACT	6	4363	163	0.449	0.259
SOIL*PAST*BACT	6	3984	132	0.510	0.396
SEED*SOIL*PAST *BACT	6	7392	222	0.135	0.104
ERROR	1066	805232	22446		

Appendix 3. Analysis of variance of growth parameters for Experiment 2. SDW - shoot dry weight; HT - shoot height; DIAM - stem diameter; RSA - root surface area; RDW - root dry weight; R/S - root/shoot dry weight ratio; TOTDW - seedling total dry weight; SEED - seed provenance; SOIL - soil source; PAST - pasteurization treatment; BACT - putative PGPR strain used. * - indicates significant main effect or interaction at p < 0.05.

SOURCE	DF	SUM-OF-SQUARES							PROBABILITY						
		SDW	HT	DIAM	RSA	RDW	R/S	TOTWT	SDW	HT	DIAM	RSA	RDW	R/S	TOTWT
SEED	1	7477	1.7	2.56	4630	24313	4.38	58843	0.000 *	0.829	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
SOIL	1	85750	6567.6	4.01	25625	140870	8.15	443184	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
PAST	1	4100	971.4	0.49	11963	30646	7.53	55414	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *	0.000 *
BACT	6	4836	432.1	0.25	359	2641	0.07	14541	0.022 *	0.070	0.024 *	0.275	0.073	0.988	0.020 *
SEED*SOIL	1	7485	1744.3	0.02	448	7846	0.03	30731	0.000 *	0.000 *	0.246	0.002 *	0.000 *	0.532	0.000 *
SEED*PAST	1	114	40.4	0.00	43	6	0.03	95	0.554	0.296	0.605	0.340	0.868	0.528	0.753
SEED*BACT	6	2972	522.7	0.17	777	1967	0.63	8637	0.168	0.029 *	0.126	0.013 *	0.197	0.243	0.178
SOIL*PAST	1	8012	193.5	0.07	2212	1934	5.44	1886	0.000 *	0.022 *	0.040 *	0.000 *	0.004 *	0.000 *	0.163
SOIL*BACT	6	872	263.0	0.03	253	1895	0.57	4488	0.848	0.311	0.921	0.503	0.218	0.307	0.590
PAST*BACT	6	2545	269.2	0.09	294	2105	0.34	8697	0.254	0.296	0.476	0.403	0.163	0.640	0.174
SEED*SOIL*PAST	1	2607	285.9	0.12	229	1792	0.03	8617	0.005 *	0.006 *	0.007 *	0.029 *	0.005 *	0.516	0.003 *
SEED*SOIL*BACT	6	2926	620.5	0.11	855	3191	0.35	12469	0.176	0.011 *	0.331	0.007 *	0.031 *	0.617	0.045 *
SEED*PAST*BACT	6	3806	422.5	0.03	495	1425	0.56	9259	0.071	0.077	0.909	0.110	0.397	0.320	0.144
SOIL*PAST*BACT	6	3288	307.4	0.12	254	3318	0.24	12888	0.122	0.217	0.285	0.502	0.025 *	0.807	0.039 *
SEED*SOIL*PAST*BACT	6	5229	620.6	0.29	713	2966	0.29	15154	0.014 *	0.010 *	0.008 *	0.021 *	0.044 *	0.725	0.016 *
ERROR	#	330764	37812.9	17.39	48299	231112	79.75	975321							
# - error df		1015	1023	1017	1014	1013	1013	1010							

Appendix 4. Effect of inoculation with putative PGPR on shoot height in all seed, soil and pasteurization treatments of Experiment 2. * - indicates treatments which differ significantly from uninoculated control at p < 0.05.
