RESPONSE OF JACK PINE (P. BANKSIANA L.) SEEDLINGS TO INOCULATION WITH PISOLITHUS TINCTORIUS (PERS.) COKER AND COUCH

by

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1.0 INTRODUCTION AND LITERATURE REVIEW

Nursery seedlings generally lack vigorous development of the ectomycorrhizae which improve seedling survival and growth after outplanting (Fortin and Pineau 1971; Marx and Artman 1979; Shoulders 1972). It is well known that high soil fertility (Bjorkmann 1942; Marx, Hatch and Mendicino 1977; Menge et al 1978; Ruehle 1980b; Tetrault et al 1978) and excessive irrigation (Gadgil 1972; Kormanik et al 1977) inhibit the formation of ectomycorrhizae, while broad spectrum soil fumigation can eliminate mycobionts within the immediate area (Beattie 1976; Marx and Barnett 1974; Wright 1957). Fungicides, nematicides, and herbicides, though now shown to be less damaging than previously thought, may reduce symbiont populations significantly (Kelley and South 1977; Marx, Bryan and Cordell 1976; Pawuk et al 1980; Rich and Bird 1974) and/or modify ectomycorrhizae directly (Wilde 1968).

Fumigated nursery soils have been successfully inoculated to produce beneficial ectomycorrhizae (Goss 1960; Jorgensen and Shoulders 1967; Richard 1975; Shoulders 1972; Vozzo and Hacskaylo 1971) which, in turn, have enhanced the growth and survival of the infected seedlings after outplanting. Several means of inoculation are available. These include the introduction of forest soil, forest litter, the roots of forest trees,basidiospores or vegetative inoculum into nursery soils. The first three represent the traditional methods and are supported by Wilde (1968) for their ease of application. They are not, however, generally encouraged because of a lack of uniformity and control over the species being introduced. In contrast, the latter two methods can be very specific with respect to the fungi, and even the isolate, involved. Fortin and Pineau (1971) suggested that this specificity made it theoretically possible to match the tree species and the intended planting site with a certain mycobiont, or combination of mycobionts, in order to obtain the best possible growth. The concept of "tailoring" forest tree seedlings to particular site requirements was later popularized by Marx (1976a).

The use of vegetative inoculum has gained widespread attention largely through the work of the Institute for Mycorrhizal Research and Development, U.S.D.A. Forest Service. Examples of successful programs using a vermiculite carrier include those reported by Berry and Marx (1976, 1978); Marx and Artman (1979); Marx and Bryan (1975) and Marx, Bryan and Cordell (1976). Richard (1975) reports that sterilized wheat grains are also an effective carrier for mycorrhizal hyphae. Zak (1975) opposed the use of vegetative inoculum as time-consuming, labourious and requiring exacting laboratory skills and equipment. Commercial production of the inoculum as projected by Beattie (1976) may remove these restrictions.

The final method, use of basidiospores, incorporates ease of collection and storage with relative simplicity of application and the desired control of quality (Fries 1943; Ruehle and Marx 1977). To date, the results of basidiospore inoculations have been erratic but promising (Marx 1976b; Marx, Mexal and Morris 1979; Marx and Ross 1970; Richard 1975; Ruehle 1980a).

A fungus which exhibits great promise as a symbiont of forest tree seedlings, and has therefore received much attention, is *Pisolithus tinctorius* (Pers.) Coker and Couch. This mycobiont has improved seedling growth on adverse sites (Berry and Marx 1976, 1978; Marx 1976a; Marx and Artman 1979; Schramm 1966), as well as on routine sites (Marx 1977a; Marx, Bryan and Cordell 1977). The preceding examples have dealt almost exclusively with the southern pines, however successes have been reported with oaks and pecans (Marx 1979a; Ruehle 1980b). *Pisolithus tinctorius* is reported to form mycorrhizae under natural conditions with many commercially important North American tree species (Grand 1976), including jack pine (Marx and Bryan 1970), the species under investigation in this study. The distribution of *P. tinctorius* is worldwide (Marx 1977b) and quarantine fears regarding its transport into Canada were dispelled by Malloch and Kuja's (1979) report of sporocarps near Sudbury and Georgian Bay.

Given the success of *P. tinctorius* inoculations in fumigated nursery soil, recent attempts have been made to ascertain its effect on container seedlings (Landis and Gillman 1976; Marx and Barnett 1974; Ruehle and Marx 1977). Container programs are characterized by 1) nearly sterile root substrates, 2) high fertility, 3) generous irrigation and 4) reliance on chemicals such as fungicides (Balmer 1977). Seedlings produced under these conditions tend to have "water roots" (Kormanik et al 1977) which are not suitable for ectomycorrhizal infection; they are, however, susceptible to attack by pathogens and to damage during handling and planting. Molin (1979) states that "most of the seedlings...examined from Northwestern container nurseries routinely lack mycorrhizae."

Despite the drawbacks, container production is expected to account for 20% of the seedlings used in reforestation in North America (Stein 1974). This percentage may well increase due to the recognized advantages of containerization (Balmer 1977). In Ontario, container production supplied 10% of the total seedlings demanded in 1974 (Reese 1974). Of this amount, 70% were jack pine. In Northwestern Ontario, specifically at the Thunder Bay Forest Station, containerized production of conifers is expected to increase in importance (A. Wynia, pers. comm.).

Several successful inoculations of containerized seedlings with mycorrhizal fungi have been reported (Hartigan 1969; Marx et al 1981, in press; Molina 1979; Richard 1975; Ruehle 1980a, b; Ruehle and Marx 1977) and subsequent growth has improved on both good and poor sites. These studies, like those involving nursery soils, have investigated primarily the southern tree species. As part of a nation wide U.S.D.A. experiment to test the effectiveness of P. tinctorius vegetative inoculum on a broader spectrum of host trees, the Forest Pathology Laboratory, School of Forestry, Lakehead University, in cooperation with the Ontario Ministry of Natural Resources, conducted the treatment of containerized jack pine at the Thunder Bay Forest Station (Navratil 1978). Seedlings from this study were used to determine the effect of P. tinctorius ectomycorrhizae on jack pine under greenhouse and Boreal forest conditions. Ectomycorrhizal seedlings, especially those with P. tinctorius associations, have exhibited greater drought resistance than have non-mycorrhizal seedlings (Goss 1960; Meyer 1973; Theodorou and Bowen 1970). Soil moisture stress was therefore included as one of the experimental variables in this study.

In many studies, physical parameters alone have been used to describe the effects of mycorrhizal inoculation. A more thorough understanding of the factors that determine physical differences can be obtained only by investigating other seedling characteristics. This study therefore includes measurements of ectomycorrhizal colonization and nutrient composition with the physical assessment.

An assessment of mycorrhizal colonization may be done visually (Marx, Bryan and Cordell 1977), through the use of a randomizing procedure (Anderson et al 1977) or by estimating enzymatic content (Iyer et al 1978). The variety of fungi involved, and the variability in appearance of induced ectomycorrhizae, make identification of all but the most common symbionts difficult. To satisfy the purposes of this study (identification of P. tinctorius ectomycorrhizae and estimation of percent colonization) the visual method was employed. Since P. tinctorius is known to have high temperature requirements (Marx, Bryan and Davey 1970), there was some question as to its ability to survive winter conditions in the Boreal forest. Isolations were made, according to the technique outlined by Marx and Bryan (1971), to confirm the association of P. tinctorius with seedling feeder roots.

Chemical analysis was undertaken to determine if P. tinctorius inoculation had affected uptake of N, P, or K over the two growing seasons. Many researchers have confirmed that ectomycorrhizae increase absorption of these elements (Bowen 1973; Langlois and Fortin 1978) and that these elements can be stored in the fungal mantle for release at times of nutrient deficiency (Stone 1950; Harley and McCready 1952).

Traditionally, only the relative amounts, or concentrations, of the elements have been reported (Morrison 1974; van Den Driessche 1974). Interpretation of these results can be confused or obscured by dilution effects (Timmer and Stone 1978). Another approach is to express nutrient composition in absolute terms, such as content per leaf or per plant. In our case the seedlings were small and were sectioned into three morphological parts designated as 1)roots, 2)aboveground growth of the first season and 3)aboveground growth of the second season. The content data reported in this paper are therefore in those terms. The seedlings were divided in this way because of the well-known differences in translocation and accumulation exhibited by the various tissues in the plant (van Den Driessche 1974). Neither content nor concentration alone, however, adequately explain the varied aspects of seedling response to the experimental variables. Both, in concert with the appropriate dry weight data, are considered sufficient for the interpretation of results (Morrow 1979).

The study was conducted in two parts: 1) controlled conditions for potted seedlings in a greenhouse at Lakehead University and 2) a field outplanting approximately 200 km northwest of Thunder Bay. It is difficult and unreliable to extrapolate the results of greenhouse trials to field conditions (Kormanik et al 1977; Mead and Pritchett 1971); the outplanting was therefore included to verify or question the validity of any conclusions drawn from the greenhouse study.

2.0 MATERIALS AND METHODS

2.1 Origin of the seedlings

Jack pine seedlings used for this study were raised in conjunction with an experiment organized by the Institute for Mycorrhizal Research and Development (I.M.R.D.), U.S.D.A. Forest Service, Athens, Georgia. The purpose of this original experiment, hereafter referred to as the I.M.R.D. study, was to determine the efficiency of *P. tinctorius* vegetative inoculum for the mycorrhizal colonization of containerized forest tree seedlings (Marx et al 1981). The School of Forestry, Lakehead University, in cooperation with the Thunder Bay Forest Station, Ontario Ministry of Natural Resources, was the only Canadian participant in this experiment. Pertinent details and results of this study are included for clarification purposes.

Five levels of *P. tinctorius* vegetative inoculum and two levels of fertilization comprised the experimental variables at the Thunder Bay Forest Station. The inoculum was of two origins, 1) "Abbott-Pt" commercially produced by the Abbott Pharmaceutical Co. of Chicago and 2) "USDA-Pt" produced by I.M.R.D., Athens, Georgia.

Fertilization refers to the amount of fertilizer (N,P,K) applied to the seedlings during the eight weeks following germination. Two fertilizer regimes were established, 1) the program currently recommended by the Ontario Ministry of Natural Resources, hereafter referred to as Normal initial fertilization or "F", and 2) a program reduced to approximately 50% of the recommended program, hereafter referred to as 1/2 Normal initial fertilization or "1/2 F". Schedules of both fertilizer regimes are presented in Table A.1 of the Appendix.

The experimental seedlings were grown in Spencer-Lemaire book planters in accordance with current cultural practice at the Thunder Bay Forest Station. The root substrate was composed of dried sphagnum, vermiculite, lime and monosuper phosphate in the proportions of 0.4 m^3: 0.2 m^3: 6 kg: 1 kg.

The range of treatments (mycorrhizal inoculation X initial fertilization) is presented in Table 2.1. Each treatment was replicated four times, with the exception of Control which was replicated six times. Each replication was comprised of approximately 100 seedlings. Inoculum levels listed in Table 2.1 designate the ratio of inoculum to root substrate. The arrangement of treatments and replicates in the Thunder Bay Forest Station greenhouse was completely randomized.

Table 2.1: I.M.R.D Study conducted at the Thunder Bay Forest Station

Inoculum Level*	Inoculum Origin	Initial Fertilization Level F	1/2 F
		Number of Seedlings/Treatment	
Control (no inoculum)		600	600
1:30	Abbott-Pt	400	400
1:15	Abbott-Pt	400	400
1:7.5	Abbott-Pt	400	400
1:15	USDA-Pt	400	400

* ratio of inoculum to root substrate

2.2 Analysis of the I.M.R.D. study

After a four month growing period, representative seedlings were shipped to I.M.R.D., Athens, Ga. for analysis. Physical attributes and ectomycorrhizal colonization were assessed. Results of the analysis are presented in Section 3.0.

2.3 Establishment of the Skea Lake Outplanting

A field outplanting, utilizing seedlings from seven of the ten treatments detailed in Table 2.1, was established in August 1978. The seven treatments outplanted were chosen on the basis of the I.M. R.D. analysis described in Section 3.0. The outplanting is located approximately 200 km northwest of Thunder Bay on a Site Class 2 (Plonski 1974) jack pine flat near Skea Lake in the Dog Lake Management Unit of the Ontario Ministry of Natural Resources.

The climate of this area is typically continental with average minimum and maximum temperatures of -35° C and 18° C respectively. The average monthly precipitation is 70 mm (En. Can. 1975a, b).

The area, which parallels a logging access road, is very uniform and was used as a landing as recently as 1977. It was scarified using a V-plough in the spring of 1978. An appraisal of nearby, undisturbed stands indicated that the area is an outwashed sand plain with an accumulation of fine materials in the surficial layer. Analysis of composite samples (Table 2.2) taken from the Ap horizon (a surface mineral horizon markedly disturbed) confirmed that the soil is a Humo-Ferric Podzol (Can. Dept. Agri. 1978). The Ap horizon was yellowish brown in colour, a sandy loam (60% sand, 35% silt, 5% clay) with single grains, loose and non-sticky,30-50 cm deep. The generally high levels of nutrients and Cation Exchange Capacity may be the result of organic material incorporated through machine travel during landing operations or scarification. As previously mentioned, disturbance of the site was severe and, at the time of planting, no vegetation was growing in the scarifier troughs.

In July 1978, the experimental seedlings were hand planted using the slit method in a 7-seedling by 7-seedling grid pattern along the scarifier troughs. Adjacent seedlings within the troughs were approximately 1 metre apart. The outplanting was designed as a complete randomized block with seven treatments, four replications per treatment and 49 trees per replication. Figure 2.1, on the following page, details the layout. Adjacent plots are a minimum of 50 metres apart to prevent accidental spread of P. tinctorius by mycelial growth or soil movement. All seedlings comprising a plot were taken from the same greenhouse container tray (i.e. replication).

Table 2.2: Outplanting site soil characteristics

Total N (%)	0.08
Available P (mg/100g)	1.87
Exchangeable:	
Ca (meq/100g)	1.65
Mg (meq/100g)	0.44
K (meq/100g)	1.14
Organic Matter (%)	6.2
pH	4.5
Cation Exchange Capacity (meq/100g)	9.75

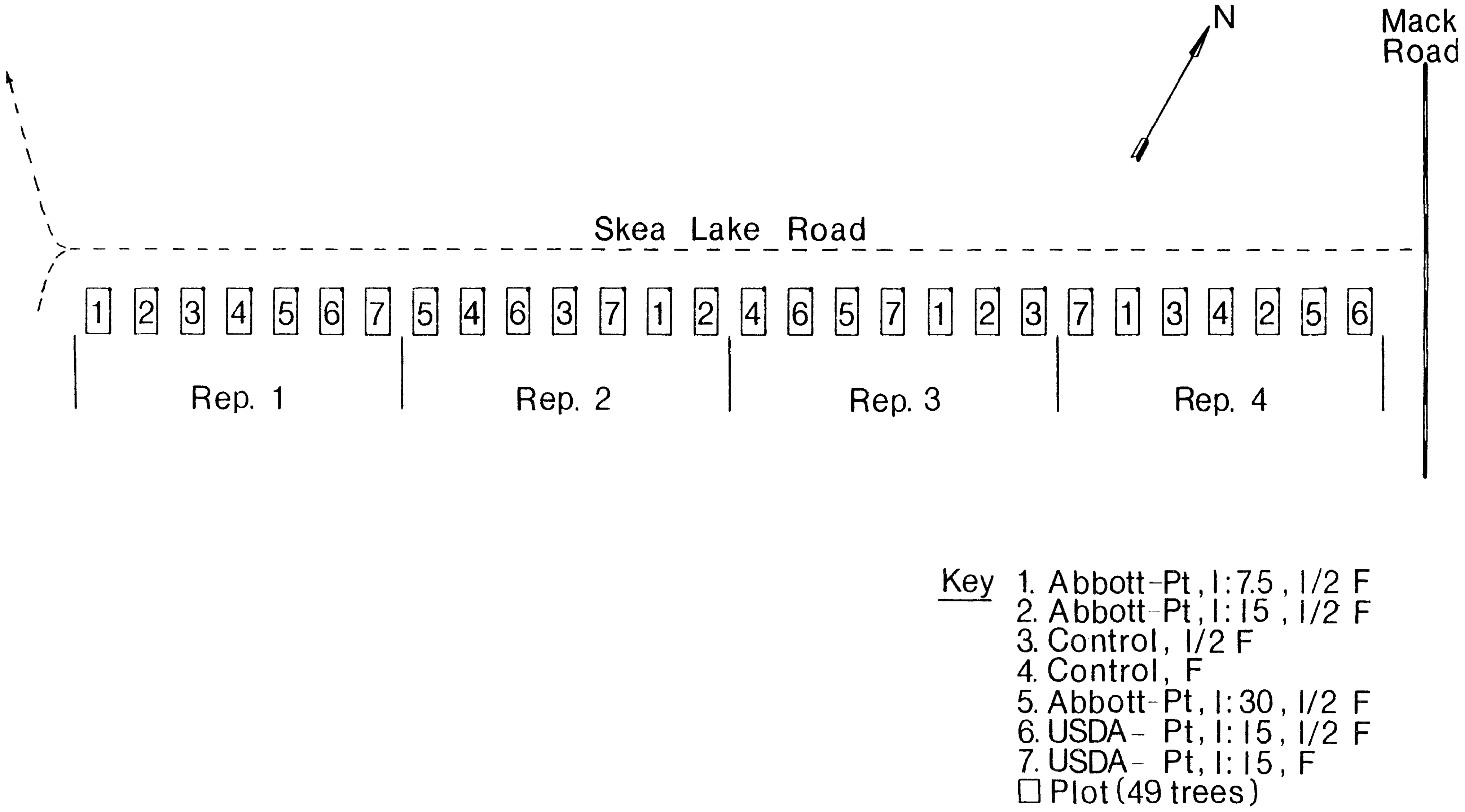


Figure 2.1 : Outplanting at Skea Lake

2.4 Establishment of the Greenhouse study at Lakehead University

The purpose of the Greenhouse study was to determine the effects of soil moisture and initial fertilization on seedlings with extensive *P. tinctorius* ectomycorrhizae. The steps taken to control these three factors (soil moisture, initial fertilization and mycorrhizal colonization) are outlined in the following paragraphs.

Two levels of mycorrhizal colonization were distinguished. "Control" seedlings, seedlings with no visible ectomycorrhizae, were selected from the control treatment groups of the original I.M.R.D. study. "Pt-colonized" seedlings were selected from the "USDA-Pt" treatment groups on the basis of a visual examination of the roots. In order to qualify as "Pt-colonized" each seedling had to have ectomycorrhizae typical of *P. tinctorius* on more than 50% of suitable root surfaces. The level of mycorrhizal colonization was therefore controlled by two factors, 1) the original I.M.R.D. study treatment and 2) a visual examination for the presence of ectomycorrhizae.

The second experimental variable was determined by the two fertilizer regimes established in the original I.M.R.D. study. As described in Section 2.1, the containerized seedlings were fertilized during the first eight weeks following germination at levels designated as Normal and 1/2 Normal initial fertilization. It should be noted that these seedlings were not fertilized again at any point in the study.

Four treatment groups were thereby constituted. These were 1) Control, 1/2 Normal initial fertilization (C-1/2F), 2) Control, Normal initial fertilization (C-F), 3) Pt-colonized, 1/2 Normal initial fertilization (Pt-1/2F) and 4) Pt-colonized, Normal initial fertilization (Pt-F). There were approximately sixty seedlings in each group.

Individual seedlings were planted in 15 cm plastic pots for the duration of the Greenhouse study. The soil used for potting was collected from the Ap horizon of the outplanting site and sieved to remove large pieces (greater then 1 cm^2) of wood and bark. The potted seedlings were hardened off outside from September to the end of December 1978. In January 1979, the seedlings were moved to a cold house in the Lakehead University greenhouse and allowed to thaw gradually over a period of ten days. Until the time of bud break, approximately one week later, the seedlings were watered daily.

Soil moisture,the third experimental variable of the Greenhouse study, was not controlled until after bud break. The seedlings were then subjected to one of two levels of induced moisture stress. Soil in one-half the pots was allowed to reach field capacity, or 1/4 bar tension, before rewatering. Soil in the other half of the pots was allowed to reach a moisture tension of 3-5 bars before each rewatering. The two soil moisture regimes will hereafter be referred to as Non-Stress (NS) and Stress (S) respectively.

Soil moisture was monitored on the basis of predetermined weight loss. Sample pots of soil were soaked with water and subjected to appropriate tensions in a Pressure Plate Extractor (#1600, Soilmoisture Equipment Co.). The resultant difference in soil weight was used as the basis for determining the need for rewatering. Experience showed that Non-Stress seedlings required watering every second day while Stress seedlings were watered, on the average, once per week.

Table 2.3 presents the details of the Greenhouse study, a 2x2x2 factorial design (soil moisture x initial fertilization x mycorrhizal colonization).

Table 2.3: Greenhouse Study conducted at Lakehead University

Soil Moisture	Stress		Non-Stress	
Myc Colonization	C	Pt	C	Pt
	1/2 Normal Fertilization			
Number of Seedlings/ Treatment	28	26	28	25
	Normal Fertilization			
	24	28	25	27

After a four month growing period in the Lakehead University greenhouse, the seedlings were removed from the pots, cleaned of soil and debris and stored as required for subsequent analysis. Four seedlings were selected for mycobiont isolations; twenty of the remaining were set aside for the physical and nutrient assessments. All seedlings destined for physical analysis were subject to two requirements: 1) that they have a single, active leader and 2) that they exhibit no damage due to disease or mechanical injury.

2.5 Selection of Outplanted seedlings for assessment

On July 28, 1979, six seedlings required for the physical, mycorrhizal and nutrient analyses, were selected and lifted from each plot. A table of random numbers, referring to seedling position in the plot, was used for the selection process. In addition, three other criteria were employed: 1) that each selected seedling have a single, active leader, 2) that each selected seedling exhibit no damage due to disease or mechanical injury, and 3) that no two selected seedlings be adjacent in planting position. The third criterium was established to minimize the impact of microsite.

Of the six seedlings removed from each plot (24 from each treatment), five were allocated to the physical and nutrient analyses. The sixth tree from each plot was reserved for the isolation of mycorrhizal fungi.

As noted in Section 2.3, the outplanting consisted of four replicates of seven treatments each. In order to make direct comparisons between the greenhouse and outplanted seedlings, data from only four of the outplanted treatments will be reported. Using the terminology of the original I.M.R.D. study (Table 2.1) these four treatments were 1) Control, 1/2 Normal initial fertilization, 2) Control, Normal initial fertilization, 3) USDA-Pt, 1/2 Normal initial fertilization, and 4) USDA-Pt, Normal initial fertilization. For the purposes of this report, however, these treatments will be referred to as 1) Control, 1/2 Normal initial fertilization (C-1/2F), 2) Control, Normal initial fertilization (C-F), 3) Pt-inoculated, 1/2 Normal initial fertilization (Pt-1/2F), and 4) Pt-inoculated, Normal initial fertilization

(Pt-F).

The distinction between Greenhouse study "Pt-colonized" and Outplanting "Pt-inoculated" seedlings is necessary. In the case of the former, visual assessment confirmed the presence and extent of colonization by P. tinctorius. For the latter, we can only be sure of the origin of the inoculum (USDA-Pt) and the level of inoculation (1:15), though the analysis of the I.M.R.D. study (Table 3.1) reports average levels of P. tinctorius ectomycorrhizae incidence among the Pt-inoculated seedlings.

2.6 Methods of seedling storage

Seedlings from both the Outplanting and Greenhouse studies were stored in one of two ways. Those which were to be used for mycobiont isolation attempts were wrapped in wet paper towelling and refrigerated for no longer than one week. Experience with other tree species (mostly southern pines) has confirmed that this method of storage does not significantly reduce the success of isolation attempts (W. Daniels, I.M.R.D., pers. comm.). Seedlings destined for physical and nutrient analyses were wrapped in wet paper towelling and frozen at -5^{o} C.

2.7 Physical assessment

In order to compare the effects of the experimental variables over two growing seasons, the seedlings were sectioned into morphological parts. Each seedling was severed at the first internode (the end point of first season growth) and at the root collar. This division resulted in each seedling having three distinct sections which were: 1) the roots, 2) the aboveground growth in the first season, and 3) the aboveground growth in the second season. Figure 2.2 illustrates these divisions which were maintained for the nutrient analysis as previously described.

The physical attributes measured were: 1) root dry weight, 2) root volume, 3) root collar diameter, 4) first season dry weight, 5) first season volume, 6) first season height increment, 7) second season dry weight, 8) second season volume, and 9) second season height increment.

Volume of seedling parts was measured by water displacement.

2.8 Mycorrhizal assessment

Visual - At the time of physical assessment each root system was visually inspected for the presence of ectomycorrhizae. The "types" of ectomycorrhizae, based on appearance, and the "relative amount", based on an estimate of the percentage of roots supporting each type, were recorded.

Mycobiont Isolation - Roots of selected seedlings were repeatedly washed; surface sterilized with 100 ppm $HgCl_2$ for two minutes and rinsed in sterile distilled water (Marx 1969). Root fragments, 10 mm long, were plated onto MMN agar. Inoculated Petrie dishes were incubated in darkness at 20^0 C. The plates were assessed every week to a maximum of six weeks.

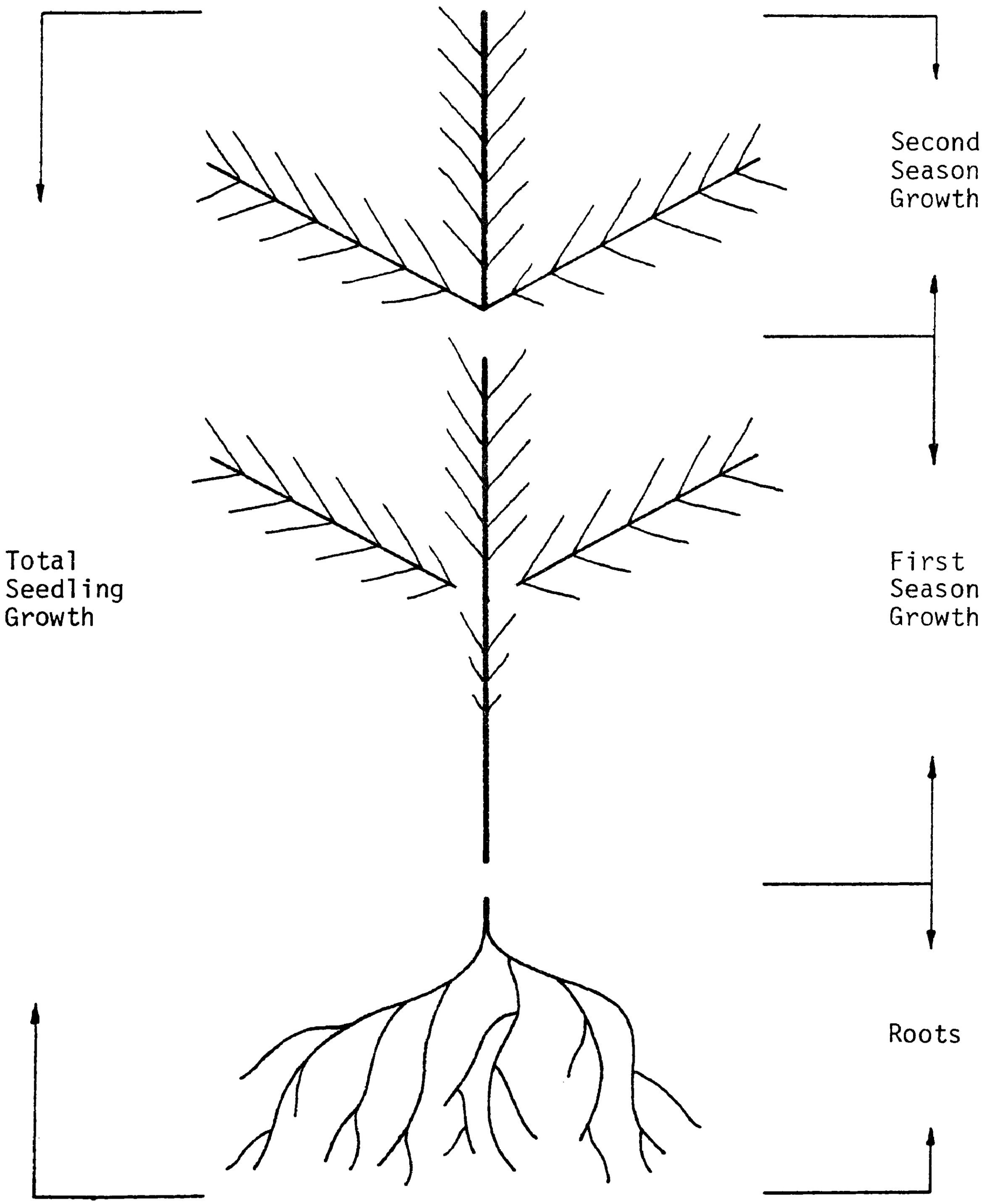


Figure 2.2: Division of the seedling into morphological parts for the physical and nutrient assessments

2.9 Nutrient analysis

Seedling tissues were dried at 20° C for 24 hours so that analysis would be on a standard oven dry basis. Plant material was thoroughly cleaned by hand and with forced air, then it was ground in a Wiley Mill to pass a 1 mm sieve. Due to the small size of many seedlings, it was necessary to group corresponding parts (i.e. roots) from each of four seedlings in order to obtain sufficient sample amounts.

For the analysis of P and K only, plant tissue samples were ashed at 500° C in a muffle furnace and the resultant material dissolved in 1*N* HCl.

Each treatment was replicated five times for the chemical analysis. Each replicate was analysed for the following nutrients by the appropriate method.

1) Nitrogen - by Semi-micro Kjeldahl digestion and distillation (Bremner 1965),
2) Phosphorus - by the Vanadate yellow method (Jackson 1958),
3) Potassium - by a Model 151 Instrumentation Laboratories AA spectrophotometer using 1.25 g tissue dry weight samples. Anion interferences were eliminated by adding Strontium Chloride (MacDonald 1977).

2.10 Soils Analysis

Soils analysis was performed on soil taken from two phases of the experiment.

The first samples were taken from the outplanting site at the time of plot establishment. Soil was collected from the Ap horizon of each plot. The analysis of this composite sample led to the description of the Skea Lake Outplanting site in Section 2.3.

In order to determine what changes may have occurred to the Skea Lake soil used in the Greenhouse study, a second set of samples was taken after the four month growing period. At the time of depotting, soil was collected from around the root system of each seedling. This soil was separated according to the experimental treatment involved; eight composite samples corresponding to the eight treatments were thereby obtained.

Tests 1 and 2, as listed below, were completed by the author in the Forest Soils Laboratory at Lakehead University. Tests 3 to 6 were conducted by the Soils Analysis Laboratory at Glendon Hall, University of Toronto.

1) Particle size - Bouyoucos Hydrometer method (Day 1965),
2) Soil Reaction - soil-water paste method (Doughty 1941),
3) Total Nitrogen - by Semi-micro Kjeldahl digestion and distillation (Bremner 1965),
4) Exchangeable Potassium, Magnesium and Calcium - by extraction with 1N ammonium acetate (pH 7.0) (Jackson 1958). Spectrophotometric determination,
5) Cation Exchange Capacity - leaching with neutral 1N ammonium acetate followed by 0.1N HCl. The ammonium recovered is determined by steam distillation (Bremner 1965),

6) Available Phosphorus - by extraction with 0.03N NH_4F and 0.1N HCl. Photometric determination (Bray and Kurtz 1945).

2.11 Statistical analysis

All physical and nutrient data were subjected to an analysis of variance. Differences between means were determined by Duncan's New Multiple Range test (Steel and Torrie 1960).

Due to the large number of observations for each physical attribute (Greenhouse study - 160 observations, Outplanting - 80 observations), data were pooled. By this method a reasonable number of degrees of freedom for the error was obtained.

For the Greenhouse study, observations from four seedlings were pooled. Five means per treatment were therefore obtained. Data from the Outplanted seedlings were pooled into four groups of five seedlings each to give four means per treatment. In both cases, pooling of data was based on the physical set-up of the experiment.

Nutrient data from the analysis of Outplanted seedlings did not require pooling. The number of treatments in the Greenhouse study, however, made it necessary to reduce the number of observations. This was done by taking the average of two observations. Ten observations per treatment were therefore reduced to five.

Basic computations for the analysis of variance were processed in Watfiv language on an IBM 360 computer. The program used was written by the author.

Concerning the analysis of variance, it should be understood that significant main effects were only considered as such when interactions involving the effect, or effects, were not significant.

3.0 RESULTS OF THE I.M.R.D. STUDY

Table 3.1 presents the results of the assessment, by the Institute for Mycorrhizal Research and Development, of the seedlings raised at the Thunder Bay Forest Station. Seedlings from this experiment were subsequently used in the Greenhouse and Outplanting studies.

The physical attributes of these seedlings can be described in three brief statements:

1) Regardless of mycorrhizal treatment, seedlings which received the higher level of fertilization (Normal) were larger in shoot length, root collar diameter and fresh weight.

2) At either fertilizer level, Control seedlings were smaller than Pt-inoculated seedlings. These differences were more evident among those seedlings raised on the lower (1/2 Normal) fertilizer schedule.

3) Among inoculated seedlings, the largest mean values were exhibited by those which received the "USDA-Pt" (1:15) and the "Abbott-Pt" (1:7.5) treatments. Both inoculum origin and the intensity of application appear to have affected growth of the containerized jack pine seedlings.

Lower fertility reduced seedling size while enhancing development of _P. tinctorius_ ectomycorrhizae. Under both fertilizer regimes, "USDA-Pt" inoculum generated the greatest numbers of _P. tinctorius_ ectomycorrhizae while colonization of "Abbott-Pt" treated seedlings declined with decreasing inoculum concentration.

Table 3.1: Assessment of I.M.R.D. Study seedlings raised at the Thunder Bay Forest Station (assessment carried out by I.M.R.D., Athens, Ga.)

Inoculum Origin Inoculum Level	Control	USDA 1:15	1:7.5	Abbott 1:15	1:30
		Normal Fertilization			
Shoot Length (cm)	11.0	12.6	12.6	12.0	12.4
Root Collar Diameter (mm)	1.4	1.6	1.8	1.5	1.4
Fresh Weight (g)					
Shoot	1.4	1.8	1.6	1.8	1.4
Root	1.1	1.4	1.4	1.2	0.9
% Ectomycorrhizae					
Pisolithus sp.	0.0	7.8	6.7	1.5	0.7
Other sp.	2.2	2.2	3.0	3.0	2.0
Total	2.2	10.0	9.7	4.5	2.7
% Seedlings with *Pisolithus* sp.	0.0	60.0	55.0	30.0	8.0
		1/2 Normal Fertilization			
Shoot Length (cm)	5.8	8.2	8.2	8.2	6.7
Root Collar Diameter (mm)	1.1	1.3	1.4	1.3	1.2
Fresh Weight (g)					
Shoot	0.5	1.0	1.0	0.8	0.7
Root	0.6	1.1	1.2	0.8	0.8
% Ectomycorrhizae					
Pisolithus sp.	0.0	35.6	15.0	7.2	1.1
Other sp.	6.4	2.9	2.9	3.2	3.3
Total	6.4	38.5	17.9	10.4	4.4
% Seedlings with *Pisolithus* sp.	0.0	85.0	73.0	38.0	10.0

The amount of ectomycorrhizae formed by symbionts other than *P. tinctorius* was consistent regardless of inoculum or fertilizer level. This suggests that: 1) natural infections, either from the air or the growing medium, did occur, and 2) the presence, or absence, of *P. tinctorius* did not influence the colonization of seedling roots by other mycobionts.

The treatments which exhibited the greatest variety of response to the experimental variables were chosen for the outplanting at Skea Lake. These treatments were: 1) Control at both fertilizer levels, 2) "USDA-Pt" at both fertilizer levels, and 3) the three "Abbott-Pt" applications raised on the 1/2 Normal fertilizer schedule.

4.0 RESULTS OF THE PHYSICAL ASSESSMENT

As outlined in Section 2.7, the seedlings were divided into three distinct morphological parts. These were: 1) roots, 2) aboveground growth in the first season, and 3) aboveground growth in the second season. Results of the physical assessment of each morphological part will be reported separately.

4.1 Greenhouse study seedlings

Completion of the analysis of variance (Table 4.1) and Duncan's Test (Table 4.2) for all physical attributes identified four general trends in the data. These were:

1) Without exception, the level of initial *P. tinctorius* colonization positively and significantly (p=0.01) affected all measured physical parameters as either a main effect or as part of a 2-way interaction.

2) Initial fertilization and soil moisture were effective primarily as factors in 2-way interactions.

3) Excluding first season data, the mean values of physical attributes of the Pt-colonized (Pt) seedlings raised under either fertilization program (1/2F or F) and subjected to Non-Stress (NS) soil moisture conditions were superior to all other treatment means (Table 4.3).

4) In no instance did the mean value of any physical characteristic of a group of Control (C) seedlings exceed significantly (p=0.05) that of the comparable mycorrhizal (Pt) group.

Table 4.1: Greenhouse Study seedlings - Analysis of Variance for the physical assessment

Physical Parameter	Blocks	SOURCE OF VARIATION Main Effects MYC (M)	 SMC (S)	 FERT (F)	 2-Way Interactions M-S	 M-F	 S-F	 3-Way M-F-S
First Season								
Volume (cm^3)	ns	**		**		*		
Dry Weight (g)	ns	**		ns		ns		
Height Increment (cm)	ns	ns		**		**		
Roots								
Volume	ns	**	**	ns	*	ns	ns	ns
Dry Weight	ns	**	**	*	ns	ns	**	ns
Root Collar Dia. (mm)	ns	**	**	ns	ns	ns	ns	ns
Second Season								
Volume	ns	**	**	ns	ns	ns	**	ns
Dry Weight	ns	**	*	**	ns	**	ns	ns
Height Increment	ns	**	**	**	ns	ns	ns	ns
Total Seedling								
Volume	ns	**	**	*	ns	ns	**	ns
Dry Weight	ns	**	**	**	**	ns	**	ns
Height	ns	**	ns	**	ns	**	**	ns

ns - not significant * - significant at p=0.05 ** - significant at p=0.01

Table 4.2: Greenhouse Study seedlings - Results of Duncan's test for the physical assessment*

Myc Colonization		Control				Pt-colonized			
Fertilization	p=	1/2 F		F		1/2 F		F	
Soil Moisture		S	NS	S	NS	S	NS	S	NS
First Season									
Volume	0.05**	b		a		a		a	
	0.01***	b		a		a		a	
Dry Weight	0.05	b		b		a		a	
	0.01	c		bc		ab		a	
Height Inc.	0.05	c		a		b		a	
	0.01	c		a		b		a	
Roots									
Volume	0.05	d	c	c	c	bc	a	b	a
	0.01	d	cd	cd	bc	bc	a	b	a
Dry Weight	0.05	d	c	c	c	c	a	b	b
	0.01	e	de	d	cd	d	a	bc	ab
Root Col. Dia.	0.05	e	cd	bcd	d	bcd	a	bc	ab
	0.01	c	b	b	b	b	a	ab	ab
Second Season									
Volume	0.05	d	bc	c	c	bc	a	bc	b
	0.01	c	b	b	b	b	a	b	ab
Dry Weight	0.05	d	d	ab	bc	c	ab	ab	a
	0.01	e	de	ab	bc	cd	ab	ab	a
Height Inc.	0.05	cd	d	b	c	b	b	a	b
	0.01	d	d	bc	cd	bc	b	a	b
Total Seedling									
Volume	0.05	d	c	c	c	bc	a	b	a
	0.01	d	c	bc	bc	bc	a	b	a
Dry Weight	0.05	d	c	c	c	c	a	b	a
	0.01	e	d	cd	d	d	a	bc	ab
Height	0.05	d	d	a	b	c	c	a	a
	0.01	d	d	a	b	c	c	a	ab

* -the same letter signifies no significant difference between means

** -differences are significant at p=0.05

***-differences are significant at p=0.01

Table 4.3: Greenhouse Study seedlings - Mean treatment values of the physical parameters

Myc Colonization	Control				Pt-Colonized			
Fertilization	1/2F		F		1/2F		F	
Soil Moisture	S	NS	S	NS	S	NS	S	NS
First Season								
Volume (cm^3)	15.1		28.4		27.1		31.4	
Dry Weight (g)	1.33		1.43		1.71		1.79	
Height Increment (cm)	8.9		18.2		10.8		17.4	
Roots								
Volume	43.2	60.6	63.2	68.2	77.2	121.6	89.6	113.8
Dry Weight	1.31	1.72	1.87	1.95	1.90	3.13	2.47	2.81
RCD (mm) *	35.0	40.6	42.2	39.6	41.4	47.2	43.6	44.4
Second Season								
Volume	28.8	50.8	43.2	43.8	44.4	65.6	49.2	54.2
Dry Weight	0.47	0.63	1.11	0.99	0.83	1.16	1.11	1.25
Height Increment	6.6	5.7	8.8	7.2	8.6	9.2	11.5	9.7
Total Seedling								
Volume	85.4	128.2	136.0	139.2	145.0	218.0	166.8	202.8
Dry Weight	2.84	3.95	4.53	4.37	4.10	6.33	5.26	5.95
Height	15.2	14.8	27.6	24.8	19.0	20.5	28.9	27.0

* Root Collar Diameter

4.1.1 First Season Growth Measurements

Volume - The analysis of variance revealed that the interaction of mycorrhizal colonization and initial fertilization significantly (p=0.01) affected seedling volume after the first growing season (Table 4.1). Figure 4.1 presents the treatment means and results of Duncan's Test. The following differences between treatment means were found:

1) The mean volumes of Pt-colonized and Control seedlings raised on the Normal fertilizer schedule (Pt-F, C-F) were the largest, though they did not differ significantly (p=0.05) from each other.

2) Significant differences occurred between the means of Pt-colonized and Control seedlings which received 1/2 Normal fertilization. The mean of seedlings in the former treatment (Pt-1/2F) was greater (p=0.01) than that of seedlings in the latter (C-1/2F).

3) The mean of Pt-1/2F seedlings was not significantly (p=0.05) less than either of the largest means.

These three observations support other studies (Marx and Barnett 1974; Marx 1976b) which have shown that mycorrhizal effects are enhanced when available nutrients are reduced. In our case, a reduction in the level of initial fertilization (to 1/2 Normal) significantly depressed the volume of Control seedlings while having minimal effect on seedlings mycorrhizal with P. tinctorius.

Dry Weight - According to the analysis of variance, only the main effect of mycorrhizal colonization influenced significantly (p=0.01) the amount of dry weight produced in the first season (Table 4.1). Duncan's Test confirmed that there were no differences due to initial fertilization between treatment means (Figure 4.2).

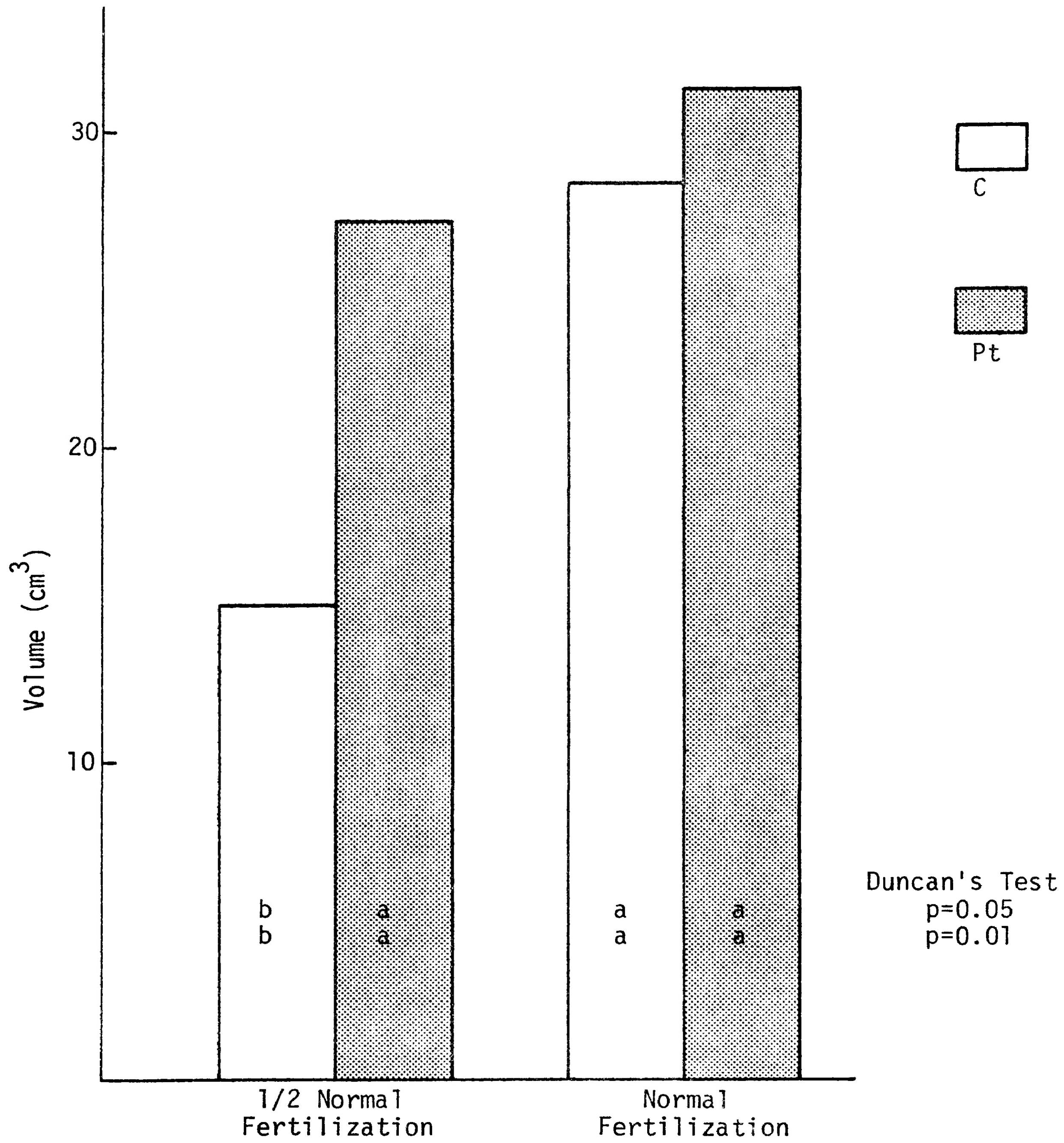


Figure 4.1: Greenhouse Study seedlings - First season volume treatment means

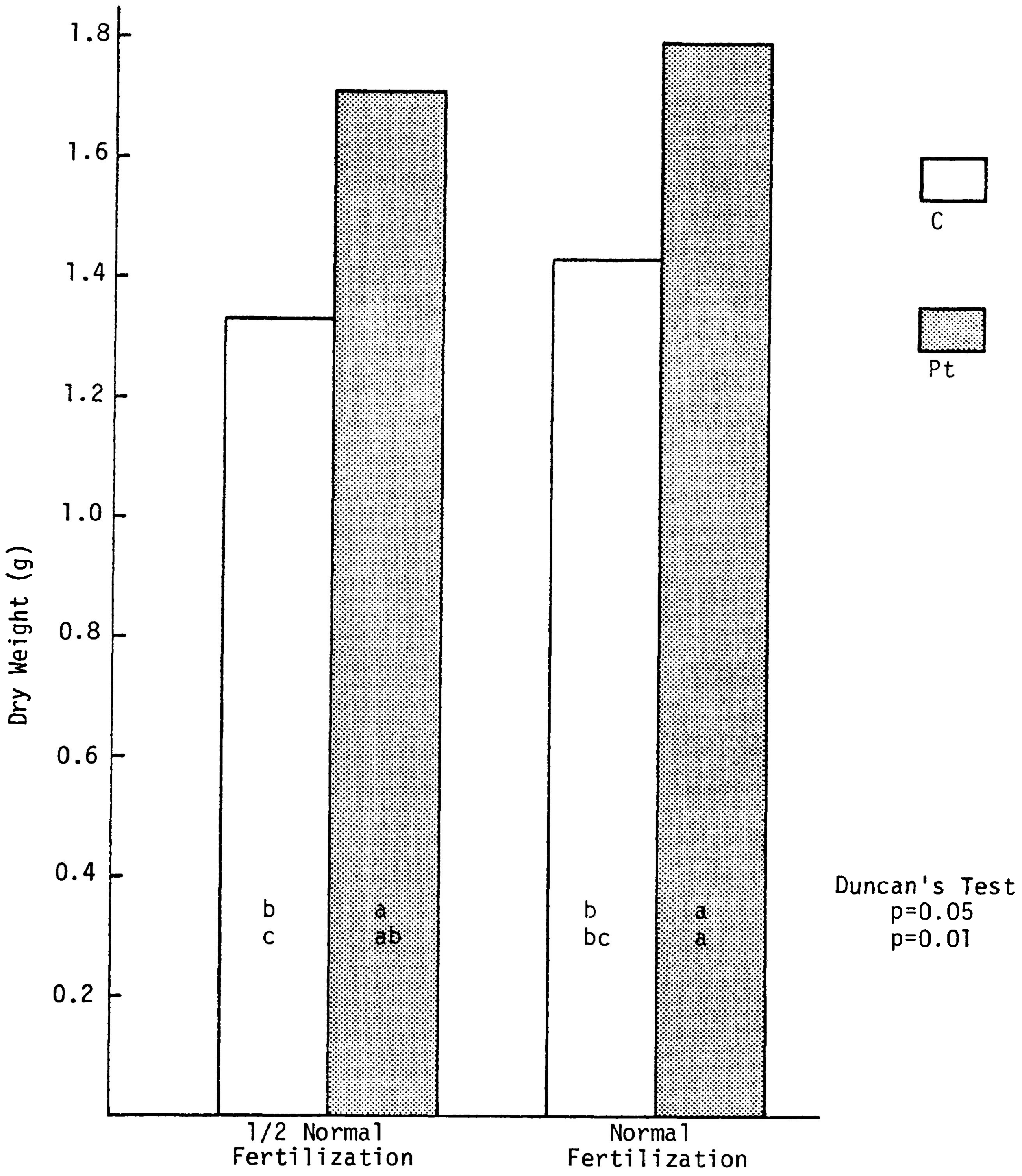


Figure 4.2: Greenhouse Study seedlings - First season dry weight treatment means

Mean dry weights of both mycorrhizal treatment groups (Pt-F, Pt-1/2F) were each significantly larger (p=0.01) than their Control counterparts' (C-F, C-1/2F). Therefore, under similar environmental conditions and fertilizer regimes, the Pt-colonized seedlings produced greater amounts of dry weight than did the non-inoculated (Control) seedlings.

Table 4.4 presents a comparison of the means of the first season volume and dry weight based on ranking (largest to smallest) and the results of Duncan's Test. If the tissues formed by each group of seedlings were essentially the same, one would expect the values for volume and dry weight to parallel one another. This was not the case for the treatments ranked second and third; C-F and Pt-1/2F respectively. While the mean volume of Control, Normally fertilized seedlings was larger than that of Pt-colonized, 1/2 Normally fertilized seedlings; the mean dry weight was significantly less. This discrepancy indicates that the relative density of tissues formed by seedlings in the two groups differed. What relationship, if any, exists between tissue density, nutrient status and future growth will be discussed in Section 7.0.

Height Increment - The analysis of variance revealed that height increment over the first season was significantly (p=0.01) affected by the interaction of initial fertilization and mycorrhizal colonization (Table 4.1). Application of Duncan's Test to the data indicates the results of this interaction; treatment means and results of the test are presented in Figure 4.3.

The means of treatment groups which received Normal initial fertilization (Pt-F, C-F) showed no difference due to mycorrhizal colonization. This is to be expected when nutrients and soil moisture are readily available.

Under 1/2 Normal initial fertilization, however, the beneficial effect of Pt-colonization was evident. As shown in Figure 4.3, the mean height increment of Pt-1/2F seedlings was significantly (p=0.01) greater than that of C-1/2F seedlings.

With height increment, as with volume and dry weight, the presence of P. tinctorius ectomycorrhizae on the roots of containerized jack pine seedlings significantly augmented growth when initial fertilization was reduced.

Table 4.4: Greenhouse Study seedlings-- A comparison of first season volume and dry weight means

Physical Parameter	Rank (largest to smallest) 1	2	3	4
Volume				
1) mean (cm^2)	31.4	28.4	27.1	15.0
2) treatment	Pt-F	C-F	Pt-1/2 F	C-1/2F
3) DMR test*	a**	a	a	b
Dry Weight				
1) mean (g)	1.79	1.71	1.43	1.33
2) treatment	Pt-F	Pt-1/2 F	C-F	C-1/2F
3) DMR test*	a	a	b	b

* differences between means significant at p=0.05

** the same letter signifies no significant difference between the treatment means

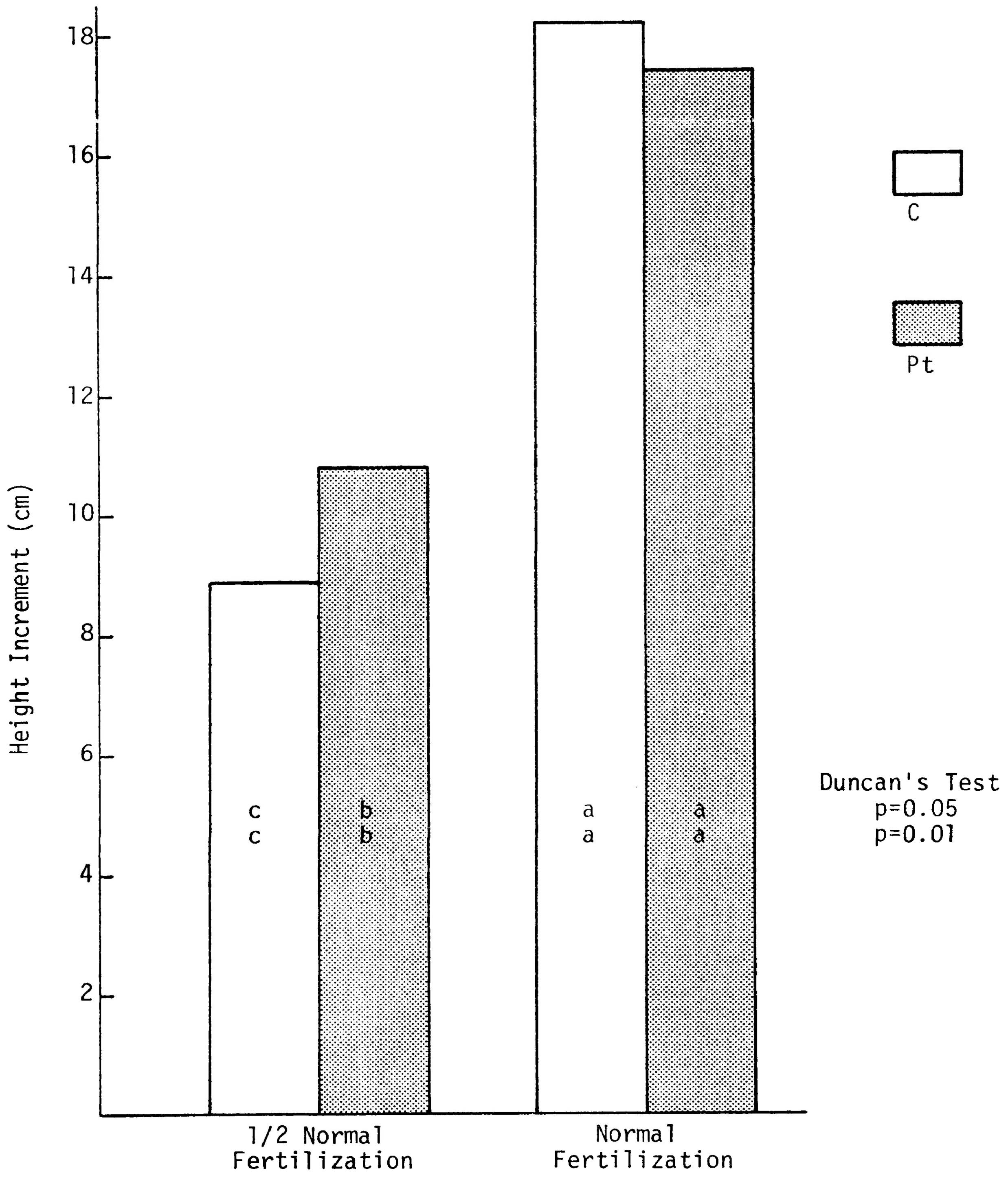


Figure 4.3: Greenhouse Study seedlings - First season height increment treatment means

At this point the following clarification should be repeated. During the first growing season only two experimental variables were controlled in the study. These were: 1) mycorrhizal colonization (Pt or C) and 2) initial fertilization (F or 1/2F). After bud break in the second season a third variable, soil moisture stress, was introduced. Soil in the greenhouse pots was maintained at either a Stress (S) or a Non-Stress (NS) level.

The remaining results of the Greenhouse study will therefore involve three experimental variables.

4.1.2 Root Measurements

Volume - The analysis of variance indicated that variation in root volume was due to the interaction between mycorrhizal colonization and soil moisture (Table 4.1). Initial fertilization did not affect this parameter. The effects of the interaction were clarified by the application of Duncan's Test for differences between treatment means (presented in Figure 4.4).

The following trends, some of which reappear throughout the analysis of the Greenhouse study data, were apparent:

1) Mean volumes of all Pt-colonized treatment groups (Pt-F-S, Pt-F-NS, Pt-1/2F-S, Pt-1/2F-NS) were significantly ($p=0.01$) larger than those of comparable Control groups (C-F-S, C-F-NS, C-1/2F-S, C-1/2F-NS).

2) Among the mycorrhizal seedlings, root volume was strongly influenced by soil moisture. The means of Pt-colonized seedlings subjected to Non-Stress moisture conditions were significantly

(p=0.01) greater than those of similar seedlings growing under Stress conditions. At both Stress and Non-Stress soil moisture levels, the effect of initial fertilization was insignificant.

3) Among the Control seedlings, the lowest mean root volume was attributed to the group raised under the poorest growing conditions: 1/2 Normal fertility and soil moisture Stress. The mean of this treatment group was significantly (p=0.05) less than those of all other groups. Means of the three remaining Control groups (C-F-S, C-F-NS and C-1/2F-NS) did not differ from one another.

Soil moisture Stress, especially in combination with lower initial fertilization, reduced root volume of both Pt-colonized and Control seedlings. A comparison of means from similar treatments, however, confirms the positive effect of P. tinctorius ectomycorrhizae at the time of transplanting. Mean root volumes of mycorrhizal seedlings exceeded those of Controls by a range of 50-100%.