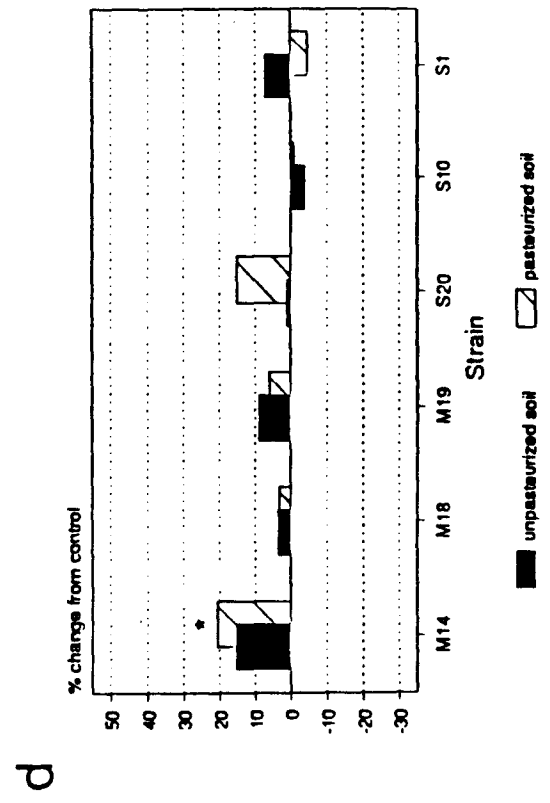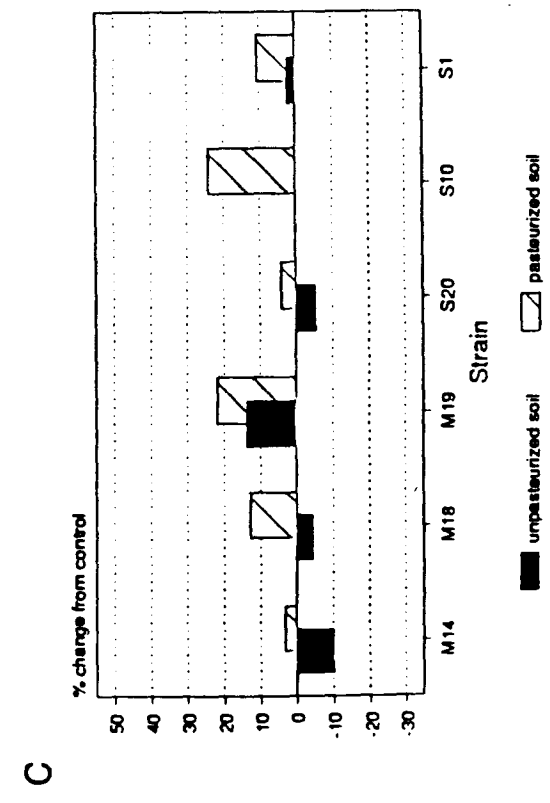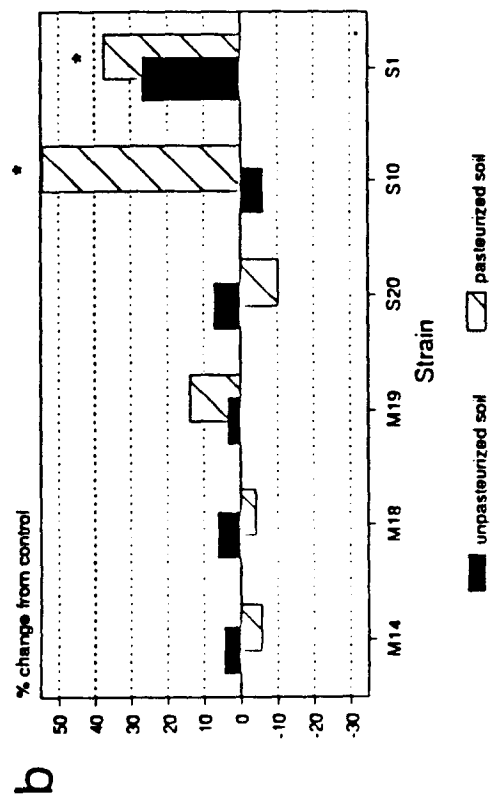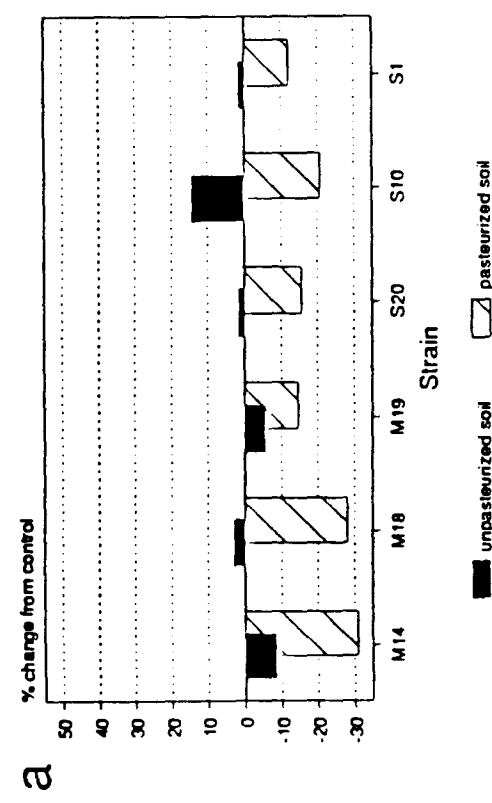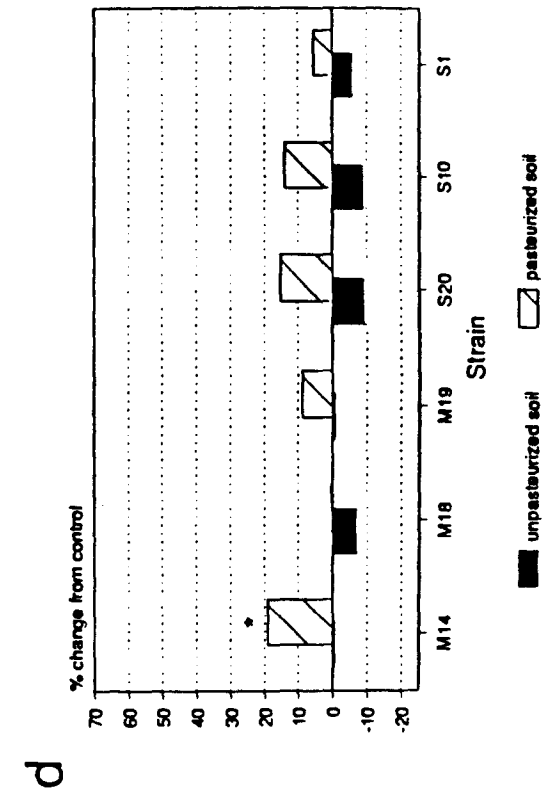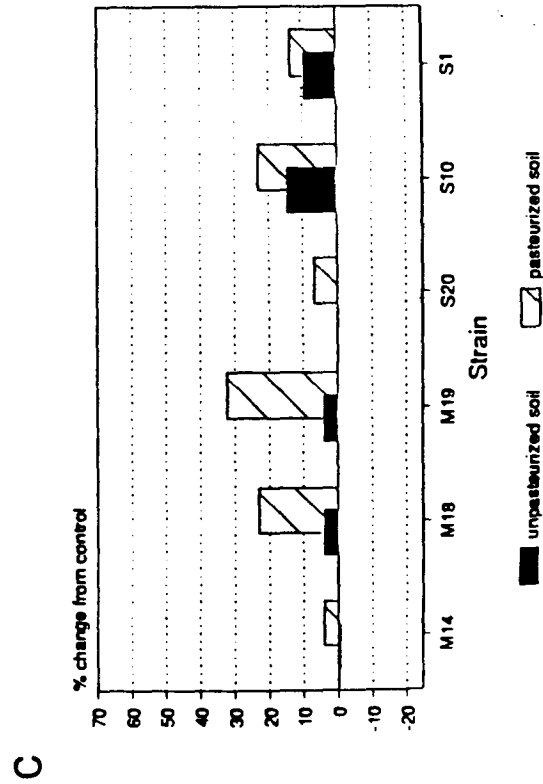