Larger root systems are reported to be advantageous to seedling performance after outplanting (Armson and Sadreika 1974). Without even considering the additional benefits of P. tinctorius colonization, the mycorrhizal stock should surpass the Control stock in growth and survival due to the larger root systems alone. This aspect will be discussed further with respect to Top/Root ratios in Sections 4.1.4 and 4.2.4.

Dry Weight - Variations in dry weight of roots were due to: 1) an interaction between soil moisture and initial fertilization and 2) the main effect of mycorrhizal colonization (Table 4.1).

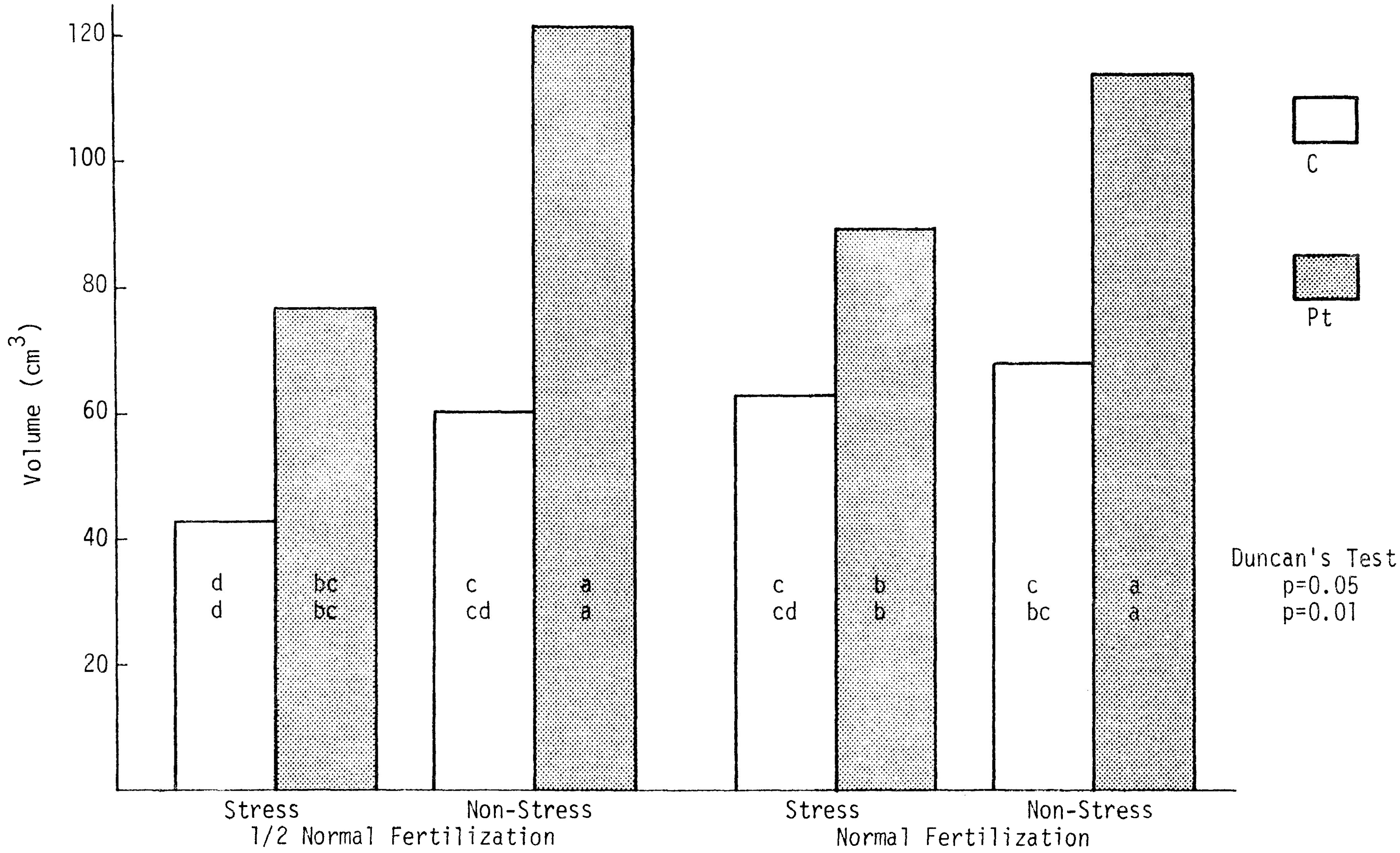


Figure 4.4: Greenhouse Study seedlings - Root volume treatment means

The effects of these two factors are illustrated by the treatments whose means rank first and seventh in order of magnitude (Table 4.3). Both treatments received the same initial fertilization (1/2 Normal) and both were subjected to the same soil moisture conditions (Non-Stress). The mean root dry weight of the Pt-colonized seedlings, however, was 80% greater than that of the Controls.

Use of Duncan's Test allows the following observations concerning root dry weight treatment means to be made; the data are presented in Figure 4.5.

1) Mean root dry weights of all Pt-colonized treatment groups were significantly (p=0.01) greater than those of comparable Controls.

2) Initial fertilization influenced the subsequent effect of soil moisture on root dry weight. This held true for both the mycorrhizal and Control seedlings. Seedlings raised on the Normal initial fertilization program and having the same level of mycorrhizal colonization were not affected by the level of soil moisture (Pt-F-S = Pt-F-NS; C-F-S = C-F-NS). Soil moisture, however, did significantly influence the root dry weight of seedlings raised on the lower fertilizer regime. For both Pt-colonized and Control seedlings, the means of those groups subjected to Stress soil moisture conditions were lower (p=0.01) than those of seedlings grown under Non-Stress conditions.

3) The combination of soil moisture Stress and 1/2 Normal fertilization reduced mean root dry weight of both Pt-colonized and Control seedlings. This reduction, however, was less for the mycorrhizal group than it was for the Controls. Pt-1/2F-S seedlings exhibited

a mean that did not differ significantly from that of Control, Normally fertilized, Non-Stress seedlings. It should be noted that these Control seedlings represent current nursery practice.

4) The dissimilarity detected between volume and dry weight of first season aboveground growth (Table 4.4) did not appear in the root system data.

The results of this portion of the Greenhouse study confirm that inoculation of containerized jack pine seedlings with P. tinctorius increased growth of the root systems. This beneficial effect was enhanced by Stressed soil moisture conditions and lower initial fertilization levels.

Root Collar Diameter - Variations in root collar diameter were due to: 1) the interaction of initial fertilization and soil moisture and 2) the main effect of mycorrhizal colonization (Table 4.1). As discussed above, root dry weight was also affected by the same two factors. The application of Duncan's Test revealed the various aspects of seedling response; most of which were similar to those recorded for both root dry weight and root volume. Root collar diameter data are presented in Figure 4.6.

Mean root collar diameters of Pt-colonized seedlings were larger than those of comparable Controls. The difference between Pt-colonized and Control means for seedlings raised on Normal initial fertilization and subjected to soil moisture Stress (Pt-F-S, C-F-S) was not significant. Differences between mycorrhizal and Control means at all other levels of fertilization and soil moisture content were large enough to be significant at $p=0.01$.

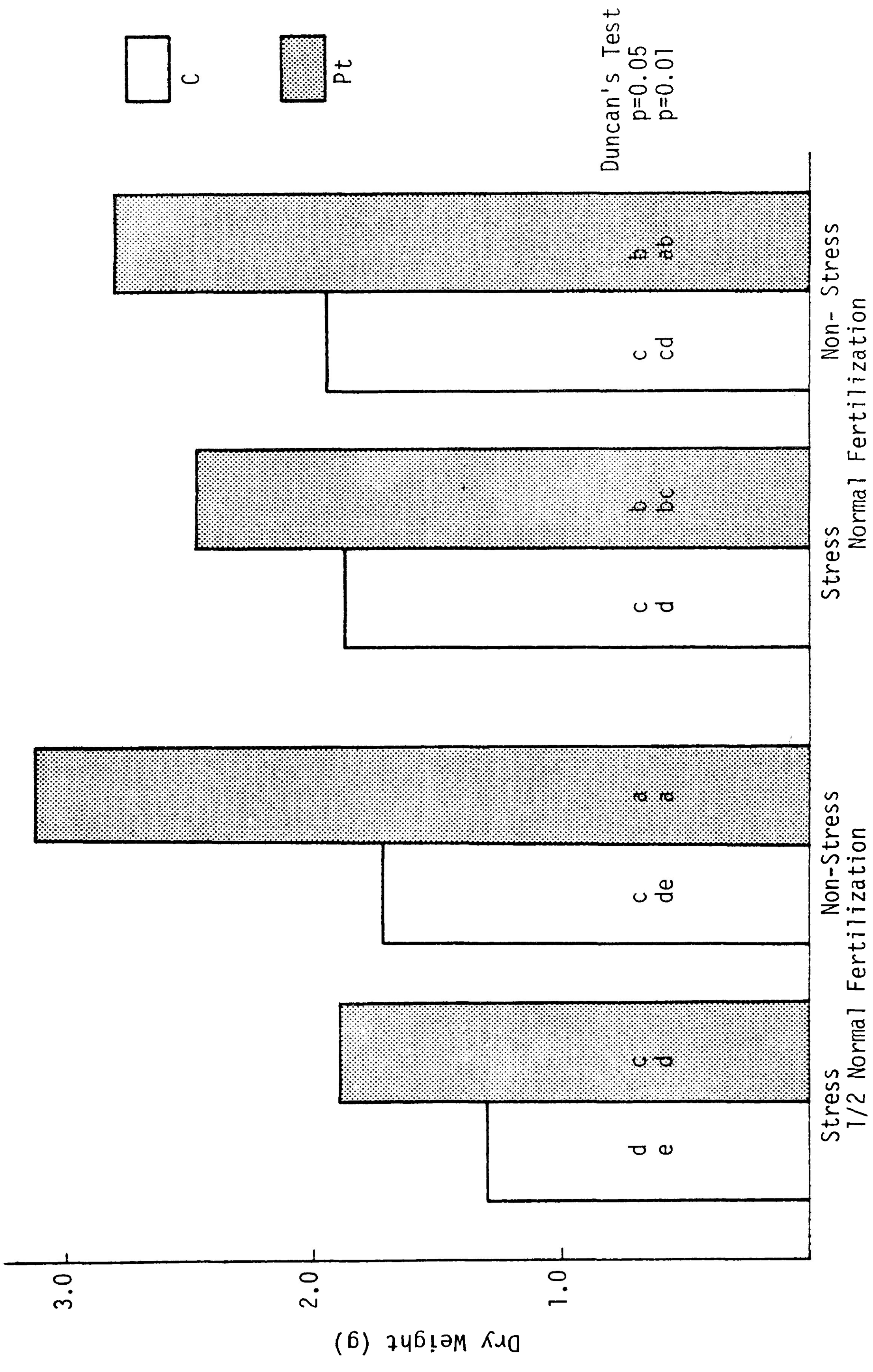


Figure 4.5: Greenhouse Study seedlings - Root dry weight treatment means

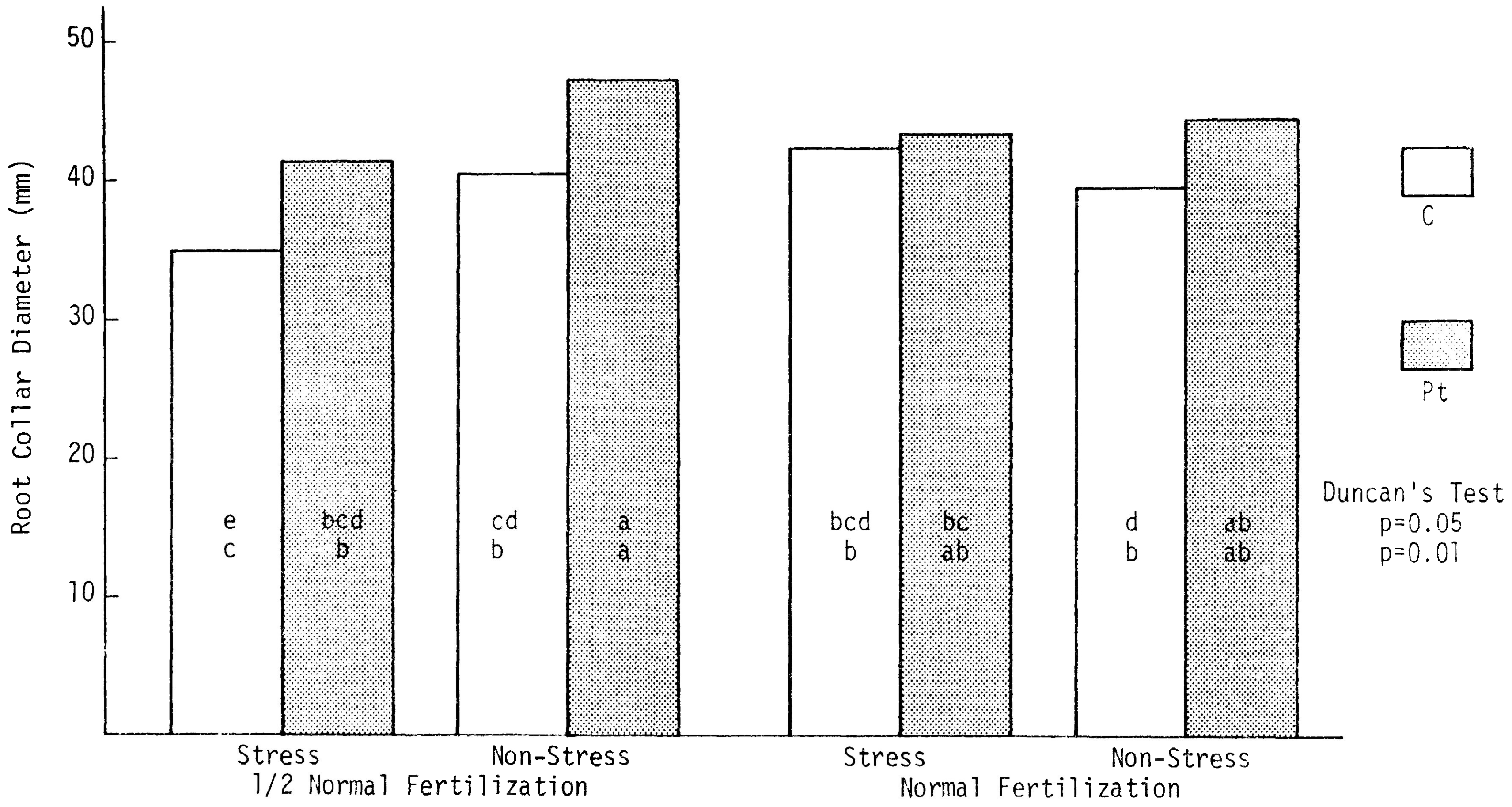


Figure 4.6: Greenhouse Study seedlings - Root collar diameter treatment means

2) Similar to root volume, the level of initial fertilization determined the influence of soil moisture on root collar diameter. No significant ($p=0.01$) differences occurred between the means of comparable seedling groups raised on Normal initial fertilization. The means of seedlings raised on 1/2 Normal fertilization, however, varied widely and significantly ($p=0.01$). Differences between Pt-colonized and Control means at this fertility level exceeded 15%. The largest mean root collar diameter was exhibited by Pt-1/2F-NS seedlings; the smallest, by C-1/2F-S seedlings.

3) Again, similar to the results of root volume and root dry weight analysis, there was no significant difference between the mean root collar diameter of Pt-1/2F-S seedlings and those of the Normally fertilized Control groups.

In summary, the initial inoculation with, and subsequent development of, P. tinctorius ectomycorrhizae on containerized jack pine seedlings resulted in larger root systems as measured by volume, dry weight and root collar diameter.

4.1.3 Second Season Growth Measurements

Strong similarities exist between the second season growth data and that of the seedling root systems. Such similarities are noted in the text. Only those responses which lend new insights are discussed in detail.

Volume - Similar to the analysis of root dry weight and root collar diameter, variations in the volume of second season growth were due to: 1) the interaction of initial fertilization and soil moisture and 2) the main effect of mycorrhizal colonization (Table 4.1). Figure 4.7 presents treatment means for second season volume and the results of Duncan's Test. The following aspects of seedling response to the experimental variables were identified:

1) With the exception of Normally fertilized seedlings subjected to soil moisture Stress, the mean volumes of Pt-colonized seedlings were larger ($p=0.01$) than those of comparable Controls.

2) Pt-colonized seedlings raised on 1/2 Normal fertilization and grown under Non-Stress soil moisture conditions exhibited a mean volume of second season growth significantly ($p=0.01$) larger than that of any treatment. The consistent superiority of the seedlings in this group (Pt-1/2F-NS) supports the claim that mycorrhizal inoculation is more effective when nutrient levels are reduced.

3) The largest and smallest mean volumes occurred among seedlings which received 1/2 Normal fertilization (Table 4.3). At this level of fertility, mycorrhizal means exceeded those of Controls by 30 and 50 % percent for Non-Stress and Stress moisture conditions respectively. The latter was the largest range in mean volume between comparable

treatments and illustrates the effect of soil conditions on inoculated versus non-inoculated seedlings.

4) Initial fertilization appeared to have affected second season volume of moisture Stressed, Control seedlings only. No significant differences occurred due to the level of initial fertilization between the means of Pt-colonized seedlings at either soil moisture level nor between those of the Controls under Non-Stress conditions. Normally fertilized, moisture Stressed, Control seedlings were significantly larger in second season volume ($p=0.01$) than those similar Controls raised on the 1/2 Normal program.

These results, combined with similar ones in Section 4.1.2.(Roots), suggest that growth of non-inoculated (Control) seedlings after transplanting was directly related to the amount of initial fertilization. The relationship appeared to be especially strong when Control seedlings were subjected to poor soil moisture conditions.

Dry Weight - Variations in dry weight of second season growth were due to: 1) the interaction of mycorrhizal colonization with initial fertilization and 2) the main effect of soil moisture (Table 4.1). Soil moisture alone, however, was less influential than the ectomycorrhizae-fertilizer interaction. This observation was confirmed by the results of Duncan's Test; the latter are presented with treatment means in Figure 4.8. The experimental variables which affected dry weight differ from those which affected volume, therefore the pattern of seedling response also differed. Similarities and differences are both outlined in the following paragraphs.

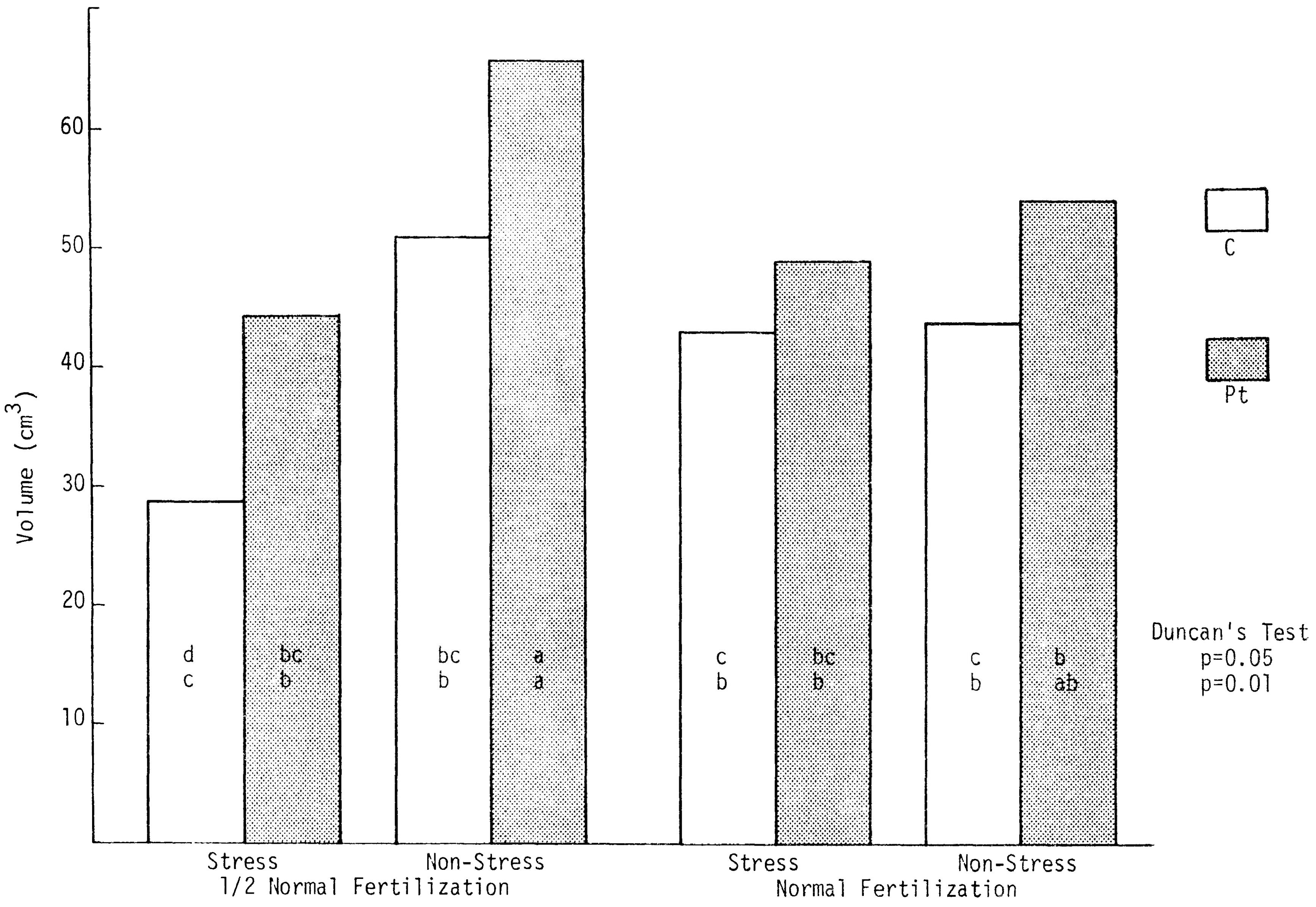


Figure 4.7: Greenhouse Study seedlings - Second season volume treatment means

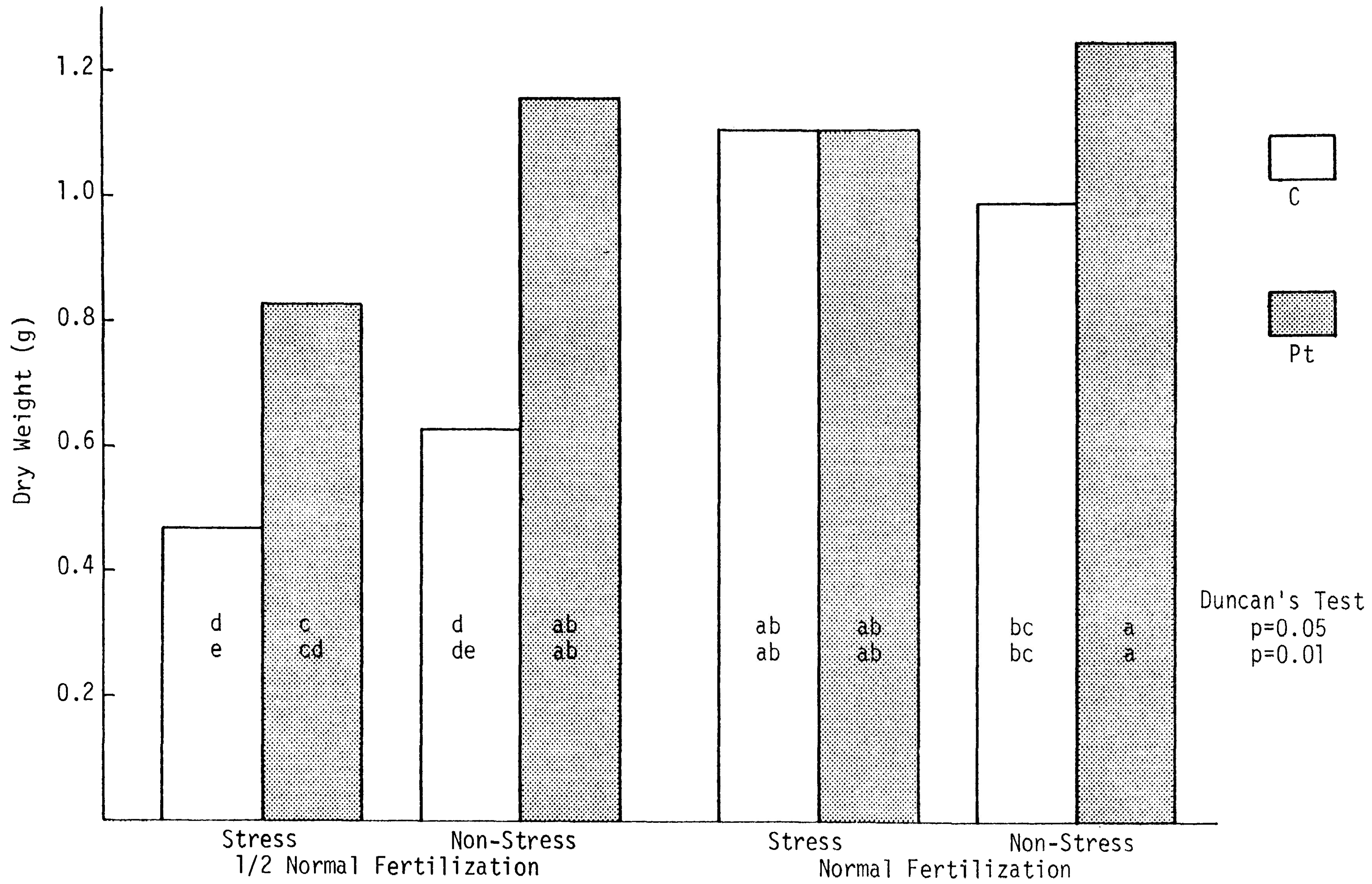


Figure 4.8: Greenhouse Study seedlings - Second season dry weight treatment means

1) with the exception of seedlings raised on Normal fertilization and subjected to Stress soil moisture conditions, the mean dry weights of Pt-colonized seedlings were significantly larger than those of comparable Controls.

2) The level of initial fertilization appears to have restricted second season dry weight of Control seedlings regardless of soil moisture conditions. No significant differences occurred between the means of Stress and Non-Stress Control seedlings raised on either fertilizer program. Such was not the case with second season volume nor with any of the root system measurements.

A comparison of Control seedlings subjected to soil moisture Stress revealed that the mean of those raised on the Normal schedule was 2.4 times that of seedlings raised on the 1/2 Normal schedule (Table 4.3). Similarily, for Control seedlings grown under Non-Stress conditions, the Normally fertilized mean was 1.6 times that of the 1/2 Normally fertilized mean. It appears that for Control seedlings, potential dry weight production in the second season was determined by the level of fertilization in the first season. These results support the observation, made earlier, that higher initial fertilization affects Control seedlings directly.

3) In contrast, mean dry weight of Pt-colonized seedlings was not directly affected by initial fertilization. Only the combination of 1/2 Normal fertility and soil moisture Stress resulted in significantly ($p=0.01$) lower dry weight production by the mycorrhizal seedlings. The mean of Pt-F-S seedlings exceeded that of Pt-1/2F-S seedlings by 40%; no such difference occurred between the means of mycorrhizal seedlings

under Non-Stress conditions. The results indicate that while dry weight production by Control seedlings was directly affected by initial fertilization, Pt-colonized seedlings required further stress before growth was depressed.

Height Increment - Second season height increment was significantly affected by the main effects of: 1) mycorrhizal colonization, 2) initial fertilization, and 3) soil moisture (Table 4.1). No interactions were detected by the analysis of variance. Seedling response to the experimental variables therefore differs from that described for volume and dry weight. The superior performance of the Pt-colonized seedlings, however, remains consistent as shown in Figure 4.9.

Duncan's Test identified the following aspects of height growth in the second season:

1) Mean height increments of Pt-colonized seedlings were, without exception, significantly ($p=0.01$) greater than those of comparable Controls.

2) In general, the means of seedlings which received Normal initial fertilization were larger than those of seedlings raised on the 1/2 Normal schedule.

3) Initial fertilization determined the subsequent effect of soil moisture on second season height increment. Under Non-Stress conditions, there were no differences between either Pt-colonized nor Control means due to initial fertilization. Distinct variations, however, existed between these groups when soil moisture was Stressed. The means of Stressed, Normally fertilized seedlings (Pt-F-S, C-F-S) were significantly ($p=0.01$) greater than those of their respective counterparts raised on the 1/2 Normal schedule (Pt-1/2F-S, C-1/2F-S).