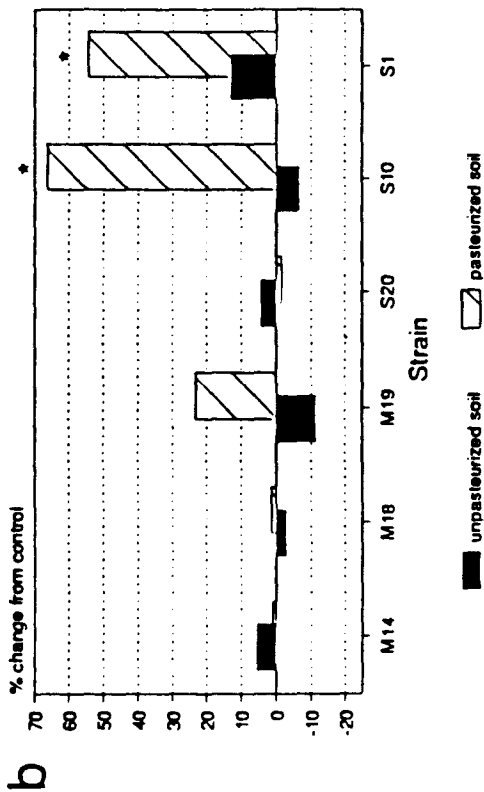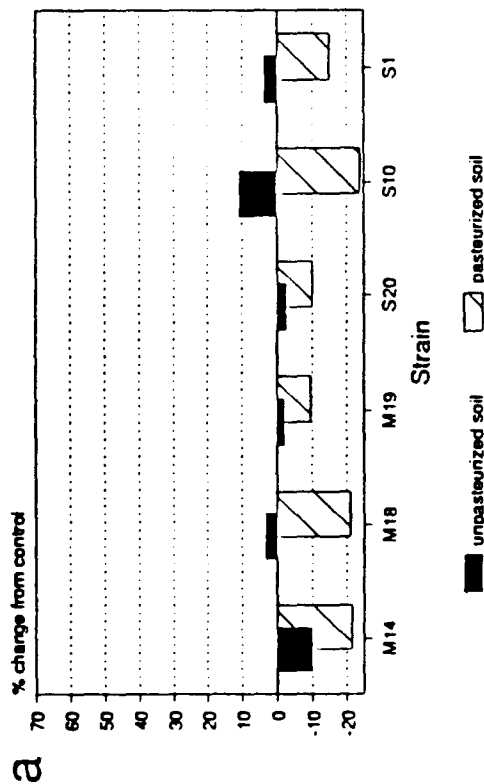
Height

83
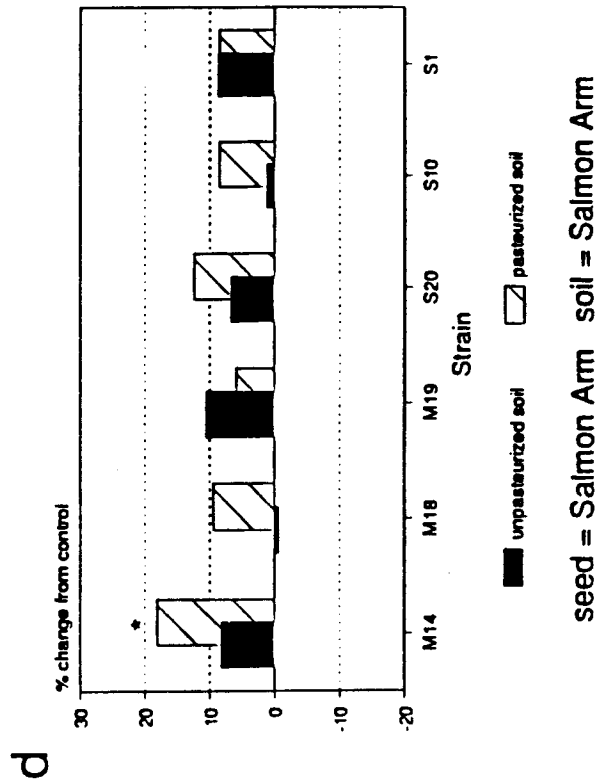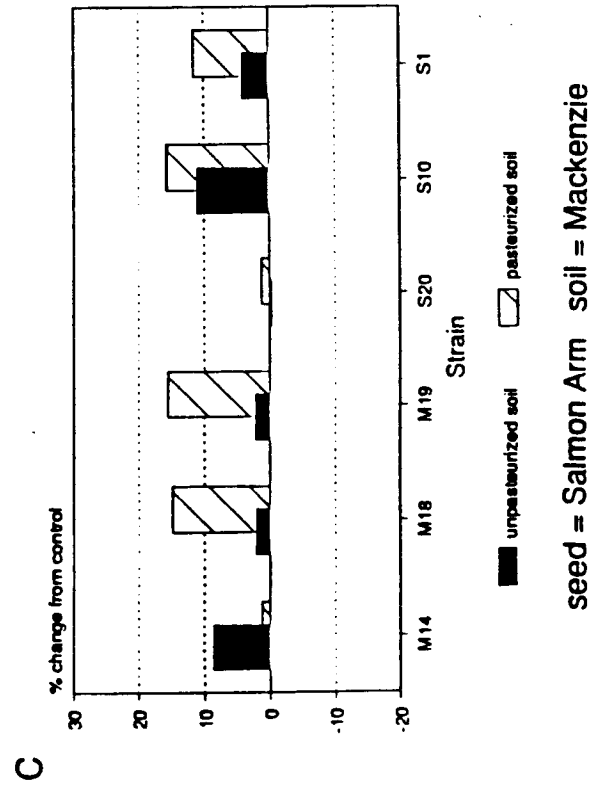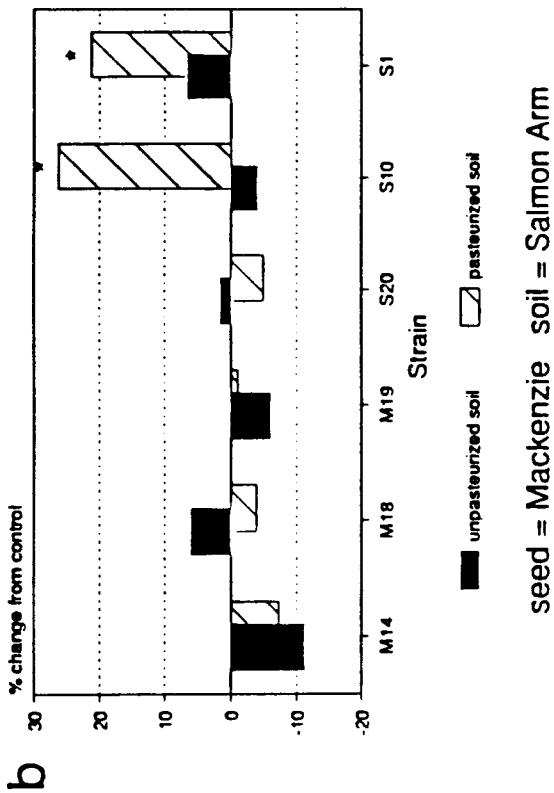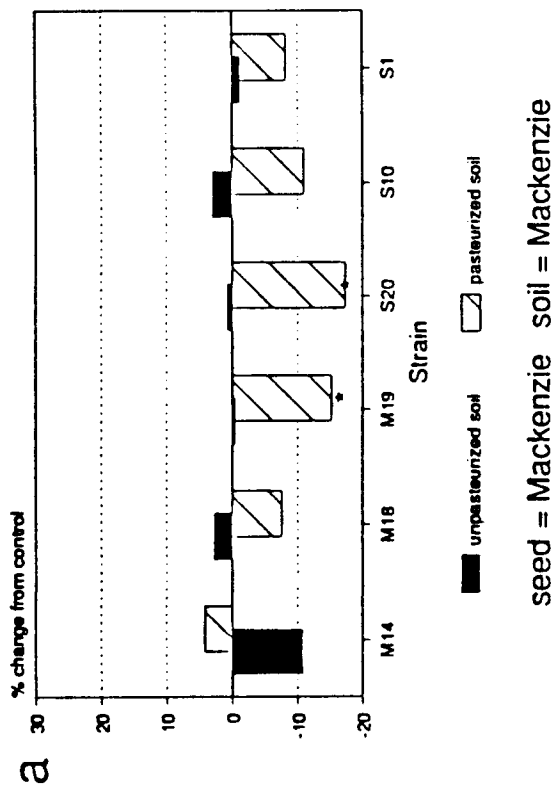
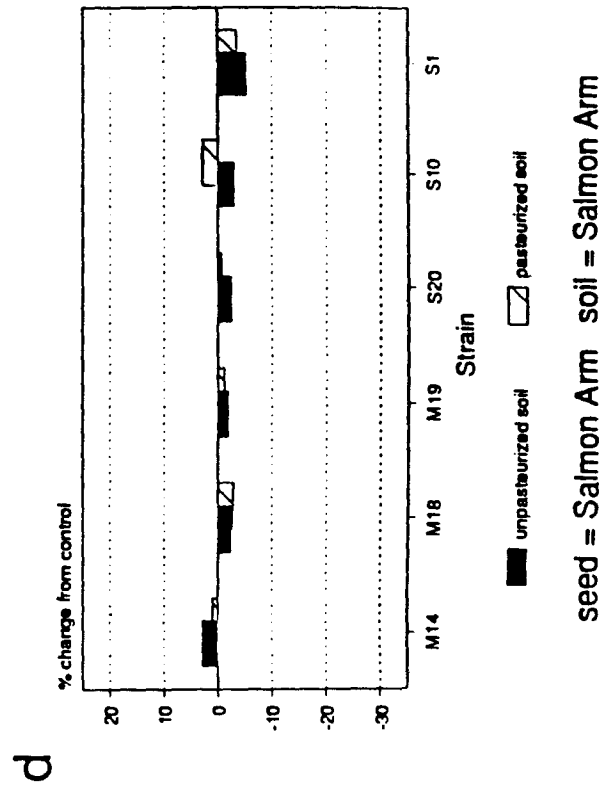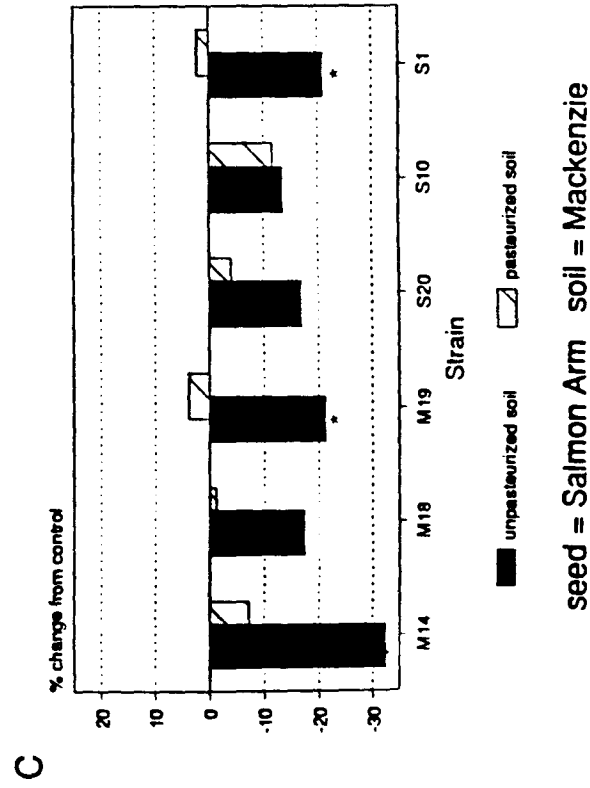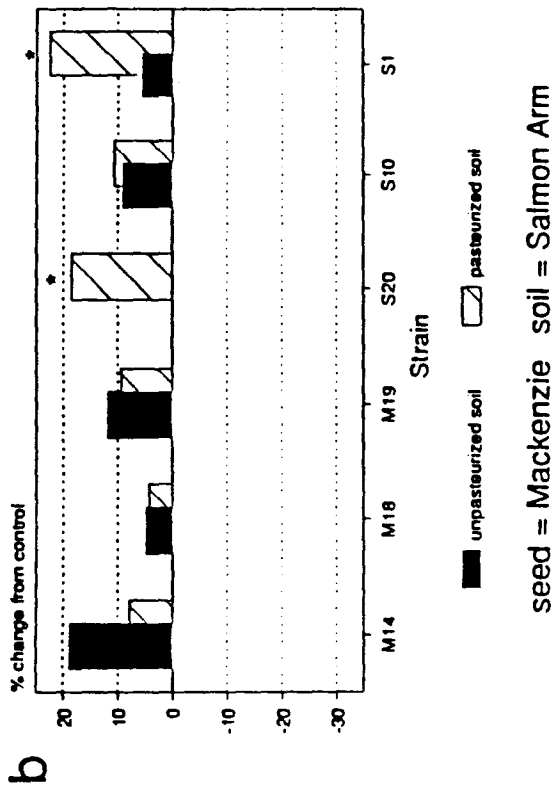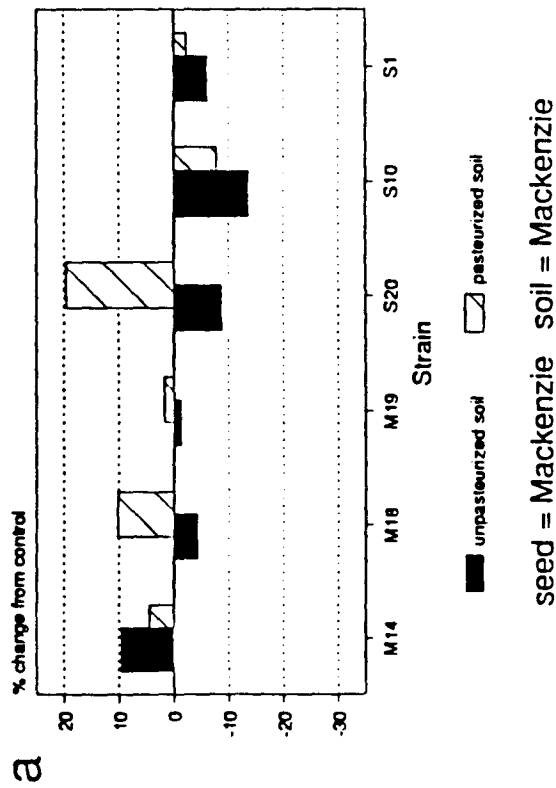Appendix 5. Effect of inoculation