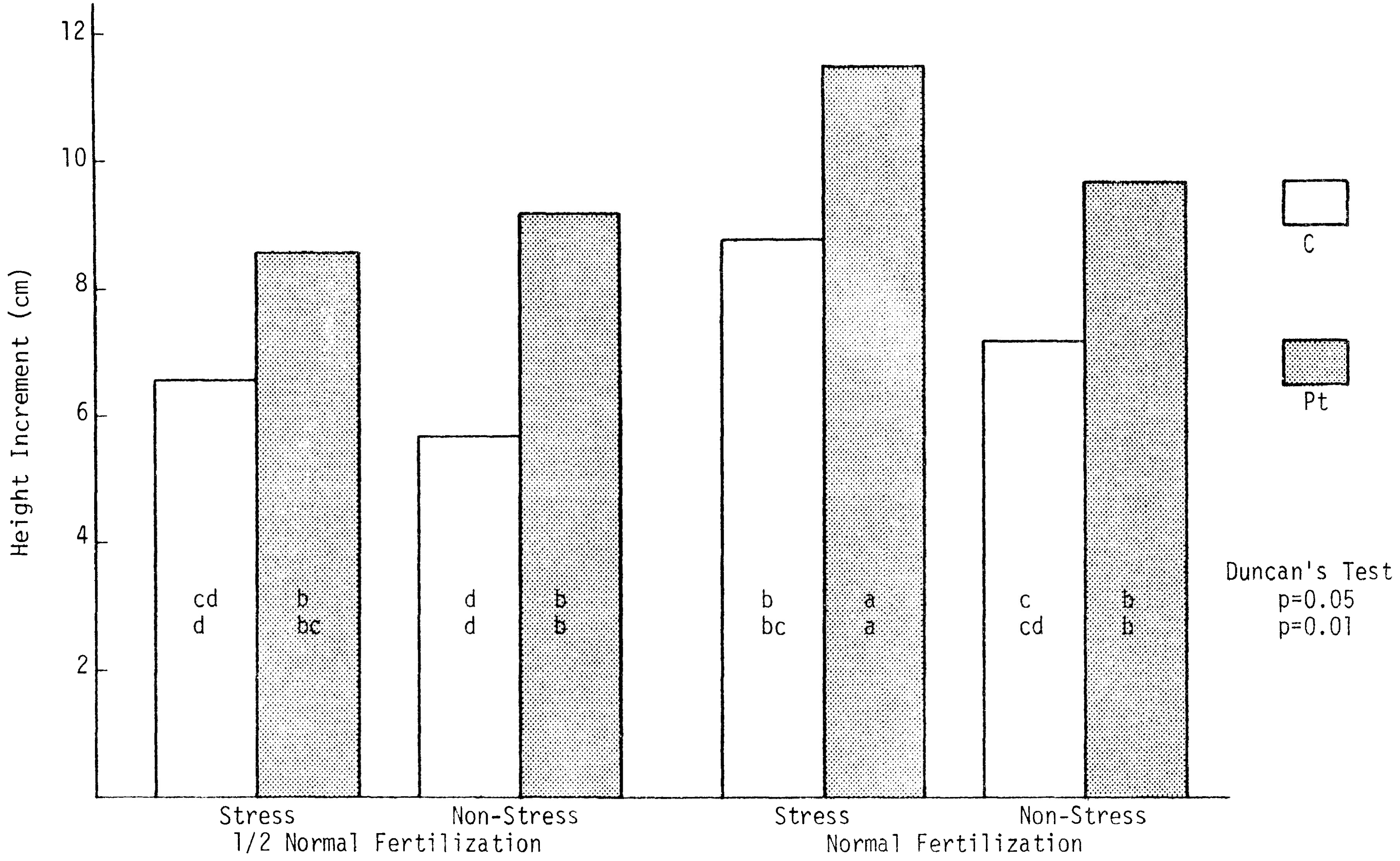


Figure 4.9: Greenhouse Study seedlings - Second season height increment treatment means

The analysis of these results indicated that the potential for height growth, limited in this study by soil moisture and initial fertilization, was best exploited by seedlings with an established *Pisolithus tinctorius* association.

A review of second season growth data, as presented in Figures 4.7, 4.8 and 4.9, reveals very little variation in the patterns of response exhibited by the Pt-colonized and Control seedlings. In general, comparable mycorrhizal and Control groups seem to have reacted to the levels of initial fertilization and moisture content in a similar manner, except that means of the former groups were usually considerably larger than those of the latter. This similarity appears throughout much of the second season data but was lacking in the analysis of root system measurements. A comparison of growth in the second season to that in the first reveals that the response of Control and Pt-colonized seedlings to the experimental variables did, in fact, differ.

Table 4.5, on the following page, presents the pertinent data.

Using height increment as an example, we note from Table 4.5 that the largest first season mean height was attributed to Control seedlings raised on the Normal fertilizer schedule (C-F). The mean increases in height recorded by these same seedlings in the second season were 47 and 41 percent respectively for Stress and Non-Stress soil moisture conditions. The first season mean height of mycorrhizal seedlings also raised on the Normal regime (Pt-F) did not differ significantly from that of the C-F seedlings previously mentioned. The Pt-F second season increment in height, however, represented increases of

Table 4.5: Greenhouse Study seedlings - Second season growth expressed as a percentage of first season growth

Fertilization	1/2 Normal				Normal			
Soil Moisture	Stress		Non-Stress		Stress		Non-Stress	
Myc Colonization	C	Pt	C	Pt	C	Pt	C	Pt
Volume								
First season	13.4	23.4	16.8	30.8	29.6	28.0	27.2	34.8
Second season	28.8	44.4	50.8	65.6	43.2	49.2	43.8	54.2
Dry Weight								
First season	1.06	1.37	1.60	2.04	1.55	1.68	1.43	1.89
Second season	0.47	0.83	0.63	1.16	1.11	1.11	0.99	1.25
Height Increment								
First season	8.6	10.4	9.1	11.3	18.8	17.4	17.6	17.3
Second season	6.6	8.6	5.7	9.2	8.8	11.5	7.2	9.7
% Increase								
Volume	215	190	302	213	146	176	161	156
Dry Weight	44	61	39	57	72	66	69	66
Height Increment	77	83	63	81	47	66	41	56

66 and 56 percent respectively for the Stress and Non-Stress treatments; much higher than the increases recorded for comparable Control groups.

Variations in the response of Pt-colonized and Control seedlings to similar fertilization and soil moisture conditions were evident for volume and dry weight as well. While the percentage of the increases fluctuated, the absolute numbers confirmed that the presence of _P. tinctorius_ ectomycorrhizae on the roots of containerized jack pine benefitted seedling growth in the second season.

4.1.4 Total Growth Measurements

The analysis of total growth confirmed the observations previously stated. In the case of dry weight and volume, seedling totals were very much influenced by the root component. This explains the similarity in the analyses of the total seedling and root system measurements.

Volume - Variation in total volume was due to: 1) the interaction of initial fertilization and soil moisture content and 2) the main effect of mycorrhizal colonization (Table 4.1). Results of Duncan's Test revealed the following differences between the treatment means (Figure 4.10):

1) The largest mean total volumes were displayed by the Pt-colonized seedlings raised under Non-Stress soil moisture conditions (Pt-F-NS, Pt-1/2F-NS). At p=0.05 and p=0.01, there were no differences between the means of the two groups. The practical implications of this result are very positive. Seedling performance can be improved and, simultaneously, fertilizer costs reduced when containerized seedlings are inoculated with the proper ectomycorrhizal fungus or fungi.

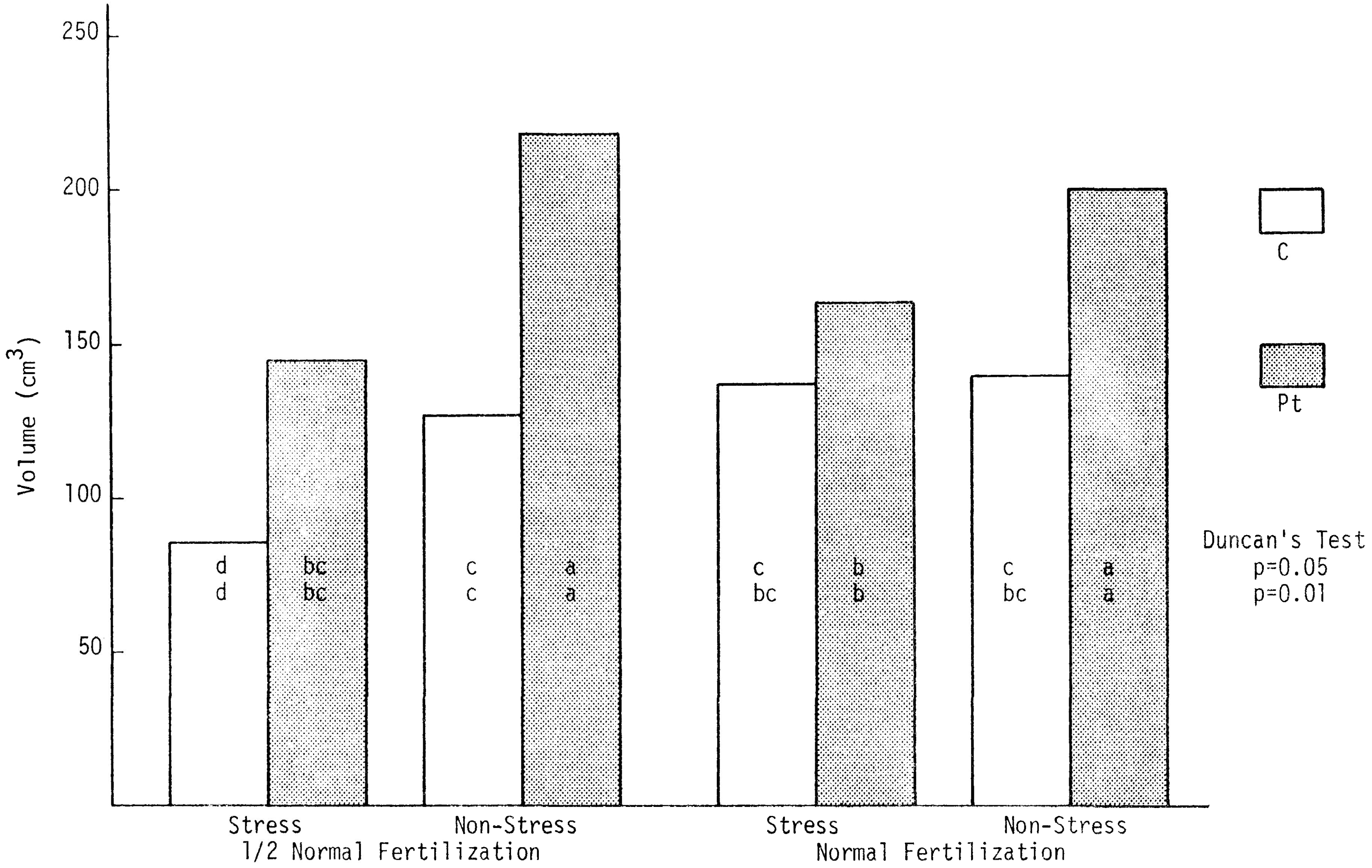


Figure 4.10: Greenhouse Study seedlings - Total seedling volume treatment means

2) At p=0.05, no difference existed between Pt-colonized seedlings grown under Stressed soil moisture conditions (Pt-F-S, Pt-1/2F-S). This equality, and that noted in the previous paragraph, suggest once again that for mycorrhizal seedlings it is the immediate limiting factor (soil moisture) rather than the pre-outplanting (fertilization) treatment which has determined seedling performance.

3) The superior quality of Pt-colonized seedlings is illustrated by a comparison of treatment means from the first and second growing seasons. Following the first season, the mean volume of Control seedlings which had received Normal fertilization did not differ significantly from the means of either Pt-colonized group (Figure 4.1). After the second growing season, however, all mycorrhizal treatments exhibited mean total volumes significantly (p=0.01) greater than those of comparable Controls. Furthermore, in absolute numbers, all mycorrhizal mean total volumes were larger than all Controls.

<u>Dry Weight</u> - Total dry weight variation was caused by two interactions: 1) initial fertilization and soil moisture and 2) mycorrhizal colonization and soil moisture (Table 4.1). Treatment means and the results of Duncan's Test are presented in Figure 4.11. The following aspects of seedling response to the experimental variables were identified:

1) The largest mean total dry weights were associated with Pt-colonized seedlings raised under Non-Stress soil moisture conditions. There was no difference between the means of Pt-F-NS and Pt-1/2F-NS seedling groups due to initial fertilization.

2) Mean total dry weights of all mycorrhizal treatments were significantly greater at p=0.05 than those of comparable Control groups. Differences between comparable means ranged from 17 to 59 percent.

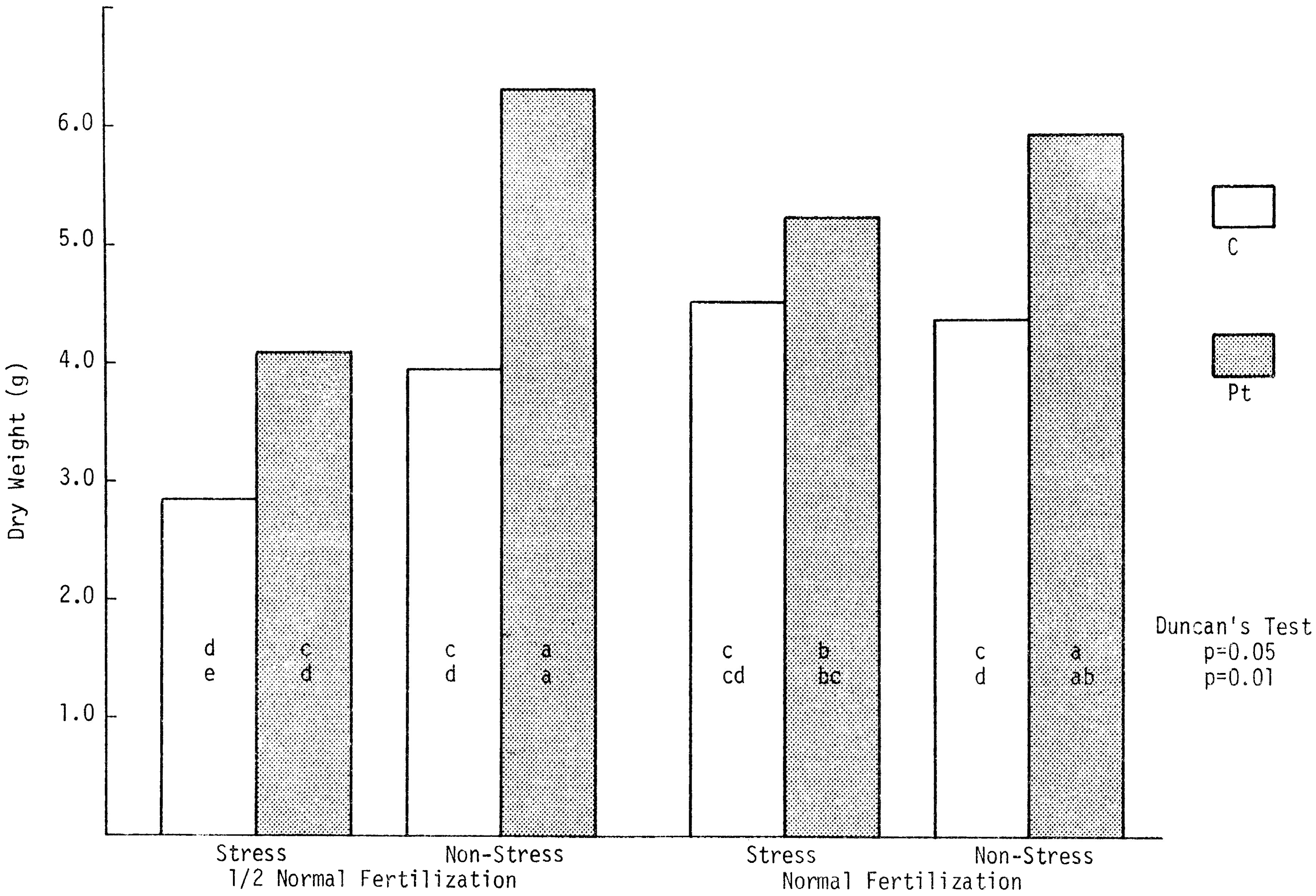


Figure 4.11: Greenhouse Study seedlings - Total seedling dry weight treatment means

3) The only Pt-colonized treatment mean not significantly greater than all Control means was that of Pt-1/2F-S seedlings. Mean total dry weight of this mycorrhizal group was, however, 50% larger than that of its direct Control counterpart, C-1/2F-S (Table 4.3).

4) Table 4.6 presents the data for Top/Root ratios based on dry weight. These ratios range from 1.02 to 1.42; the former representing a Pt-colonized group of seedlings, the latter, a Control. It is interesting to note that the treatment groups with the three largest total dry weight means (Pt-1/2F-NS, Pt-F-NS, and Pt-F-S), also exhibited the lowest and therefore the most desirable Top/Root ratios. According to Armson and Sadreika (1974), Top/Root ratios approaching 1:1 are desirable for nursery stock as this ratio represents a "well-balanced" tree.

Table 4.6: Top/Root ratios of Greenhouse Study seedlings

Soil Moisture	Stress		Non-Stress	
Myc Colonization	C	Pt	C	Pt
	1/2 Normal Fertilization			
Top D.W. (g)	1.53	2.20	2.23	3.20
Root D.W. (g)	1.31	1.90	1.72	3.13
Total D.W. (g)	2.84	4.10	3.95	6.33
Top/Root ratio	1.17	1.16	1.30	1.02
	Normal Fertilization			
Top D.W. (g)	2.66	2.79	2.42	3.14
Root D.W. (g)	1.87	2.47	1.95	2.81
Total D.W. (g)	4.53	5.26	4.37	5.95
Top/Root ratio	1.42	1.13	1.24	1.12

Height - The superiority of Pt-colonized seedlings was not as consistent in terms of total height as it was with total volume and total dry weight. This was due to the potent effect of initial fertilization in the first growing season. Height increment during that time accounts for an average of 75% of the total height growth over the two seasons. It is therefore difficult to explain total height growth in terms of 'growth since potting' or to expect soil moisture to have had much effect on this parameter.

The analysis of variance revealed that variation between treatments was due to the interactions between 1) mycorrhizal colonization and initial fertilization and 2) soil moisture and initial fertilization (Table 4.1). These results, as well as those of Duncan's test, substantiate the statements made in the preceding paragraph. Total height treatment means and the results of Duncan's test are presented in Figure 4.12, found on the following page.

1) Mean total heights of Pt-colonized treatment groups exceeded those of comparative Control groups.

2) Between seedling groups raised at 1/2 Normal fertility, the difference in total height due to Pt-colonization was significant at p=0.01. Such differences did not occur between seedling groups which received Normal initial fertilization.

3) Soil moisture alone was not responsible for any significant variation between total height means of similarily fertilized and colonized seedlings.

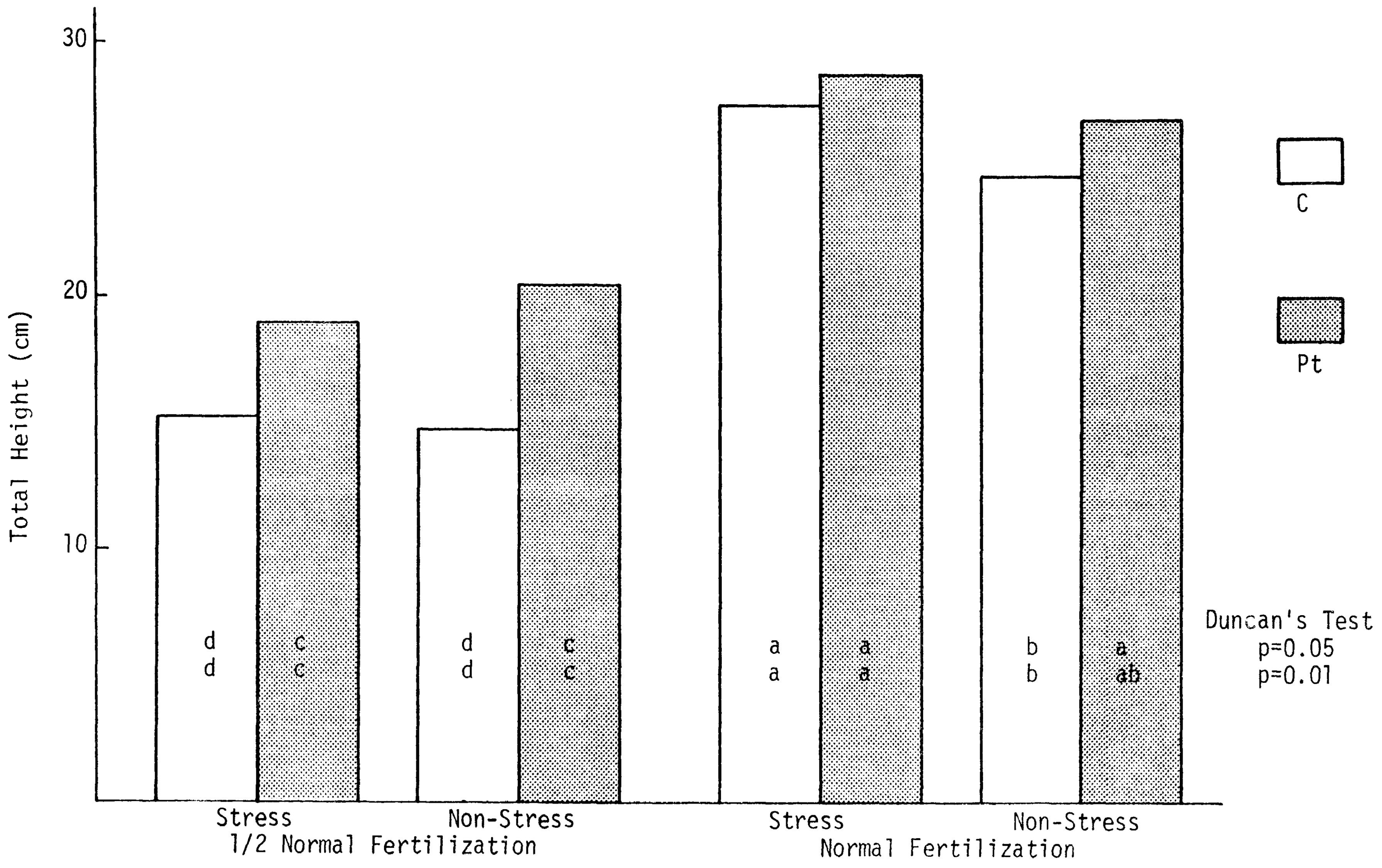


Figure 4.12: Greenhouse Study seedlings - Total seedling height treatment means

4.2 Outplanting at Skea Lake

Outplanted seedlings were classified according to initial fertilization and mycorrhizal inoculation as described in Section 2.1. The treatment groups were 1) Pt-inoculated, 1/2 Normal initial fertilization (Pt-1/2F), 2) Pt-inoculated, Normal initial fertilization (Pt-F), 3) Control, 1/2 Normal initial fertilization (C-1/2F) and 4) Control, Normal initial fertilization (C-F).

Results of the analysis of seedling data from the field outplanting were extremely uniform. Table 4.7 presents the results of the analysis of variance; Table 4.8 the results of Duncan's test and Table 4.9 the treatment means. The following is a summary of general trends exhibited by these seedlings.

1) For all seedling measurements in both the first and second growing seasons, the means of Pt-inoculated treatments were larger than those of comparable Control treatments (Table 4.9). These differences, however, were not necessarily significant (Table 4.8).

2) Significant differences between Pt-inoculated and Control treatments occurred consistently at the 1/2 Normal initial fertilization level. All parameters, except height increment in the first and second seasons, exhibited this characteristic.

3) In comparison to the Greenhouse Study seedlings, those outplanted at Skea Lake had much smaller root systems, much larger aboveground parts and were bigger in size overall.

Table 4.7: Outplanted seedlings - Analysis of Variance for the physical assessment

Physical Parameter	Blocks	SOURCE OF VARIATION MYC (M)	FERT (F)	M-F
First Season				
Volume (cm^3)	ns	ns	ns	ns
Dry Weight (g)	ns	ns	ns	ns
Height Increment (cm)	ns	ns	**	ns
Roots				
Volume	ns	**	ns	ns
Dry Weight	ns	**	ns	ns
RCD (mm) +	ns	**	ns	ns
Second Season				
Volume	ns	**	ns	ns
Dry Weight	ns	**	ns	ns
Height Increment	ns	**	ns	ns
Total Seedling				
Volume	ns	**	ns	ns
Dry Weight	ns	**	ns	ns
Height	ns	**	ns	ns

ns - not significant **- significant at p=0.01

\+ - Root Collar Diameter

Table 4.8: Outplanted seedlings - Results of Duncan's test for the physical assessment*

Significance Level	p=0.05				p=0.01			
Myc Colonization	Control		Pt-Inoculated		Control		Pt-Inoculated	
Fertilization	1/2F	F	1/2F	F	1/2F	F	1/2F	F
First Season								
Volume (cm^3)	b	ab	a	a	b	ab	ab	a
Dry Weight (g)	b	ab	a	a	b	ab	ab	a
Height Increment (cm)	b	ab	ab	a	a	a	a	a
Roots								
Volume	b	a	a	a	b	ab	a	a
Dry Weight	b	a	a	a	b	a	a	a
RCD (mm) *	b	a	a	a	b	ab	a	a
Second Season								
Volume	c	b	a	ab	b	ab	a	a
Dry Weight	c	bc	a	ab	b	ab	a	a
Height Increment	b	ab	a	a	b	ab	ab	a
Total Seedling								
Volume	c	b	a	ab	b	ab	a	a
Dry Weight	b	a	a	a	b	ab	a	a
Height	c	b	b	a	b	a	a	a

* - the same letter signifies no significant difference between treatment means

Table 4.9: Outplanted seedlings - Mean treatment values of the physical parameters

Myc Colonization Fertilization	Control 1/2 F	 F	Pt-Inoculated 1/2-F	 F
<u>First Season</u>				
Volume (cm^3)	21.5	33.5	40.5	45.5
Dry Weight (g)	0.58	1.00	1.22	1.43
Height Increment (cm)	8.6	14.2	10.8	16.6
<u>Roots</u>				
Volume	21.3	35.5	47.5	41.3
Dry Weight	0.45	0.89	1.08	1.07
RCD (mm) *	37.0	49.2	57.2	54.8
<u>Second Season</u>				
Volume	84.5	149.0	216.0	191.0
Dry Weight	1.99	3.70	5.52	4.60
Height Increment	7.5	11.9	14.2	16.3
<u>Total Seedling</u>				
Volume	127.3	218.0	304.0	277.8
Dry Weight	3.03	5.59	7.82	7.10
Height	16.1	26.2	25.0	32.9

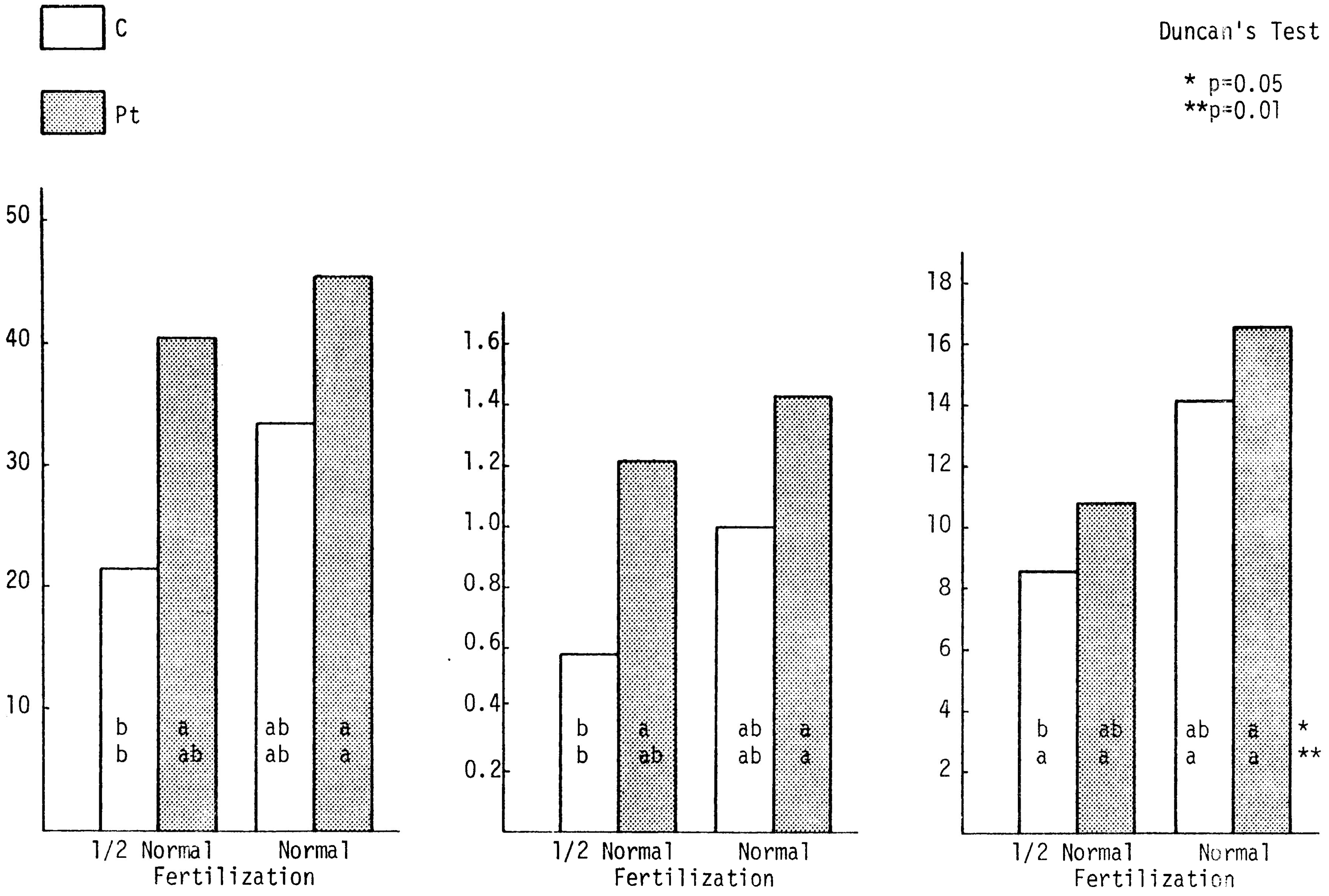
* Root Collar Diameter

4.2.1 First Season Growth Measurements

The analysis of variance for measures of first season growth of the Outplanted seedlings revealed minimal variation among the treatments (Table 4.7). Volume and dry weight were unaffected by any experimental variable. Height increment, however, was significantly (p=0.01) influenced by the level of initial fertilization. Duncan's test distinguished the following trends in seedling response.

1) Significant differences (p=0.01) occurred between volume and dry weight means of Pt-inoculated and Control seedlings raised on 1/2 Normal initial fertilization (Figure 4.13 a,b). Mean volume and dry weight values of the mycorrhizal treatments were 2 times those of the Controls. Among seedlings which received Normal initial fertilization, the pattern of response was similar but differences between treatment means were not as large.

2) Mean height increments of both Pt-inoculated and Control seedlings raised on Normal initial fertilization were larger than those of comparable seedlings raised on the 1/2 Normal schedule (Figure 4.13 c). These differences, however, were not significant at p=0.05.



a. Volume (cm^3)

b. Dry Weight (g)

c. Height Increment (cm)

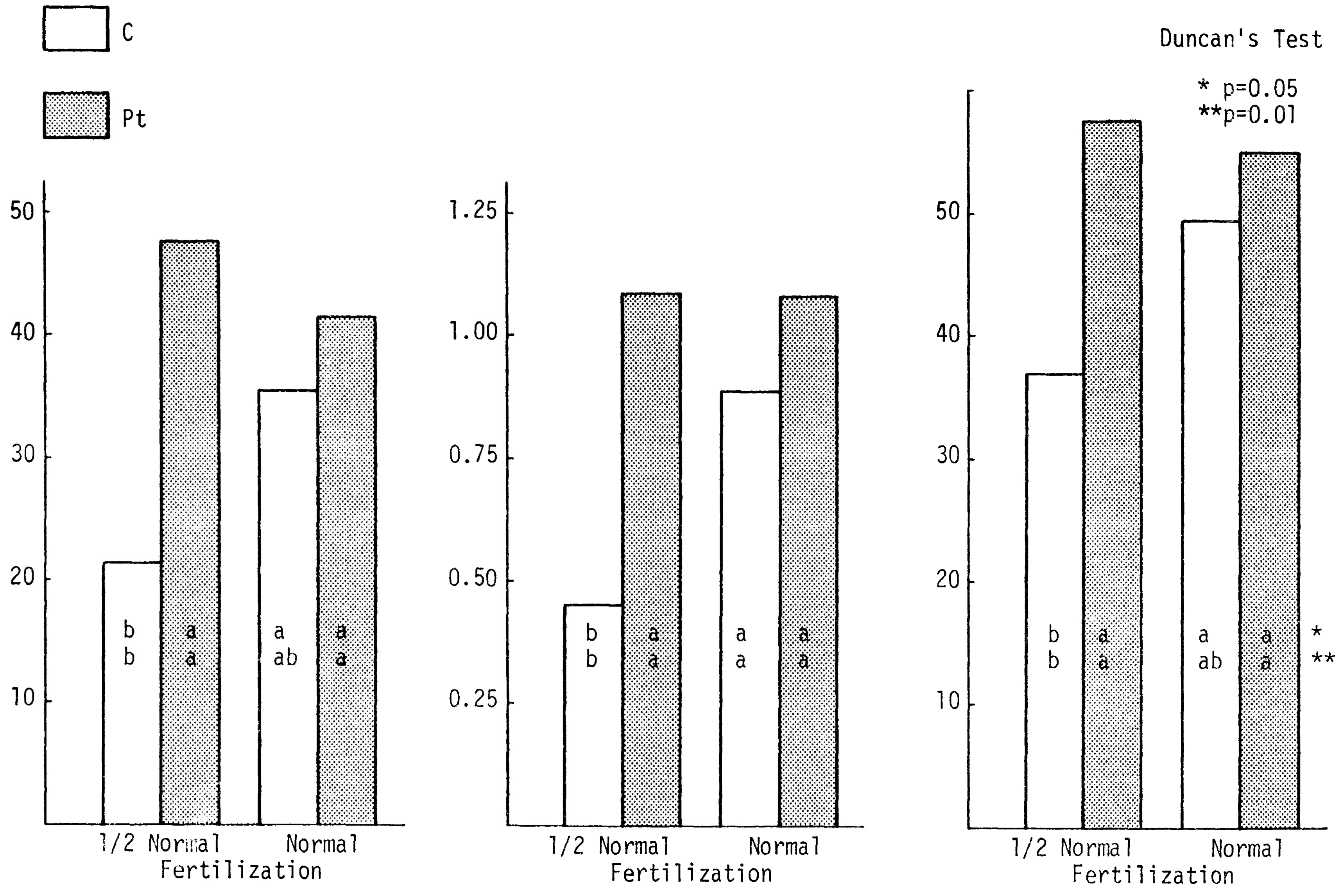
Figure 4.13: Outplanted seedlings - First season measurement treatment means

4.2.2 Root Measurements

The analysis of variance revealed that only the main effect of mycorrhizal inoculation was responsible for the variation in root volume, root dry weight and root collar diameter (Table 4.7). For all three parameters, the means of inoculated seedlings were larger than those of Controls (Figure 4.14). Duncan's test identified the following relationships between seedling response and the experimental variables (Table 4.8).

1) Once again, significant differences between Pt-inoculated and Control seedlings existed only at the 1/2 Normal initial fertilization level. Mycorrhizal means at this level exceeded Control means by 55% for root collar diameter, 116% for volume and 110% for dry weight. Furthermore, these mycorrhizal means also exceeded those of Control seedlings raised on the Normal initial fertilization schedule, though not significantly so.

2) The level of initial fertilization affected the root development of Control seedlings but not that of Pt-inoculated seedlings. Mean root collar diameter, root volume and root dry weight of non-inoculated seedlings which received the lower amount of fertilization were significantly smaller at $p=0.05$ than the means of Normally fertilized non-inoculated seedlings.



a. Volume (cm^3)

b. Dry Weight (g)

c. Root Collar Diameter (mm)

Figure 4.14: Outplanted seedlings - Root measurement treatment means

4.2.3 Second Season Growth Measurements

As in the first season, seedling growth expressed through volume and dry weight followed one pattern while height increment followed another. Each pattern will therefore be discussed separately.

Volume and Dry Weight - Results of the statistical analysis of second season volume and dry weight data parallel those presented for the root systems in Section 4.2.2 in that only the main effect of mycorrhizal colonization was responsible for any variation between treatments (Table 4.7).

Significant differences at p=0.01 between Pt-inoculated and Control seedlings occurred only at 1/2 Normal initial fertilization levels (Figure 4.15 a, b). Volume and dry weight means of this mycorrhizal group exceeded those of the comparable Control group by 156 and 177 percent respectively. Furthermore, mean volume and dry weight of Pt-inoculated, 1/2 Normally fertilized seedlings were larger, though not significantly so, than those of both mycorrhizal and Control seedlings raised on Normal initial fertilization.

Height Increment - While the analysis of variance indicated that only the main effect of mycorrhizal inoculation significantly affected height growth in the second season (Table 4.7), the influence of initial fertilization was apparent (Figure 4.15 c).

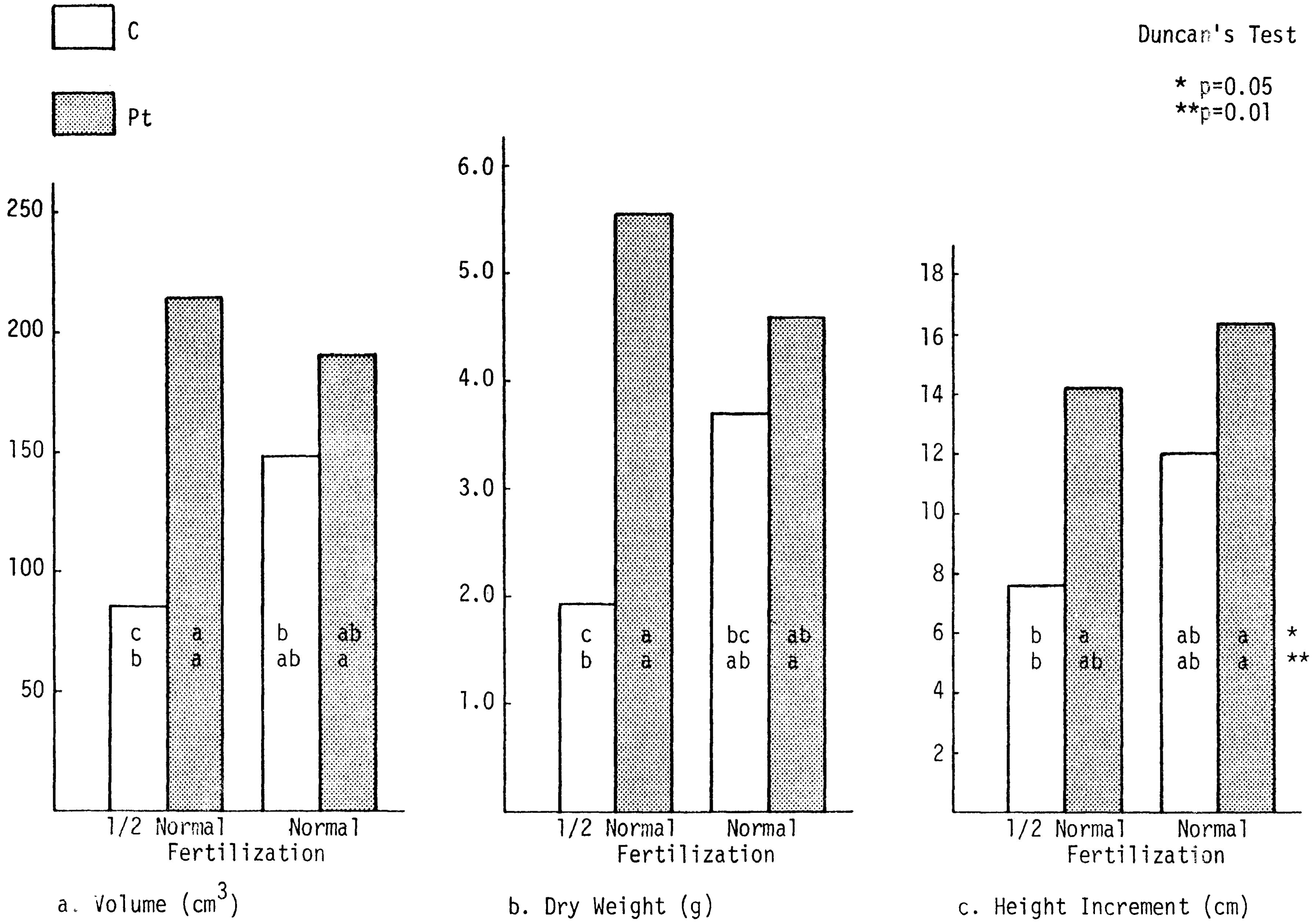


Figure 4.15: Outplanted seedlings - Second season measurement treatment means

Duncan's test identified the following aspects of seedling response in terms of height increment.

1) Similar to volume and dry weight, significant (p=0.05) differences between inoculated and Control seedlings occurred only when initial fertilization was at the 1/2 Normal level. In this instance the mycorrhizal mean was almost twice that of the Control.

2) The effect of initial fertilization, although not significant, was evident. Mean height increments of both Pt-inoculated and Control groups raised on Normal initial fertilization were larger than for the same seedlings raised on the 1/2 Normal schedule. While this difference appeared between absolute numbers (Table 4.9), another existed between the relative increases in seedling height. Table 4.10 presents this data.

Table 4.10: Outplanted seedlings- Second season growth as a percentage of first season growth

Fertilization	1/2 Normal		Normal	
Myc Colonization	C	Pt	C	Pt
Volume	340	460	370	320
Dry Weight	400	520	450	420
Height Increment	90	130	80	100

The mean height increment of Pt-inoculated, 1/2 Normally fertilized seedlings, while not significantly different from that of Pt-inoculated, Normally fertilized seedlings, was less by approximately 2 cm (Table 4.9). In terms of relative increase over the first season's growth, however, the Pt-1/2F seedlings exhibited a superior performance after transplanting (Table 4.10). Furthermore, for volume and dry weight measurements as well, the greatest increases in growth were made by the initially smaller, 1/2 Normally fertilized but Pt-inoculated seedlings.

4.2.4 Total Seedling Growth Measurements

Once again, the pattern of seedling response to the experimental variables as expressed through volume and dry weight differred from that of height.

Volume and Dry Weight - Total volume and dry weight means followed very closely the pattern of second season growth (Table 4.9). This is understandable given the relative amounts of seedling tissues attributed to the roots on one hand and the first and second season aboveground parts on the other; the latter being by far the larger of the two. The analysis of variance revealed that only the main effect of mycorrhizal colonization was responsible for variation between the treatments (Table 4.7).

Duncan's test identified the following relationships between treatment means (Table 4.8).

1) Significant differences between similar Pt-inoculated and Control seedlings occurred only at the 1/2 Normal initial fertilization level (p=0.05). The means of these mycorrhizal seedlings exceeded those of the Control group by at least 100% (Figure 4.16 a, b).

2) Mean volume and dry weight of Pt-inoculated, 1/2 Normally fertilized seedlings were larger than those of seedlings raised on Normal fertilization regardless of mycorrhizal inoculation, though this difference was not significant at p=0.05. It was apparent from this data and that of second season growth and the root systems, that lower levels of initial fertilization actually favoured the seedlings inoculated with P. tinctorius.

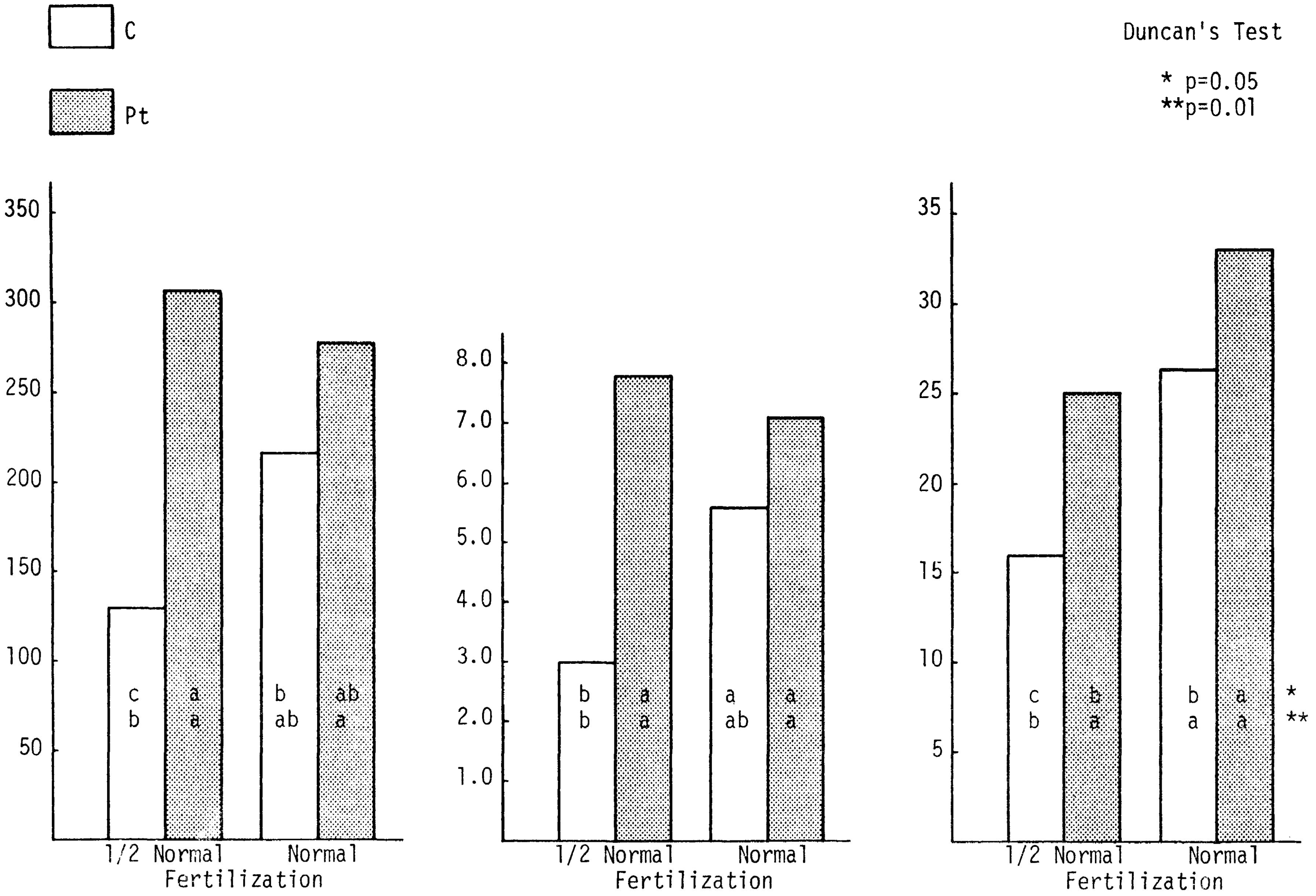
3) Volume and dry weight data of Control groups, however, confirmed the direct influence of initial fertilization on non-inoculated seedlings. Mean values for Control seedlings raised on the higher fertilizer regime exceeded significantly (p=0.05) those of seedlings raised on the 1/2 Normal schedule by an average of 75%.

It should be recalled that Top/Root ratios of seedlings in the Greenhouse Study were between 1.0 and 1.5 after two growing seasons (Table 4.6). Values for the outplanted seedlings were much higher (5.2 to 6.3) indicating the difference in root size between the potted and outplanted seedlings (Table 4.11). Smith (1962) states that a Top/Root ratio of greater than 4:1 prior to outplanting is undesirable. Very little information exists, however, concerning changes in seedling T/R ratio after outplanting and therefore there are no guidelines by which to judge the results in Table 4.11.

One approach may be to consider the vigour of these seedlings in the second growing season. The seedling group with the highest T/R ratio (Pt-1/2F) was also the group which recorded the greatest relative increases in seedling size (Table 4.10). One must conclude, in this case, that the relatively smaller root systems of Pt-inoculated seedlings raised on the 1/2 Normal initial fertilizer program have not limited growth.

Table 4.11: Top/Root ratios of Outplanted seedlings

Fertilization	1/2 Normal		Normal	
Myc Colonization	C	Pt	C	Pt
Top D.W. (g)	2.58	6.74	4.70	6.03
Root D.W. (g)	0.45	1.08	0.89	1.07
Total D.W. (g)	3.03	7.82	5.59	7.10
Top/ Root ratio	5.73	6.24	5.28	5.64



a. Volume (cm^3)

b. Dry Weight (g)

c. Total Height (cm)

Figure 4.16: Outplanted seedlings - Total seedling measurement treatment means

Total Height - The analysis of variance revealed that variation in total height was due to the main effects of mycorrhizal inoculation and initial fertilization (Table 4.7). These results were further clarified by Duncan's test of the treatment means (Figure 4.16 c).

1) Significant differences between Pt-inoculated and Control treatments occurred at each level of fertilization. The means of mycorrhizal seedlings were 25 to 56 percent greater than comparable Control means at the higher and lower fertilizer regimes respectively ($p=0.05$).

2) Also,significant ($p=0.05$) differences in total height between seedlings raised on Normal and 1/2 Normal fertilizer schedules were detected for both mycorrhizal and Control groups. Means of Normally fertilized seedlings exceeded those of seedlings raised on the lower regime by 32 and 63 percent for Pt-inoculated and Control seedlings, respectively.

3) There was no difference between mean total height of Control, Normally fertilized seedlings and that of Pt-inoculated, 1/2 Normally fertilized seedlings. This results reinforces the claim that vigourous containerized seedlings can be produced when lower levels of initial fertilization are combined with suitable mycorrhizal inoculations.

5.0 RESULTS OF THE NUTRIENT ASSESSMENT

5.1 Soils Analysis

Several tests were conducted to determine if greenhouse conditions had altered the original characteristics of the soil removed from the outplanting site and used for potting the seedlings. Results of this analysis are presented in Table 5.1. It should be noted that the data for "Outplanting Site" represents the soil prior to transplanting of the seedlings and that for "Greenhouse Study" represents the soil after completion of the second growing season in the Lakehead University greenhouse. Each element of the analysis will be discussed separately.

Soil Reaction - pH of the soil at the outplanting site was slightly higher than that of any of the greenhouse samples. The range of pH values is low compared to those reported to be most favourable for ectomycorrhizal development. Modess (1941) suggested a range of 4.0 to 6.0 for nursery soils; Marx and Zak (1965) more recently recommended a range of 5.0 to 5.5 for artificial growing mediums.

The values in Table 5.1 show minimal variation which suggests that none of the greenhouse treatments altered soil reaction markedly.

Total Nitrogen - The values in Table 5.1 exhibit very little difference between the outplanting and greenhouse study samples.

Available Phosphorus - Levels of P measured in the greenhouse samples are slightly less than that of the outplanting. Frequent watering in the greenhouse may have resulted in P leaching. This observation is supported by the fact that available P measured in the Stress soil moisture treatments was slightly higher than that measured in the Non Stress treatments. There appears to be very little difference, if any, between mycorrhizal and Control samples.

Exchangeable Potassium - The amount of K found in the outplanting sample was the highest and the range of values among the treaments narrow. Some leaching may have occurred in the greenhouse, however, no pattern with respect to watering was evident. Another factor adding to the variability was the amount of this nutrient in the irrigation water.

Exchangeable Calcium - The concentration of Ca in the greenhouse samples increased over that of the outplanting by an average of 50%. The increase may be attributed to high Ca levels in the irrigation water. No other trends existed among this data.

Exchangeable Magnesium - Mg levels in the greenhouse samples increased slightly over that of the outplanting.

Cation Exchange Capacity - The range of values for CEC was comparatively large; although all greenhouse values were lower than that of the outplanting. This situation may be due to the rapid loss or breakdown of organic material under greenhouse conditions. Both the lowest and highest greenhouse values were attributed to Pt-colonized treatments; the former being associated with soil moisture Stress, the latter, with

Non Stress conditions. No other patterns were evident.

The lack of alteration in soil characteristics, especially among the greenhouse study samples, supports the argument that variations in seedling size and nutrient status were due to the direct effects of the experimental variables rather than indirect changes in the soil.

Table 5.1: Results of the soils analysis - Outplanting and Greenhouse Study

Soil Sample	pH	Total N (%)	Available P (mg/100g)	Exchangeable Cations K	(meq/100g) Ca	Mg	C.E.C. (meq/100g)
Outplanting	4.5	0.08	1.87	0.14	1.65	0.44	9.75
Greenhouse Study							
1. C-1/2F-S	4.1	0.09	1.57	0.09	2.35	0.64	8.00
2. C-F-S	4.3	0.08	1.47	0.09	2.35	0.64	7.50
3. C-1/2F-NS	3.9	0.09	1.37	0.11	3.04	0.82	8.38
4. C-F-NS	4.0	0.08	1.33	0.09	2.53	0.67	6.00
5. Pt-1/2F-S	4.2	0.09	1.67	0.13	2.54	0.69	5.75
6. Pt-F-S	3.9	0.08	1.60	0.11	2.45	0.62	7.63
7. Pt-1/2F-NS	4.0	0.09	1.43	0.07	2.46	0.64	7.75
8. Pt-F-NS	4.1	0.09	1.37	0.07	2.60	0.69	8.75

5.2 Nutrient composition of the Greenhouse Study seedlings

Treatment means for the nutrient assessment of the Greenhouse Study seedlings are presented in Table 5.2. The analysis of variance for both nutrient concentration and content data (Tables 5.3 and 5.4) revealed few similarities in seedling response as a result of treatments. Results of Duncan's test are found in Tables 5.5 and 5.6, as well as in the appropriate bar graphs (Figures 5.1 to 5.18).

For the nutrient concentration data, the factors most frequently significant (p=0.01) were initial fertilization and soil moisture (Table 5.3). Mycorrhizal colonization was significant as a main effect in only three instances and not at all as part of any 2-way interactions. Only the root tissue data exhibited evidence of 3-way interactions.

In contrast to concentration, nutrient content was significantly (p=0.01) influenced by the main effect of mycorrhizal colonization in 8 out of 9 possible cases (Table 5.4). The combined effects of soil moisture and initial fertilization were again evident. Root tissue data alone exhibited 3-way interactions in the analysis of variance.

The variability in significant main effects and interactions was not surprising. Nutrient status tends to vary with the stage of seedling development, age of the foliage and time of year as well as with soil and environmental conditions (van Den Driessche 1974). Notwithstanding these variations, several trends were noticeable from the data.

Concerning nutrient concentration of the seedlings:

1) For all three nutrients the concentration levels of Pt-colonized seedlings were generally lower than those of comparable Controls. In seven instances (of a total of 36) these differences were significant (p=0.05, Table 5.5).

2) Seedling response to Stressed soil moisture conditions appeared to depend on the level of initial fertilization. Both Pt-colonized and Control seedlings tended to follow the same pattern in this respect. At 1/2 Normal fertilization, soil moisture Stress increased nutrient concentration while at Normal fertilization, Stress decreased concentrations. The former response was more consistent (8 out of 9 possible cases) than the latter (5 out of 9 possible cases).

3) Generally (6 cases out of 9), the level of initial fertilization did not affect nutrient concentration under Non-Stress soil moisture conditions. In those cases where it did, seedling response was erratic.

4) Nitrogen concentration of 1/2 Normally fertilized seedlings varied more than did those of seedlings raised on the Normal regime (Figures 5.1, 5.7 and 5.13). This suggests that the effects

of both mycorrhizal colonization and soil moisture were enhanced or exaggerated when the rate of initial fertilization was reduced. It may be that the quality and/or the quantity of ectomycorrhizae was altered by first season fertilization or that translocation of nutrients was influenced by the availability of water in the second season.

5) The range of mean P concentrations in each seedling part was narrow and few significant differences were identified (Figures 5.5, 5.11 and 5.17). This suggests that P was abundantly available in the soil solution and that the experimental variables were not at levels extreme enough to have affected uptake of this element.

6) Concentrations of K varied widely compared to those recorded for N and P (Figures 5.3, 5.9 and 5.15). In all three seedling parts, the mean K concentration of C-1/2F-S seedlings significantly exceeded those of comparable mycorrhizal seedlings. No other element, nor combination of treatments, exhibited such consistently significant results (Table 5.5).

Table 5.2: Greenhouse Study seedlings - Treatment means for nutrient concentration and content

Myc Colonization		Control				Pt-Colonized			
Fertilization		1/2 Normal		Normal		1/2 Normal		Normal	
Soil Moisture		S	NS	S	NS	S	NS	S	NS
First Season									
Conc*	N	1.01	0.86	0.79	0.77	1.10	0.90	0.78	0.78
	K	0.55	0.50	0.38	0.48	0.42	0.44	0.36	0.44
	P	0.20	0.21	0.18	0.21	0.22	0.21	0.18	0.18
Content**	N	4.73	6.10	8.72	7.59	9.03	10.38	8.71	9.74
	K	2.60	3.56	4.22	4.72	3.48	4.98	4.02	5.48
	P	0.97	1.53	2.04	2.04	1.78	2.42	1.98	2.24
Roots									
Conc	N	1.20	1.01	1.07	1.00	1.15	0.92	1.00	0.91
	K	0.51	0.36	0.18	0.32	0.31	0.35	0.25	0.36
	P	0.26	0.23	0.21	0.23	0.26	0.21	0.24	0.21
Content	N	15.65	17.16	19.63	19.54	21.75	28.78	24.56	25.54
	K	6.71	6.05	3.27	6.14	5.82	10.90	6.17	10.21
	P	3.43	3.89	3.87	4.33	4.82	7.49	5.29	5.84
Second Season									
Conc	N	1.86	1.46	1.72	1.70	1.79	1.54	1.68	1.59
	K	0.69	0.70	0.56	0.64	0.57	0.69	0.52	0.56
	P	0.23	0.20	0.24	0.21	0.23	0.20	0.22	0.18
Content	N	20.64	23.22	28.84	24.19	24.54	31.45	27.18	30.18
	K	7.62	11.06	9.36	9.20	7.88	14.00	8.39	10.71
	P	2.52	3.23	3.95	2.97	3.13	4.02	3.49	3.46

* Concentration measured as % oven dry weight

** Content measured as mg/seedling part

Table 5.3: Greenhouse Study seedlings - Analysis of variance for the nutrient concentration data

Tissue and Element	Blocks	SOURCE OF VARIATION Main Effects Myc (M)	SMS (S)	Fert (F)	2-Way Interaction M-S	M-F	S-F	3-Way Interaction M-S-F
First Season								
N	ns	**	**	**	ns	ns	**	ns
K	ns	**	ns	**	ns	ns	**	ns
P	ns	ns	ns	ns	ns	ns	ns	ns
Roots								
N	ns	ns	**	**	ns	ns	ns	**
P	ns	ns	ns	**	**	**	**	**
K	ns	ns	ns	**	ns	ns	ns	ns
Second Season								
N	ns	ns	**	ns	ns	ns	**	ns
K	ns	**	**	**	ns	ns	ns	ns
P	ns	ns	**	ns	ns	ns	ns	ns

ns - not significant
** - significant at p=0.01

Table 5.4: Greenhouse Study seedlings - Analysis of variance for the nutrient content data.