with putative PGPR on stem diameter in all seed, soil and pasteurization treatments of Experiment 2. * - indicates treatments which differ significantly from uninoculated control at p < 0.05.
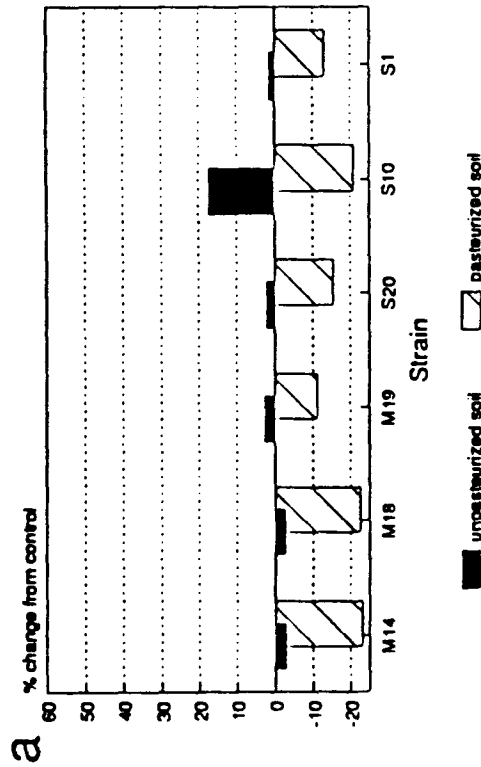