Tissue and Element	Blocks	SOURCE OF VARIATION Main Effects Myc (M)	SMS (S)	Fert (F)	2-Way Interaction M-S	M-F	S-F	3-Way Interaction M-S-F
First Season								
N	ns	**	ns	ns	ns	**	ns	ns
K	ns	**	**	**	ns	ns	ns	ns
P	ns	**	**	**	ns	**	ns	ns
Roots								
N	ns	**	**	ns	ns	ns	ns	ns
K	ns	**	**	**	**	ns	ns	**
P	ns	**	**	ns	**	*	ns	**
Second Season								
N	ns	**	ns	**	**	ns	**	ns
K	ns	ns	**	ns	**	ns	**	ns
P	ns	ns	ns	ns	ns	ns	**	ns

ns - not significant * - significant at p=0.05 ** - significant at p=0.01

Table 5.5: Greenhouse Study seedlings - Results of Duncan's test for nutrient concentration

Myc Colonization		Control				Pt-Colonized			
Fertilization		1/2 Normal		Normal		1/2 Normal		Normal	
Soil Moisture	p=	S	NS	S	NS	S	NS	S	NS
First Season									
N	0.05	ab	c	d	d	a	bc	d	d
	0.01	ab	c	c	c	a	bc	c	c
K	0.05	a	ab	de	bc	cde	bcd	e	bcd
	0.01	a	ab	de	ab	bcd	bcd	e	bcd
P	0.05	ab	ab	b	ab	a	ab	b	b
	0.01	a	a	a	a	a	a	a	a
Roots									
N	0.05	a	c	bc	cd	ab	de	cd	e
	0.01	a	cd	bc	cd	ab	d	cd	d
K	0.05	a	b	d	b	b	b	c	b
	0.01	a	b	d	bc	bc	b	cd	b
P	0.05	a	ab	b	ab	a	b	ab	b
	0.01	a	ab	b	ab	a	b	ab	b
Second Season									
N	0.05	a	f	bc	bc	ab	ef	cd	de
	0.01	a	e	abc	bc	ab	de	bcd	cde
K	0.05	a	a	c	ab	bc	a	c	c
	0.01	a	a	bc	ab	bc	a	c	bc
P	0.05	a	ab	a	ab	a	ab	a	b
	0.01	a	a	a	a	a	a	a	a

Table 5.6: Greenhouse Study seedlings - Results of Duncan's test for nutrient content

Myc Colonization		Control				Pt-Colonized			
Fertilization		1/2 Normal		Normal		1/2 Normal		Normal	
Soil Moisture	p=	S	NS	S	NS	S	NS	S	NS
First Season									
N	0.05	d	cd	ab	bc	ab	a	ab	a
	0.01	d	cd	ab	bc	ab	a	ab	ab
K	0.05	e	d	bcd	abc	d	ab	cd	a
	0.01	d	c	bc	abc	c	ab	bc	a
P	0.05	d	c	ab	ab	bc	a	ab	ab
	0.01	cd	cd	abc	abc	bc	a	abc	ab
Roots									
N	0.05	f	ef	de	de	cd	a	bc	b
	0.01	d	d	cd	cd	bc	a	ab	ab
K	0.05	b	b	c	b	b	a	b	a
	0.01	b	b	c	b	b	a	b	a
P	0.05	f	ef	ef	bc	cd	a	bc	b
	0.01	e	de	de	cde	cd	a	bc	b
Second Season									
N	0.05	e	de	ab	cd	bcd	a	bc	ab
	0.01	bc	bc	ab	bc	bc	a	ab	a
K	0.05	d	b	bcd	cd	d	a	d	bc
	0.01	d	b	cd	bcd	d	a	d	bc
P	0.05	c	b	a	bc	bc	a	ab	ab
	0.01	c	b	a	bc	abc	a	ab	abc

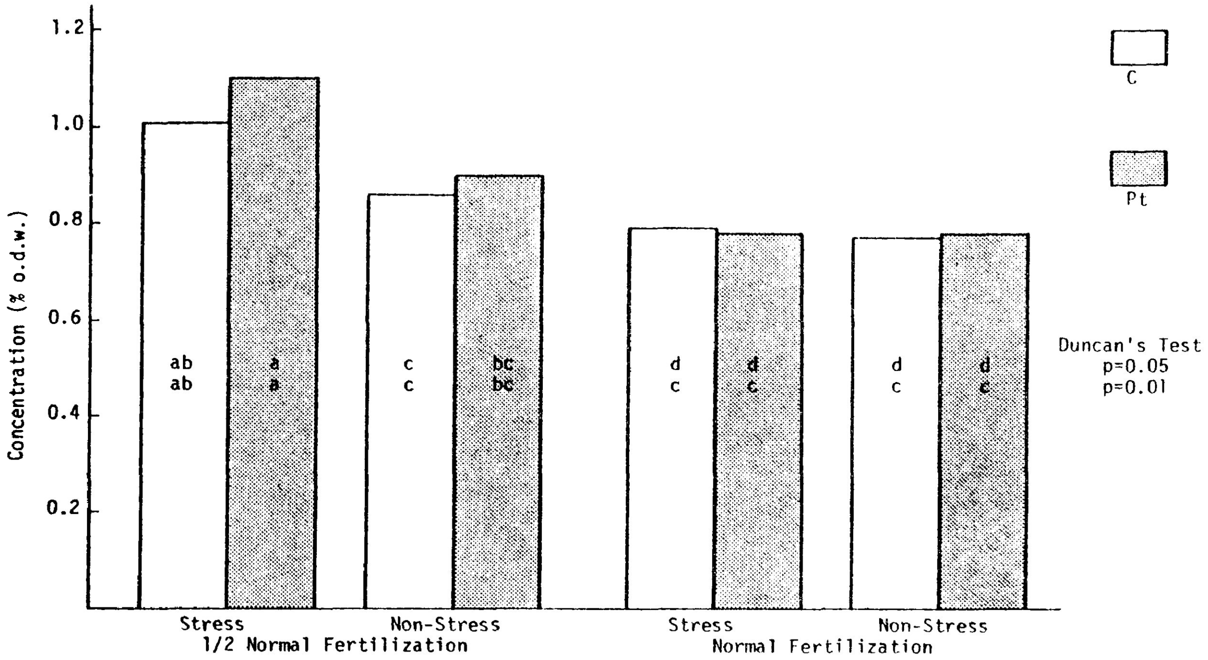


Figure 5.1: Greenhouse Study seedlings - Concentration of N in the first season tissues

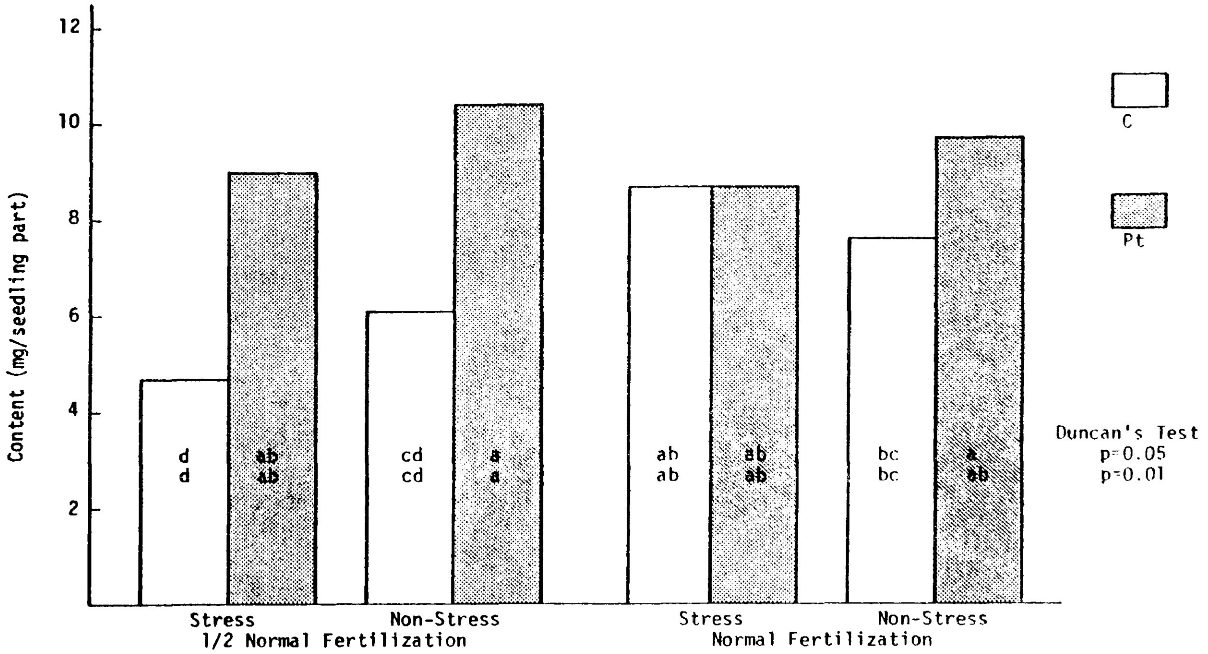


Figure 5.2: Greenhouse Study seedlings - Content of N in the first season tissues

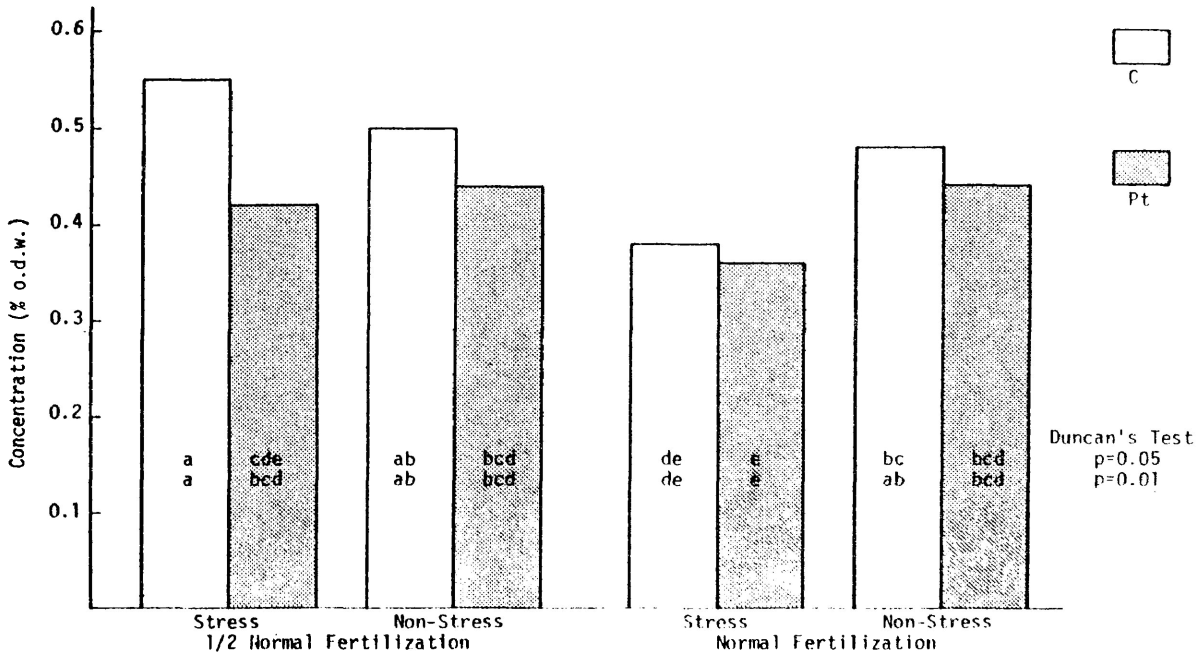


Figure 5.3: Greenhouse Study seedlings - Concentration of K in the first season tissues

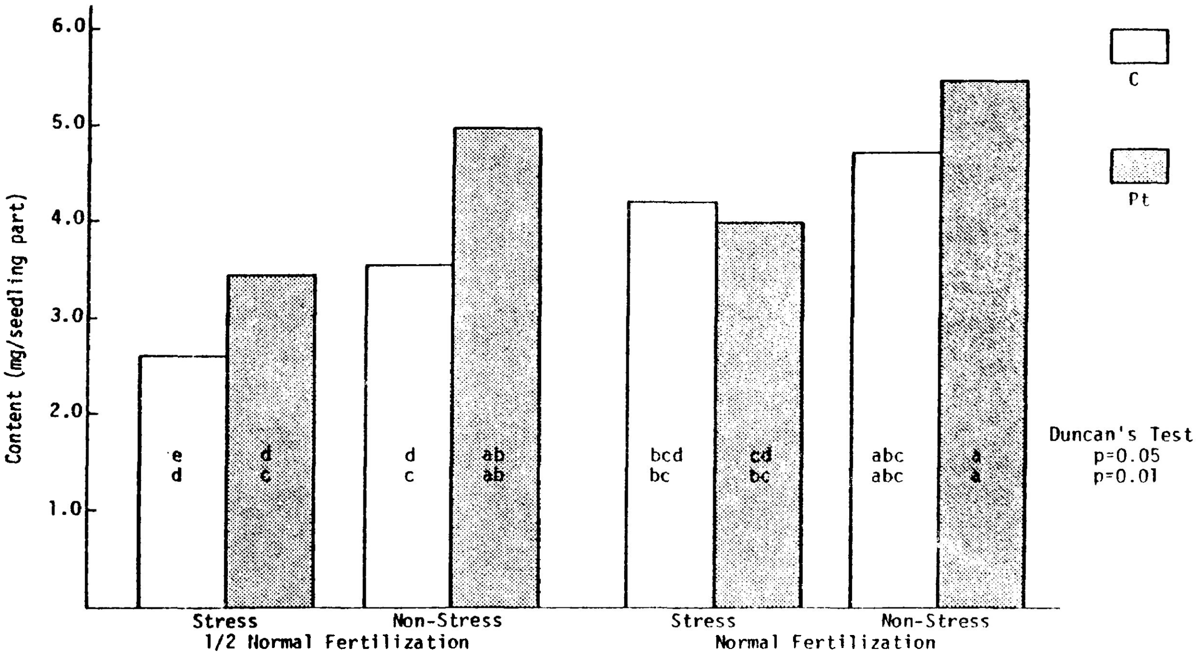


Figure 5.4: Greenhouse Study seedlings - Content of K in the first season tissues

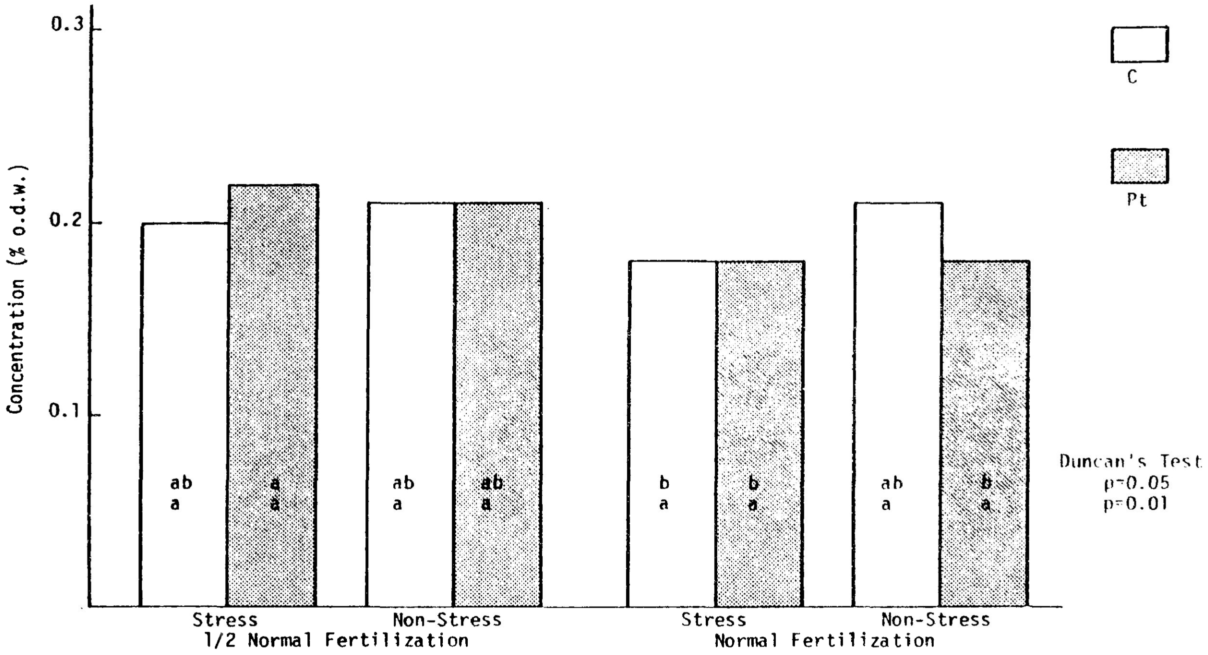


Figure 5.5: Greenhouse Study seedlings - Concentration of P in the first season tissues

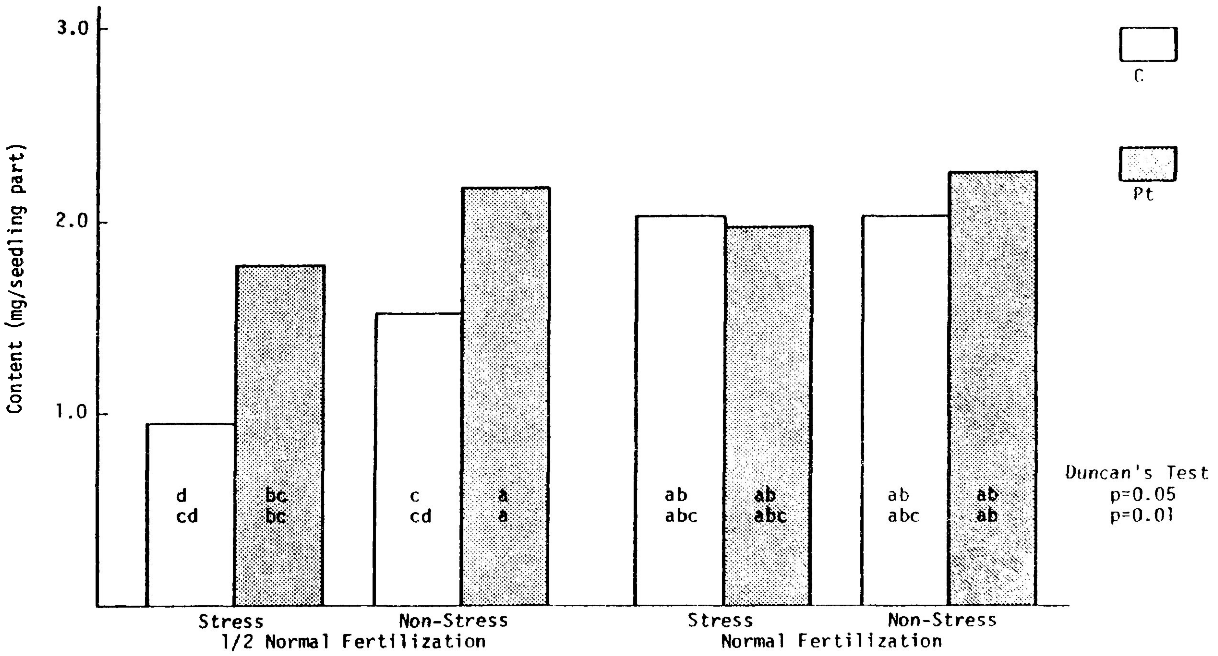


Figure 5.6: Greenhouse Study seedlings - Content of P in the first season tissues

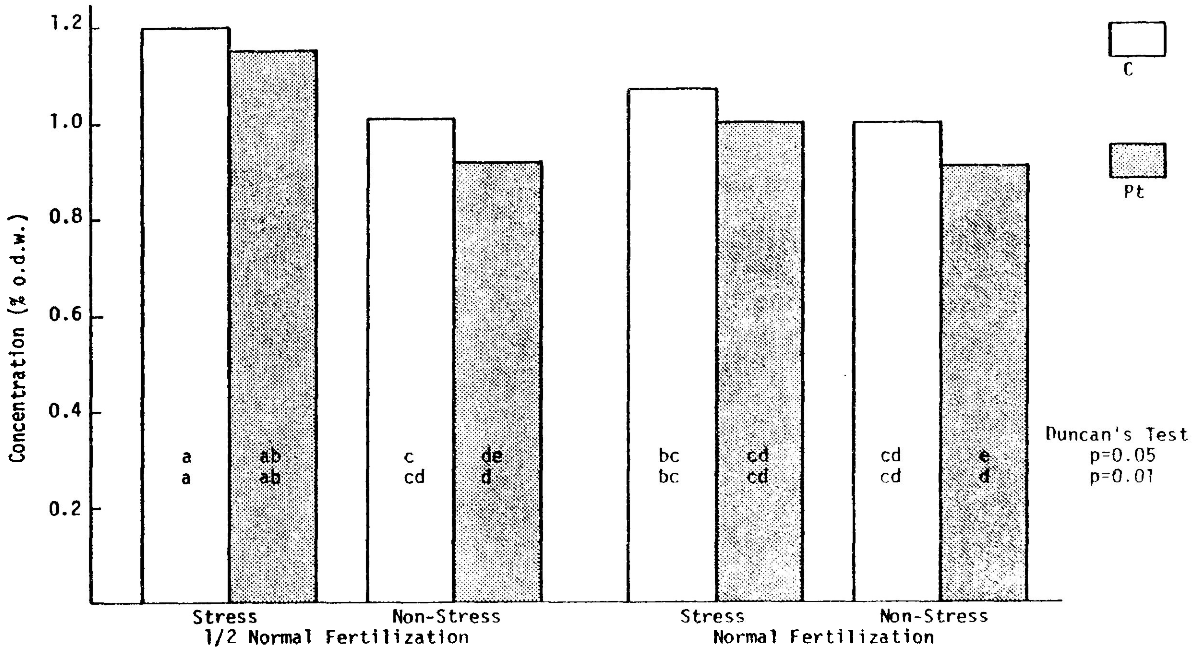


Figure 5.7: Greenhouse Study seedlings - Concentration of N in the root tissues

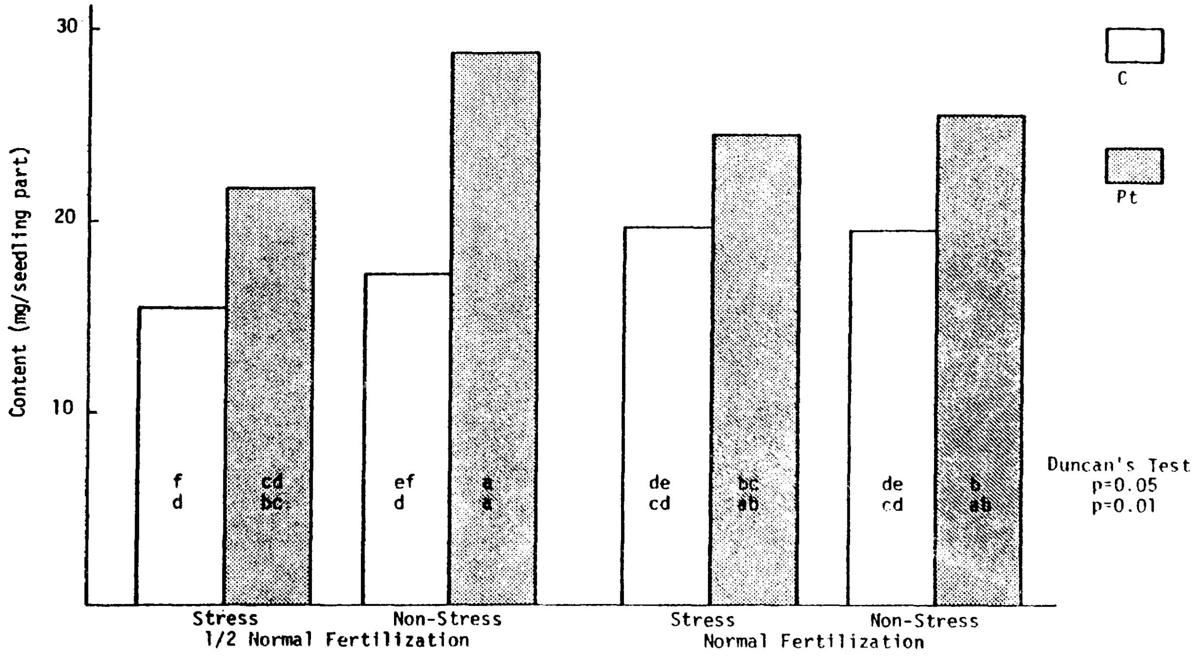


Figure 5.8: Greenhouse Study seedlings - Content of N in the root tissues

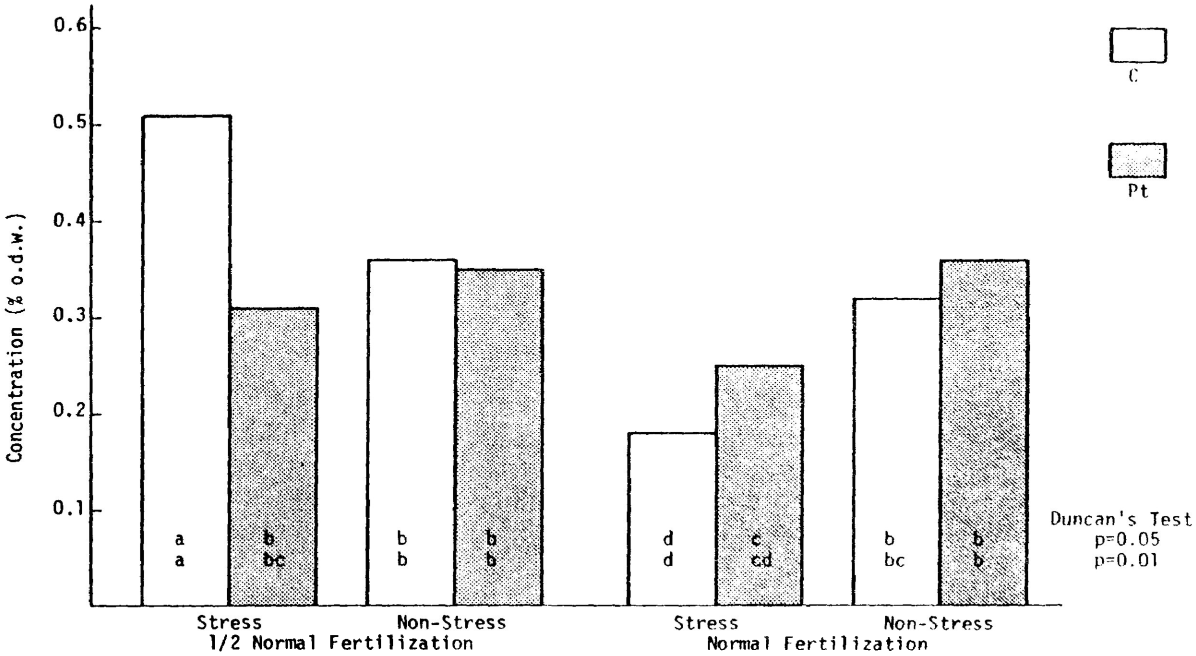


Figure 5.9: Greenhouse Study seedlings - Concentration of K in the root tissues

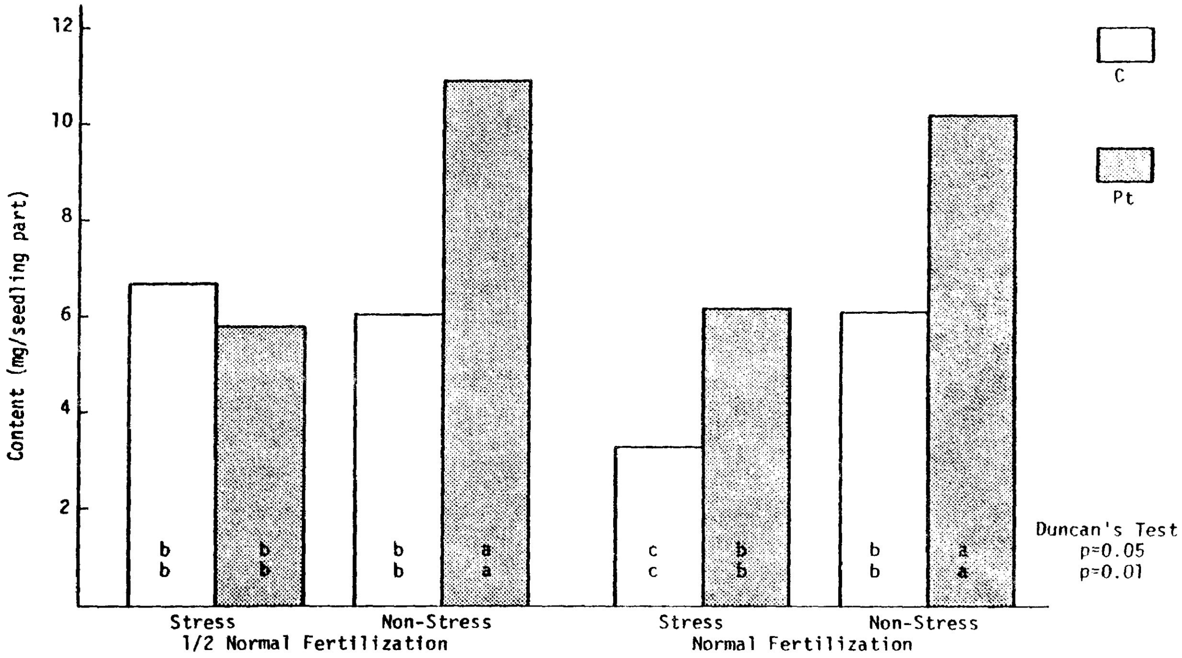


Figure 5.10: Greenhouse Study seedlings - Content of K in the root tissues

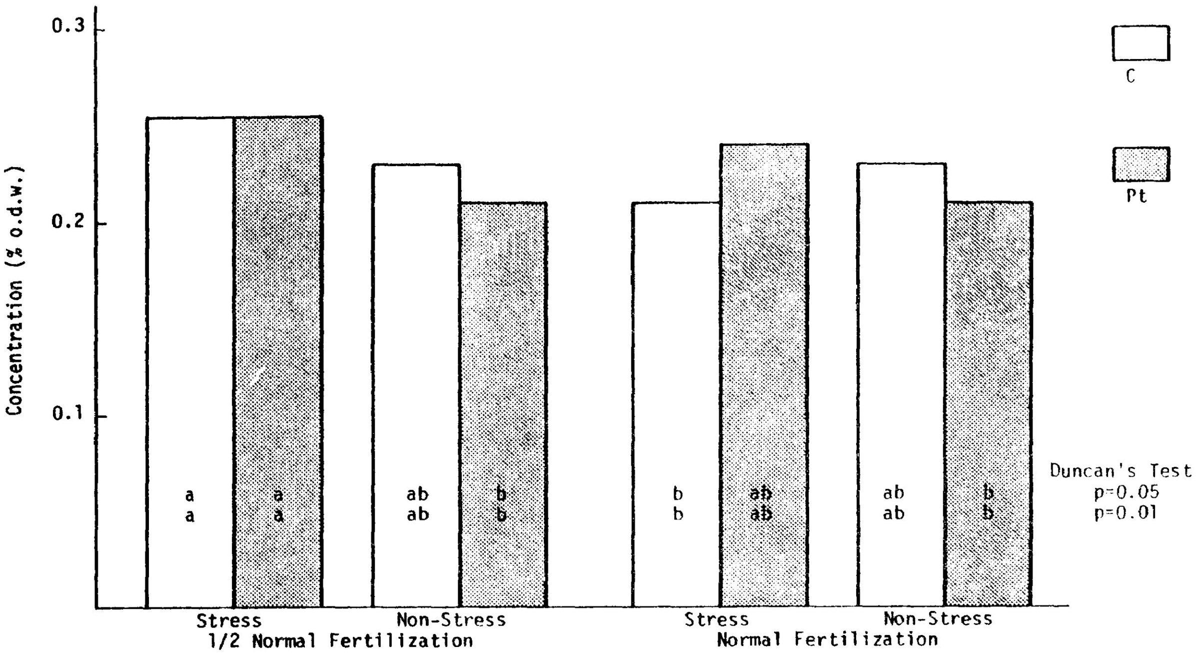


Figure 5.11: Greenhouse Study seedlings - Concentration of P in the root tissues

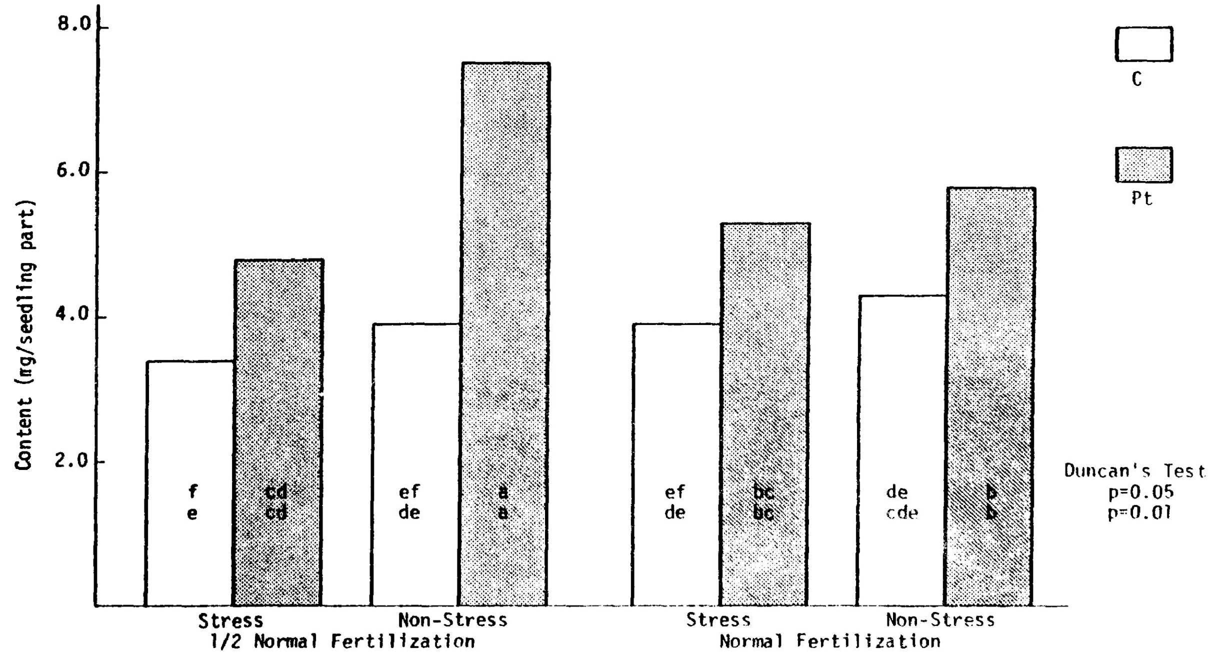


Figure 5.12: Greenhouse Study seedlings - Content of P in the root tissues

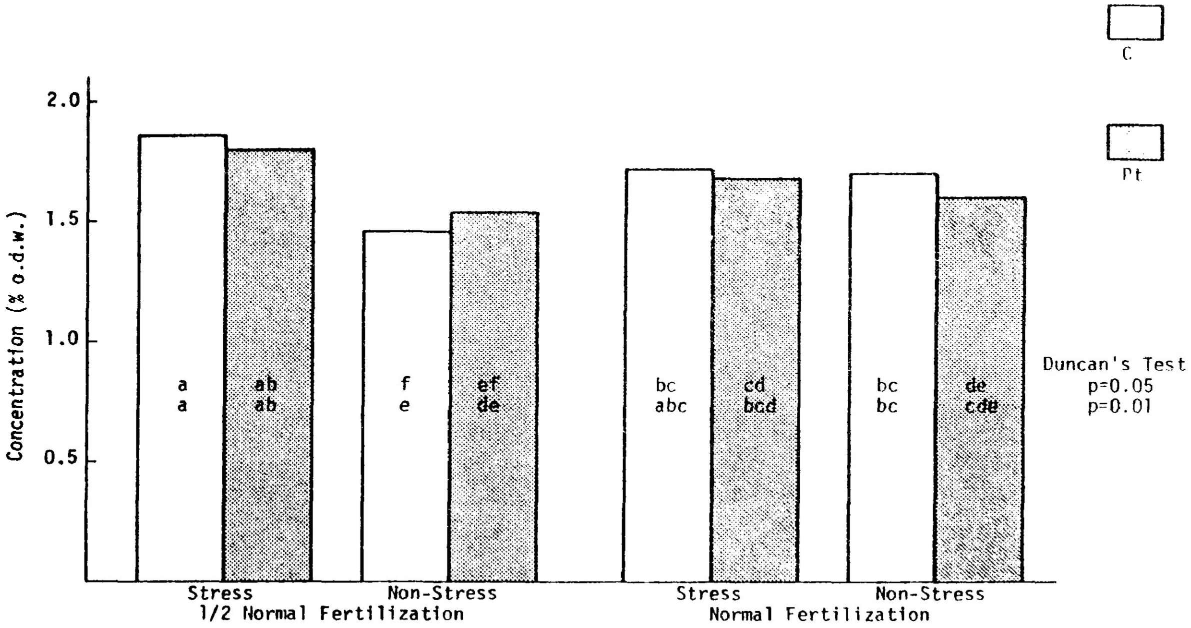


Figure 5.13: Greenhouse Study seedlings - Concentration of N in the second season tissues

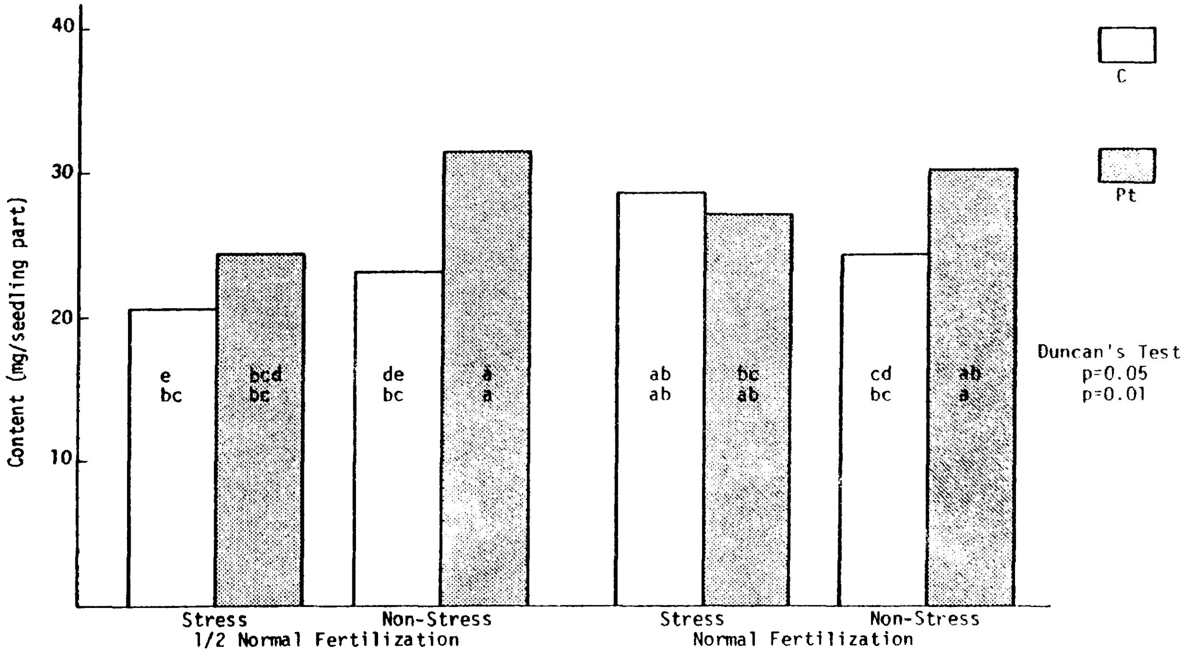


Figure 5.14: Greenhouse Study seedlings - Content of N in the second season tissues

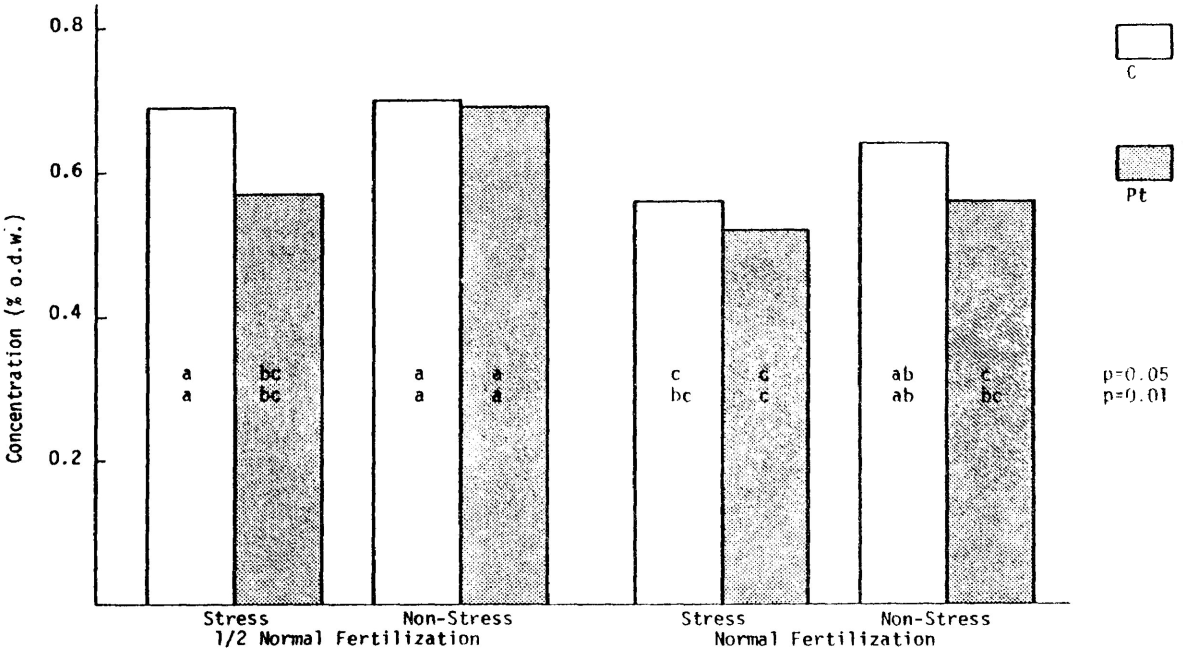


Figure 5.15: Greenhouse Study seedlings - Concentration of K in the second season tissues

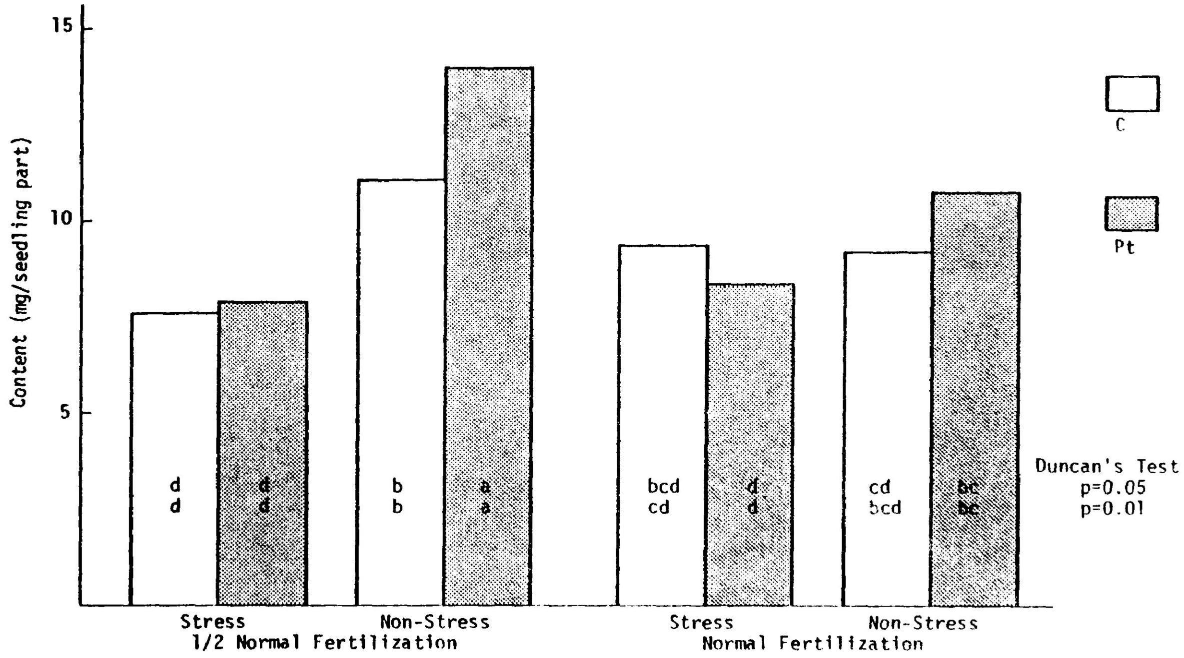


Figure 5.16: Greenhouse Study seedlings - Content of K in the second season tissues

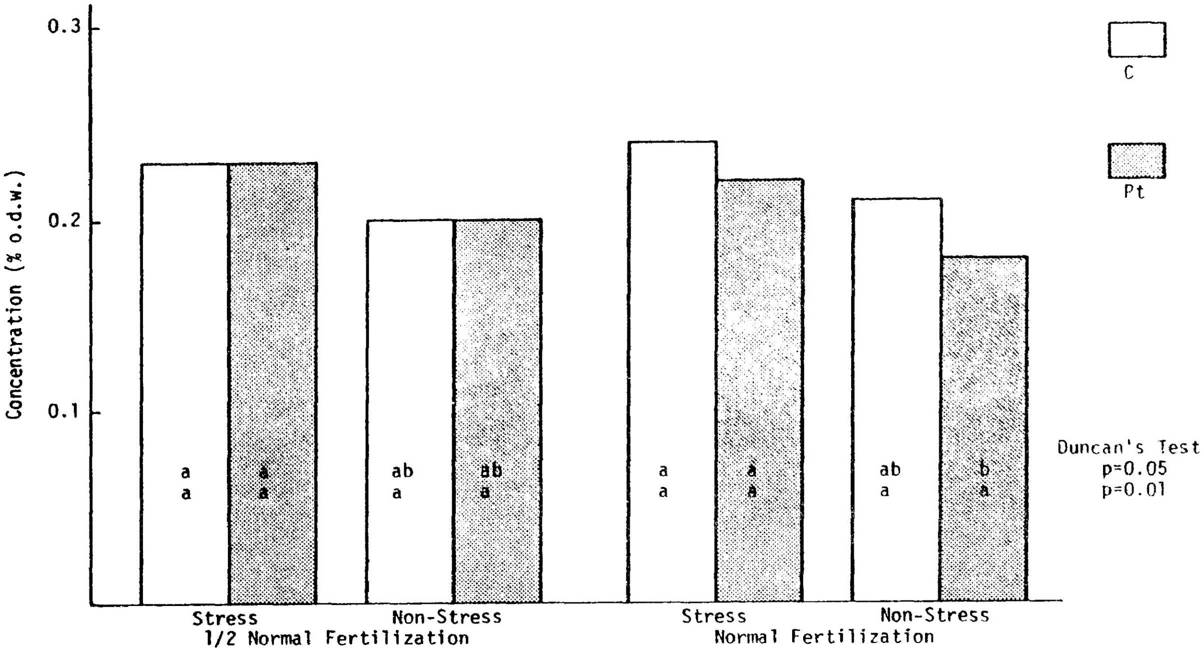


Figure 5.17: Greenhouse Study seedlings - Concentration of P in the second season tissues

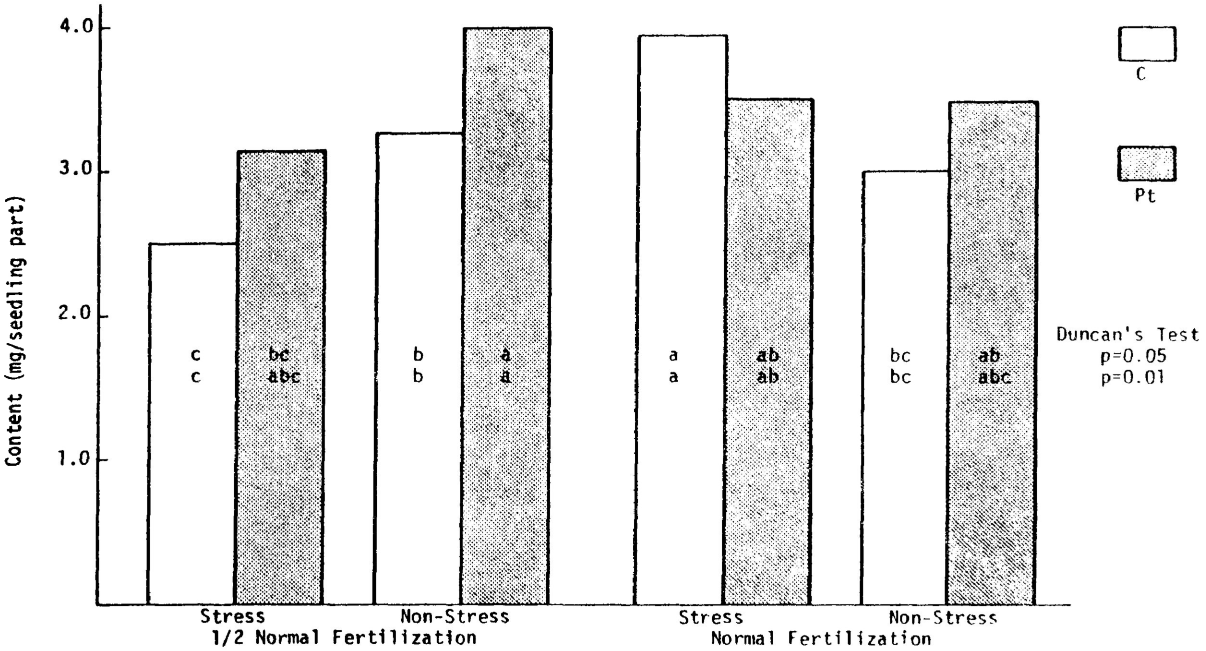


Figure 5.18: Greenhouse Study seedlings - Content of P in the second season tissues

Concerning nutrient content data of the greenhouse seedlings (Figures 5.2, 5.4, 5.6, 5.8, 5.10, 5.12, 5.14, 5.16 and 5.18):

1) For all elements and all comparable treatment pairs (except "Normal fertilization, Stress soil moisture" in the first season), mean nutrient contents of the Pt-colonized seedlings were larger than those of the Controls (Table 5.2). The differences were significant (p=0.05) in 23, out of a possible 36, cases (Table 6.5). The presence of a P. tinctorius association therefore enhanced the uptake of N, P, and K which, in turn, was reflected in higher contents in both the new and old tissues.

2) For all nutrients, except K in the root tissues, the range of mean content values was larger among seedlings raised on the lower fertilizer schedule. This indicates, as it did with the concentration data, that initial fertilization affected the subsequent influence of the other experimental variables.

3) The effect of soil moisture depended on the levels of initial fertilization and mycorrhizal colonization. The higher initial fertilizer program tended to increase nutrient content of Control seedlings regardless of soil moisture levels. This difference was significant in 8, out of a possible 16, cases (Table 5.6). In contrast, nutrient content of Pt-colonized seedlings growing under similar soil moisture conditions was minimally affected by the level of initial fertilization (the difference was significant at p=0.05 in only 2 of 16 cases). Generally, mean content values of Non-Stress, Pt-colonized seedlings were larger than those of similar seedlings grown under Stress soil moisture conditions (Table 5.2)

4) The highest mean nutrient contents were consistently exhibited by Pt-colonized seedlings raised on 1/2 Normal initial fertilization and subjected to Non-Stress soil moisture conditions (8 out of 9 cases); the lowest, by similarily fertilized Control seedlings subjected to soil moisture Stress (again, 8 out of 9 cases). In both instances the mean dry weight data correspond (Table 4.1)

In summary, the following statements describe the response of the greenhouse seedlings to the experimental variables as expressed through nutrient concentration and content:

1) The presence of a P. tinctorius association at the time of transplanting to the pots reduced mean nutrient concentration and enhanced mean nutrient content.

2) The extent of the mycorrhizal effect was dependant on the level of initial fertilization. More significant differences between comparable mycorrhizal and Control seedlings occurred among those raised on the 1/2 Normal fertilizer regime.

3) The effect of soil moisture was complicated and depended on the level of initial fertilization, the presence or absence of P. tinctorius ectomycorrhizae and the nutrient element involved.

4) As mentioned previously, mean content values corresponded very closely to mean dry weight values (Table 4.3). As dry weight increased, so did nutrient content. This relationship was evident for all nutrients in all seedling parts (Table 5.7). At the same time, nutrient concentration tended to decrease as dry weight increased. The correlations between dry weight and concentration were not nearly as strong as those

between dry weight and content. Figure 5.19, on the following page, illustrates these two relationships for N in the root tissues. Trends for the other elements in the various tissues were similar, as can be seen from Table 5.7 below.

Table 5.7: Greenhouse Study seedlings - Results of the regression of mean nutrient content and concentration on dry weight.

Element	Concentration			Content		
	b_o*	b_1**	r***	b_o	b_1	r
First Season						
N	1.179	-0.193	0.48	0.444	4.868	0.79
K	0.600	-0.097	0.47	0.217	2.482	0.82
P	0.219	-0.013	0.24	0.117	1.263	0.85
Roots						
N	1.338	-0.142	0.84	6.200	7.167	0.97
K	0.414	-0.039	0.25	0.368	3.049	0.74
P	0.278	-0.022	0.64	0.384	2.091	0.96
Second Season						
N	1.807	-0.148	0.31	14.574	12.404	0.92
K	0.760	-0.153	0.59	6.757	3.199	0.42
P	0.237	-0.025	0.34	2.038	1.386	0.77

* - y-intercept
** - slope
*** - correlation coefficient

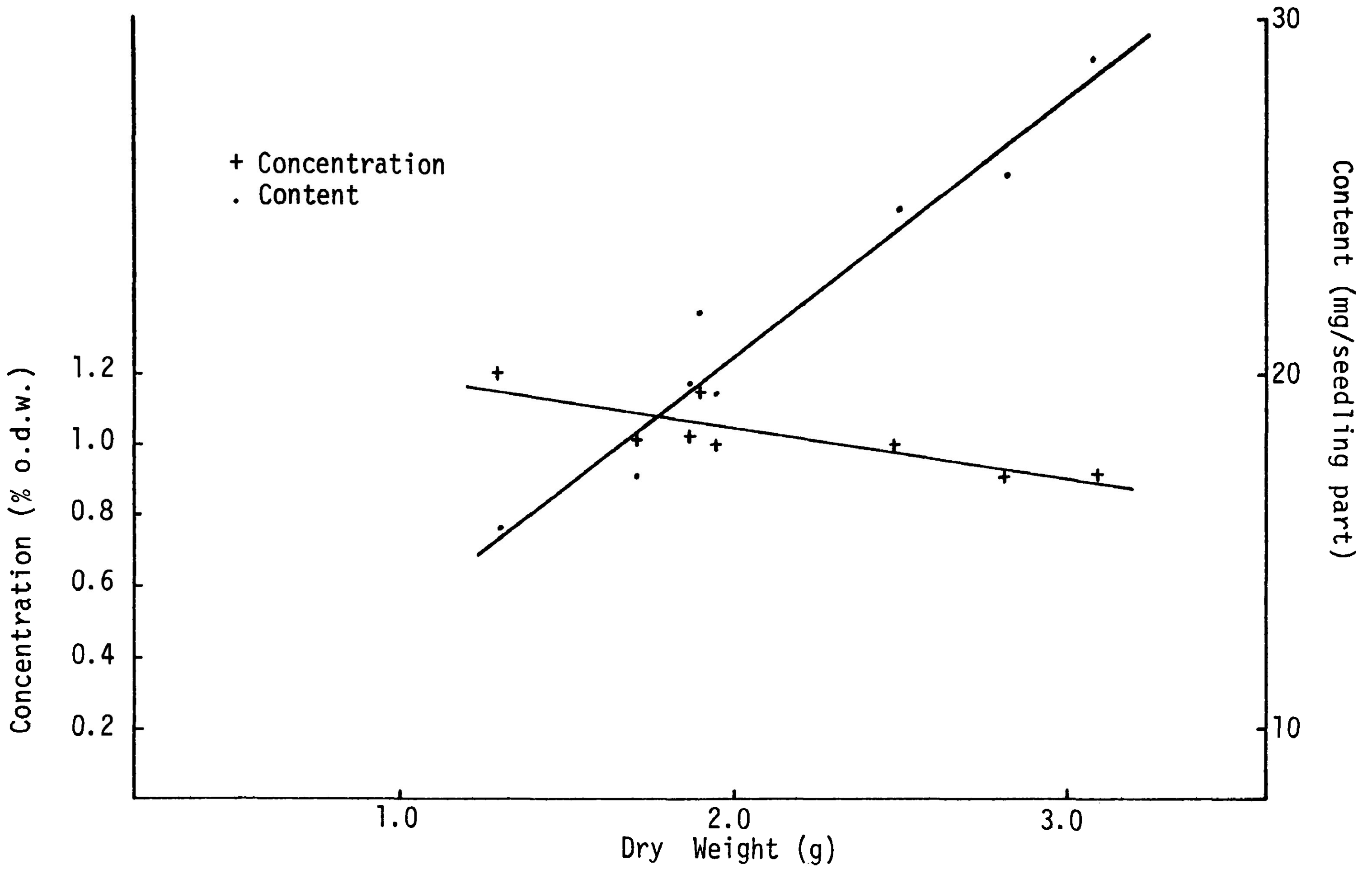


Figure 5.19: Greenhouse Study seedlings - Content and Concentration of N vs. Dry Weight of roots

5.3 Nutrient composition of the Outplanted seedlings

Treatment means for the concentration and content data of the outplanted seedlings are presented in Table 5.8. The results of the analysis of variance for these measurements was not uniform as can be seen from Table 5.9. Results of Duncan's test (Table 5.10), however, suggest several trends; some of which are similar to those outlined for the Greenhouse study seedlings. Figures 5.20 to 5.28 illustrate the outplanted seedling nutrient data, and the following statements describe seedling response to the experimental variables:

1) For both concentration and content, initial fertilization determined the subsequent effect of mycorrhizal colonization on nutrient status. Differences between Pt-inoculated and Control seedlings were larger and more apt to be significant (p=0.05) when the seedlings were raised on the 1/2 Normal fertilizer schedule (13 cases out of a possible 18).