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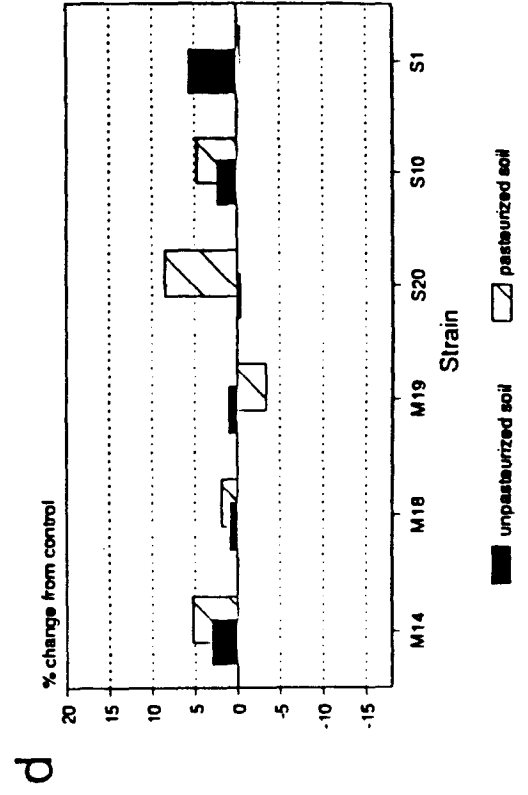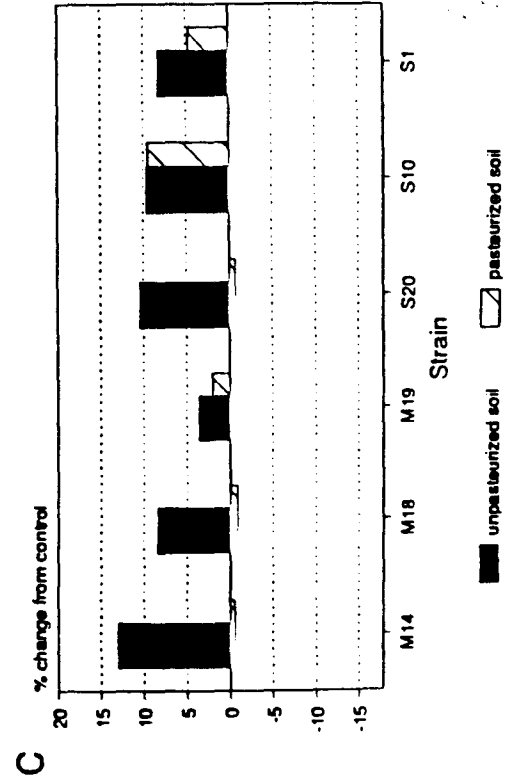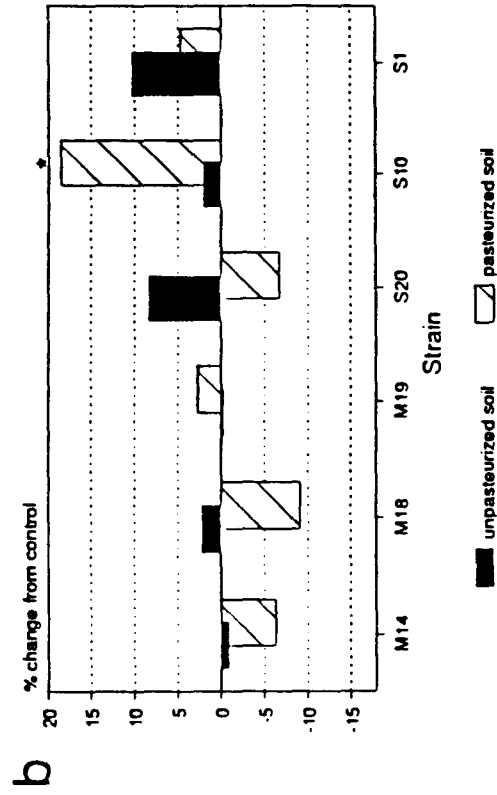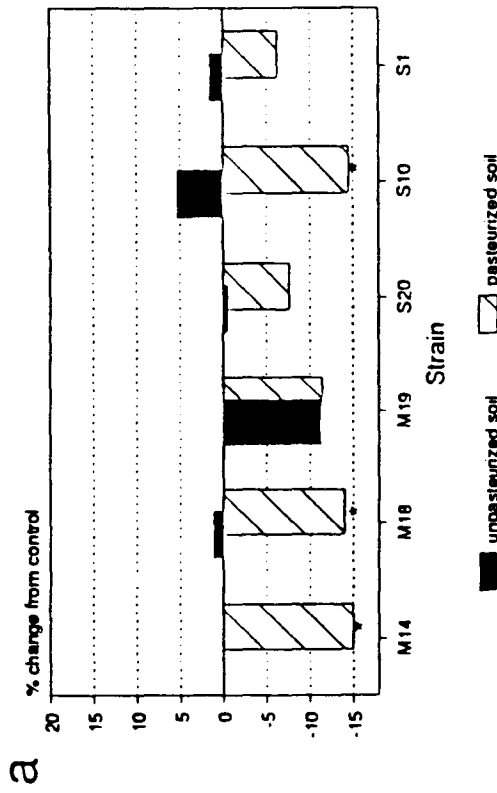
Appendix 6. **Effect of inoculation with putative PGPR on seedling root surface area in all seed, soil and pasteurization treatments of Experiment 2.** * · indicates treatments which differ significantly from uninoculated control at p < 0.05.
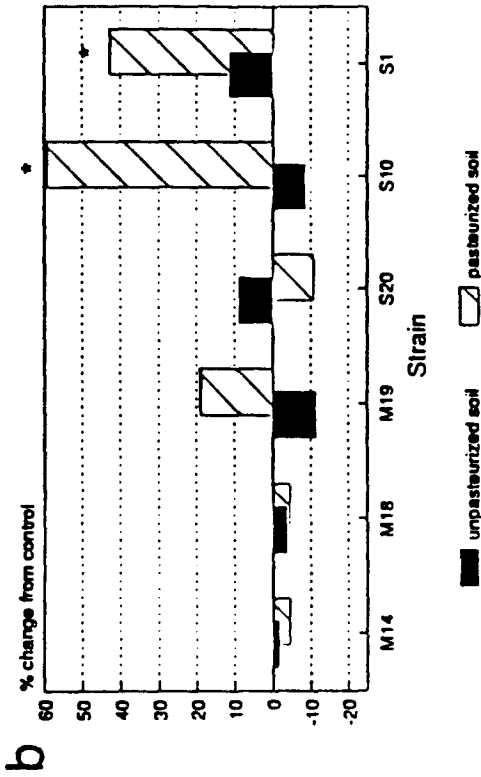
Root Surface Area

Appendix 7. Effect of inoculation with putative PGPR on seedling root dry weight in all seed, soil and pasteurization treatments of Experiment 2. * indicates treatments which differ significantly from uninoculated control at p < 0.05.

Root Dry Weight

Appendix 8. Effect of inoculation with putative PGPR on root/shoot dry weight ratio in all seed, soil and pasteurization treatments of Experiment 2. * - indicates treatments which differ significantly from uninoculated control at p < 0.05.
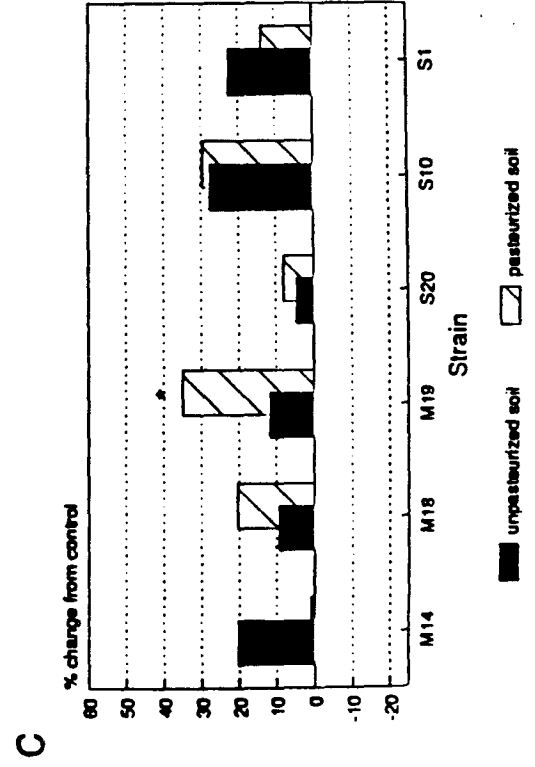
Root/Shoot Dry Weight Ratio

Total Dry Weight

Appendix 9. Effect of inoculation with putative PGPR on seedling total dry weight in all seed, soil and pasteurization treatments of Experiment 2. * indicates treatments w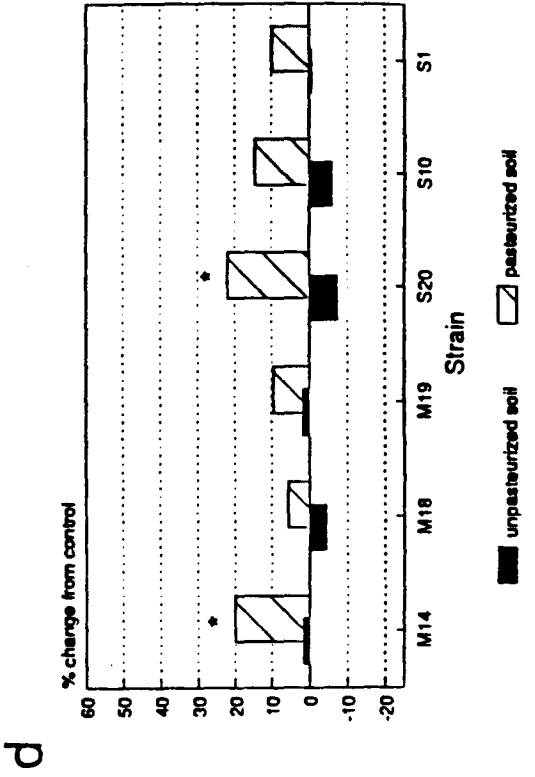hich differ significantly from uninoculated control at p < 0.05.