2) Among concentration means, those of the Pt-inoculated seedlings were consistently (16 out of 18 possible cases) lower than those of the comparable Controls (Table 5.8). Of these, eight cases were significant at p=0.05 (Table 5.10).

3) Among content means, those of the Pt-inoculated seedlings consistently (17 out of 18 possible cases) exceeded those of comparable Controls. Of these, thirteen cases were significant at p=0.05.

Table 5.8: Outplanted seedlings - Treatment means for nutrient concentration and content

Myc Colonization	Concentration*				Content**			
	Control		Pt-Inoculated		Control		Pt-Inoculated	
Fertilization	1/2F	F	1/2F	F	1/2F	F	1/2F	F
<u>First Season</u>								
N	0.90	0.70	0.80	0.65	5.16	7.00	9.68	8.94
K	0.48	0.41	0.36	0.45	2.76	4.08	4.38	6.19
P	0.12	0.12	0.11	0.09	0.70	1.21	1.25	1.19
<u>Roots</u>								
N	1.41	1.01	1.03	0.97	4.80	8.95	11.19	10.40
K	0.54	0.51	0.44	0.46	2.48	4.56	4.76	5.02
P	0.23	0.23	0.20	0.21	1.06	2.04	2.09	2.28
<u>Second Season</u>								
N	1.81	1.76	1.67	1.76	35.47	65.38	91.59	81.28
K	0.65	0.71	0.74	0.59	12.94	26.62	40.29	26.97
P	0.23	0.23	0.22	0.21	4.49	8.45	12.19	9.76

*Concentration measured as % oven dry weight
**Content measured as mg/seedling part

Table 5.9: Outplanted seedlings - Analysis of variance for nutrient concentration and content

	SOURCE OF VARIATION							
Tissue and Element		Concentration				Content		
	Blocks	Myc (M)	Fert (F)	M-F	Blocks	Myc (M)	Fert (F)	M-F
First Season								
N	ns	**	**	ns	ns	**	ns	ns
K	ns	ns	ns	**	ns	**	**	ns
P	ns	ns	ns	ns	ns	ns	ns	ns
Roots								
N	ns	ns	**	ns	ns	**	**	**
K	**	**	ns	ns	ns	**	**	**
P	ns	ns	ns	ns	ns	**	**	ns
Second Season								
N	ns	ns	ns	ns	ns	**	ns	**
K	ns	ns	ns	**	ns	**	ns	**
P	ns	ns	ns	ns	ns	**	ns	*

ns - not significant * - significant at p-0.05 ** - significant at p=0.01

Table 5.10: Outplanted seedlings - Results of Duncan's test for nutrient concentration and content

Myc Colonization		Concentration				Content			
		Control		Pt-Inoculated		Control		Pt-Inoculated	
Fertilization		1/2F	F	1/2F	F	1/2F	F	1/2F	F
First Season									
N	0.05	a	bc	b	c	c	b	a	a
	0.01	a	bc	b	c	c	bc	a	ab
K	0.05	a	b	b	ab	c	b	b	a
	0.01	a	ab	b	a	c	b	b	a
P	0.05	a	a	a	a	b	a	a	a
	0.01	a	a	a	a	b	ab	a	ab
Roots									
N	0.05	a	bc	b	c	c	b	a	a
	0.01	a	b	b	b	c	b	a	ab
K	0.05	a	a	b	b	b	a	a	a
	0.01	a	ab	c	bc	b	a	a	a
P	0.05	a	a	a	a	b	a	a	a
	0.01	a	a	a	a	b	a	a	a
Second Season									
N	0.05	a	ab	b	ab	c	b	a	a
	0.01	a	a	a	a	c	b	a	ab
K	0.05	b	ab	a	c	c	b	a	b
	0.01	bc	ab	a	c	c	b	a	b
P	0.05	a	a	a	a	c	b	a	ab
	0.01	a	a	a	a	b	ab	a	a

4) For all elements in all seedling parts, the highest mean concentrations (5 cases out of a possible 9) were exhibited by Control seedlings raised on the lower fertilizer schedule (C-1/2F). In contrast, seedlings from this treatment recorded the lowest mean nutrient contents in all of 9 possible comparisons. Furthermore, the mean dry weights of C-1/2F seedlings were also consistently the lowest (Table 4.7). It would appear that these seedlings were capable of accumulating nutrients (i.e. high concentrations) but not of producing tissues (i.e. smallest dry weight).

5) The lowest mean concentrations, as well as the greatest mean contents, were exhibited by Pt-inoculated seedlings. The former attribute being more characteristic of Normally fertilized seedlings (5 out of 9 cases); the latter of seedlings raised on the lower fertilizer regime (6 out of 9 cases). Once again, dry weights corresponded very closely to nutrient contents and to the inverse of nutrient concentration. In comparison to the Control seedlings, these seedlings appeared capable of increased uptake (i.e. higher contents) and enhanced tissue production (i.e. larger dry weights). Lower concentration levels were therefore the result of the dilution effect, not of poor uptake mechanisms.

6) N concentration was lowest in first season tissues (0.65-0.90%, Figure 5.20), increased in the roots (0.96-1.41%, Figure 5.23) and peaked in second season tissues (1.66-1.81%, Figure 5.26).

Significant differences did exist (Table 5.10) although the range of values was narrow (Table 5.8).

N content means of roots (Figure 5.23) and first season tissues (Figure 5.20) were very close (4.8-11.2 mg/seedling) in comparison to the range of second season values (35.0-92.0 mg/seedling, Figure 5.26). Dry weight enhanced the differences between the seedling parts, however, the ranking of means and the results of Duncan's test (p=0.05) were exactly the same for all three morphological parts. The ratio of N content in the roots to that in first season and second season tissues was computed and the data are presented in Table 5.11. Ratios of Control and Pt-inoculated seedlings raised on either fertilization schedule were extremely similar. This indicates that regardless of mycorrhizal colonization or initial fertilization, the percentage of N being translocated to the newer tissues was uniform. This, in turn, emphasizes the importance of nutrient uptake and the role a mycorrhizal association could play in improving and expanding the uptake mechanisms.

7) Potassium was the element which consistently did not agree with the general trends. For example, in second season tissues, both the highest and the lowest concentration means were attributed to Pt-inoculated seedlings, 1/2 F and F treatments respectively (Figure 5.27). In the roots (Figure 5.24) and first season tissues (Figure 5.21) Pt-1/2F seedlings recorded the lowest K

concentration means while similarily fertilized Controls recorded the highest ones. In compliance with the other elements, C-1/2F seedlings exhibited the lowest mean K contents; in contrast, however, the highest K contents in roots and first season tissues were were attributed to Pt-F seedlings. K content of second season tissues was greatest in Pt-1/2F seedlings in agreement with the general trends.

Table 5.11: Ratios of nutrient content in the Outplanted seedlings

Nutrient	Treatment	Roots	:	First Season	:	Second Season
N	C - 1/2F	1	:	1.08	:	7.39
	C - F	1	:	0.78	:	7.31
	Pt- 1/2F	1	:	0.86	:	8.18
	Pt- F	1	:	0.86	:	7.82
K	C - 1/2F	1	:	1.11	:	5.22
	C - F	1	:	0.89	:	5.84
	Pt- 1/2F	1	:	1.09	:	8.46
	Pt- F	1	:	1.23	:	5.37
P	C - 1/2F	1	:	0.66	:	4.24
	C - F	1	:	0.59	:	4.14
	Pt- 1/2F	1	:	0.60	:	5.83
	Pt- F	1	:	0.52	:	4.28

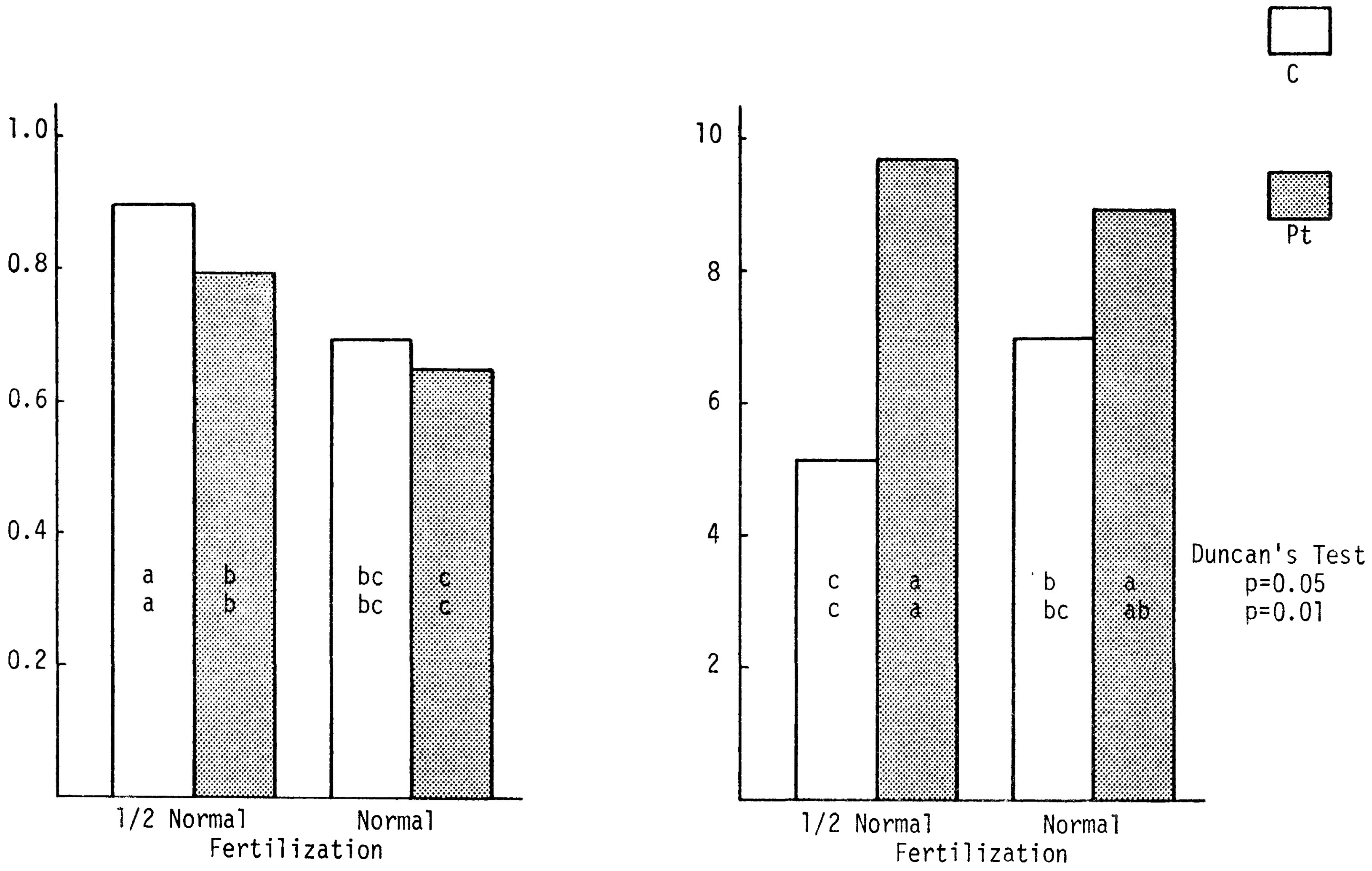
In the root tissues (Figure 5.24) only the mean K content of C-1/2F seedlings differed significantly (p=0.01) from those of the other treatments. This implies that the combination of a lack of _P. tinctorius_ ectomycorrhizae and reduced initial fertilization was necessary in order to depress K content markedly. Most likely, the lower K content was related to reduced tissue production; C-1/2F seedlings also exhibited the lowest mean root dry weight (Table 4.7)

Nutrient data of first season tissues was similar to that of the roots. C-1/2F seedlings exhibited the highest K concentration, the lowest K content (Figure 5.21) and the smallest first season dry weight(Figure 4.2). For comparison, concentrations of Control and Pt-inoculated seedlings did not differ, however, content and dry weight of the latter exceeded that of the former significantly (p=0.01). Interestingly, the ratio computations presented in Table 5.11 show this difference between the roots and first season tissues of Normally fertilized seedlings. It must however be kept in mind that translocation of the mobile elements (such as K), especially during periods of new growth, is directed toward the newer tissues (van Den Driessche 1974) and will therefore affect this ratio. Subsequently, the root:first season content ratio is an indication of nutrient abundance. A higher ratio implies a larger first season residual after the newer tissues have been supplied which, in turn, implies a higher initial uptake.

Mean K contents of second season tissues (Table 5.8) followed the general trends; the computed ratios, however, showed a dramatic increase in the amount translocated by Pt-1/2F seedlings to the newer tissues. This was accompanied by higher concentration and dry weight values (Table 4.7) which indicated that not only were Pt-1/2F seedlings absorbing and distributing larger amounts of K, they were also producing larger amounts of tissue. The superior performance of Pt-1/2F seedlings, especially after the second growing season, has been documented throughout this report. Perhaps the ability to translocate and absorb larger amounts of K was one of the factors which contributed to this superiority.

8) Within each seedling part, the concentration of P did not vary according to treatment (Figures 5.22, 5.25, and 5.28). The range of mean concentration in root and second season tissues was from 0.20% to 0.24% oven dry weight; approximately twice that of the first season tissues (0.088% to 0.125% o.d.w.). Content of P, however, varied widely depending on the seedling part and the treatment. In first season and root tissues, contents ranged from 0.7 to 2.0 mg per seedling, while those of second season tissues rose to a range of 4.0 to 12.0 mg per seedling. These differences are clearly illustrated by the ratio computations in Table 5.11. Once again, the roots:second season P content ratio of Pt-1/2F seedlings was larger than all others. The explanation employed for K content may be valid in this case as well. Soil P, however, is a more mobile ion than soil K and this may account for the smaller difference in content ratios.

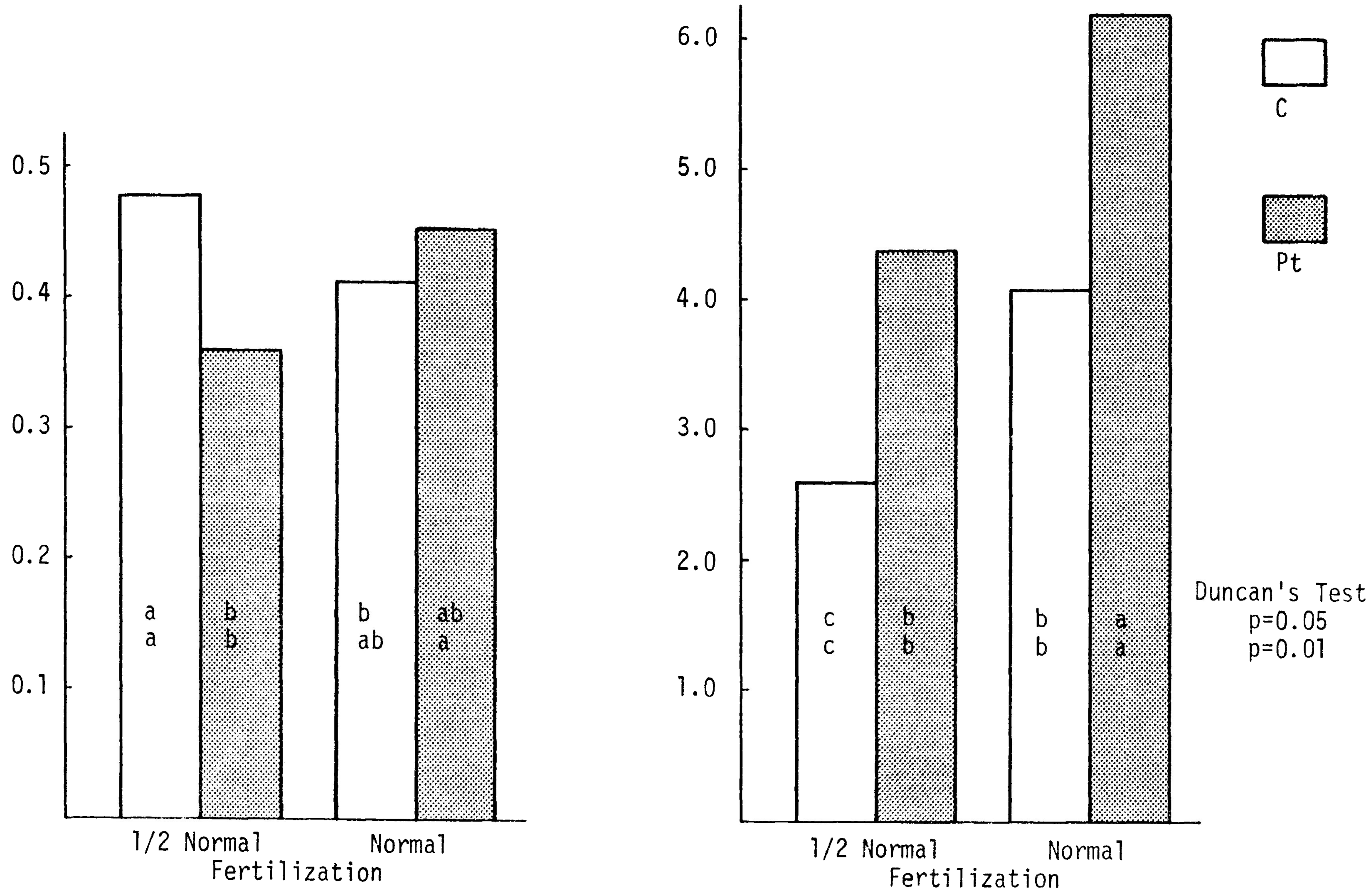
In summary, it appears that the combination of Pt-inoculation and 1/2 Normal fertilization affected the uptake and distribution characteristics of both P and K. Nitrogen was not influenced by the experimental variables, however, that may have been a result of an abundance of the element in the soil.



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

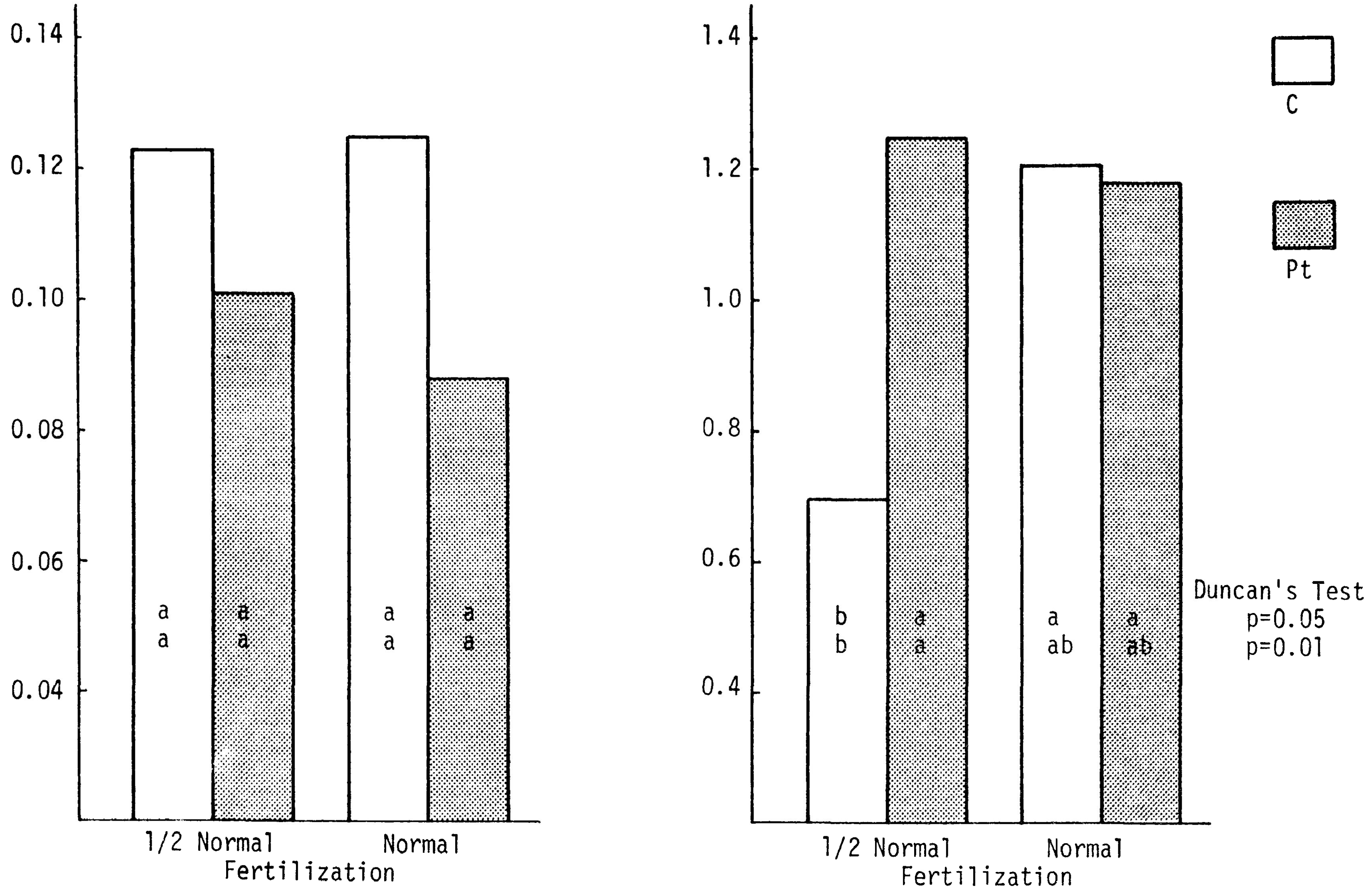
Figure 5.20: Outplanted seedlings - Concentration and Content of N in the first season tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

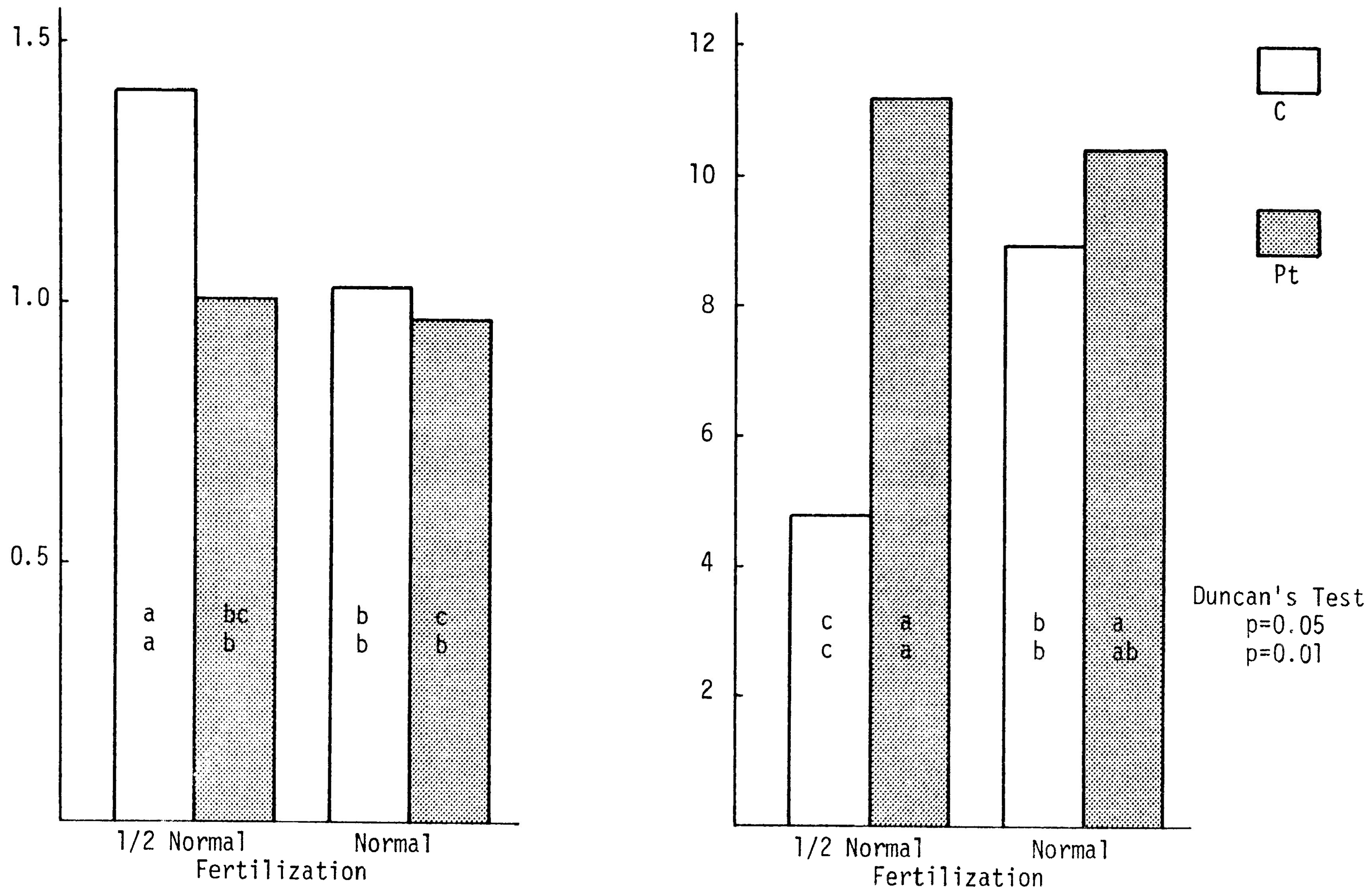
Figure 5.21: Outplanted seedlings - Concentration and Content of K in the first season tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

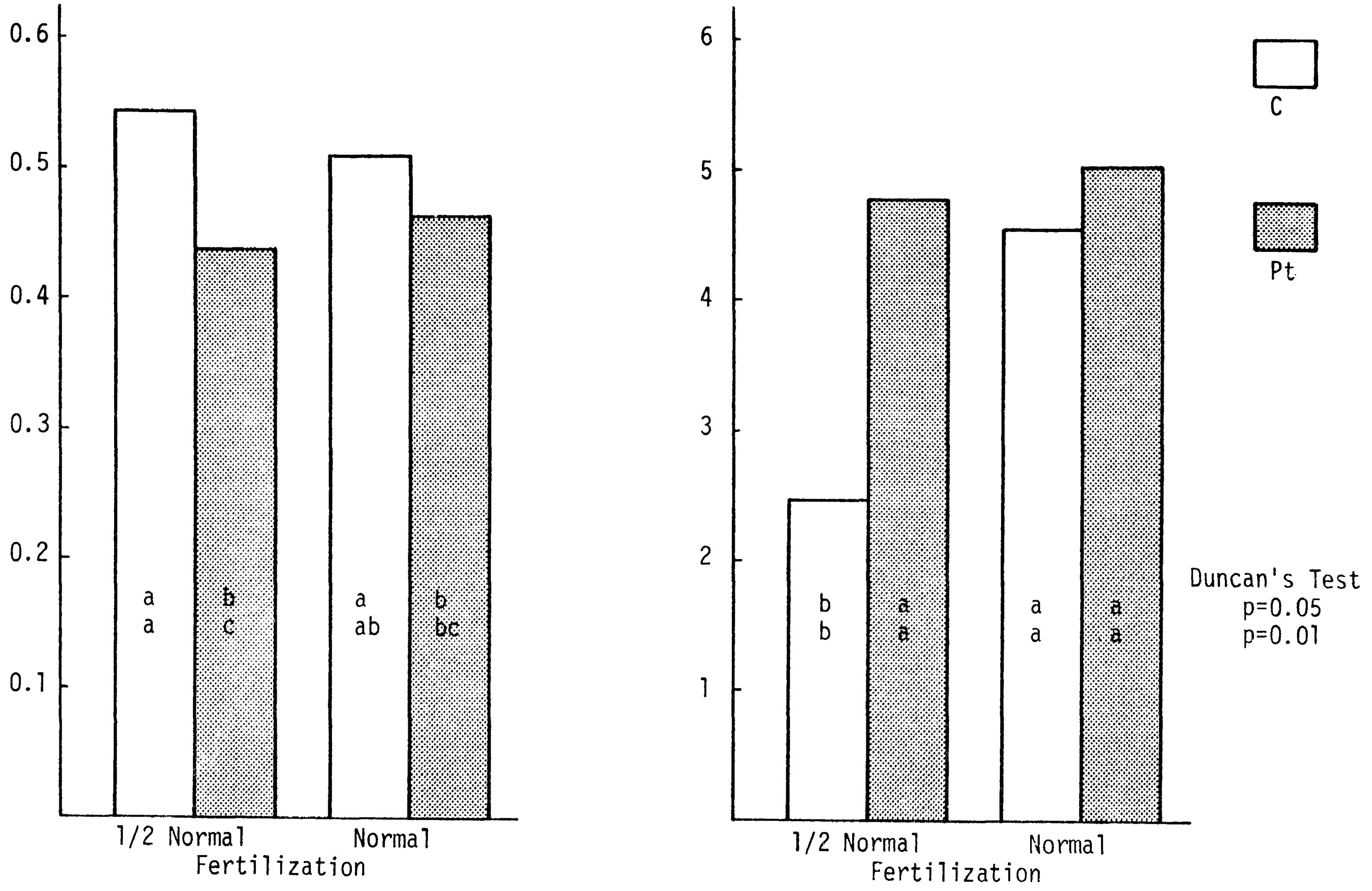
Figure 5.22: Outplanted seedlings - Concentration and Content of P in the first season tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

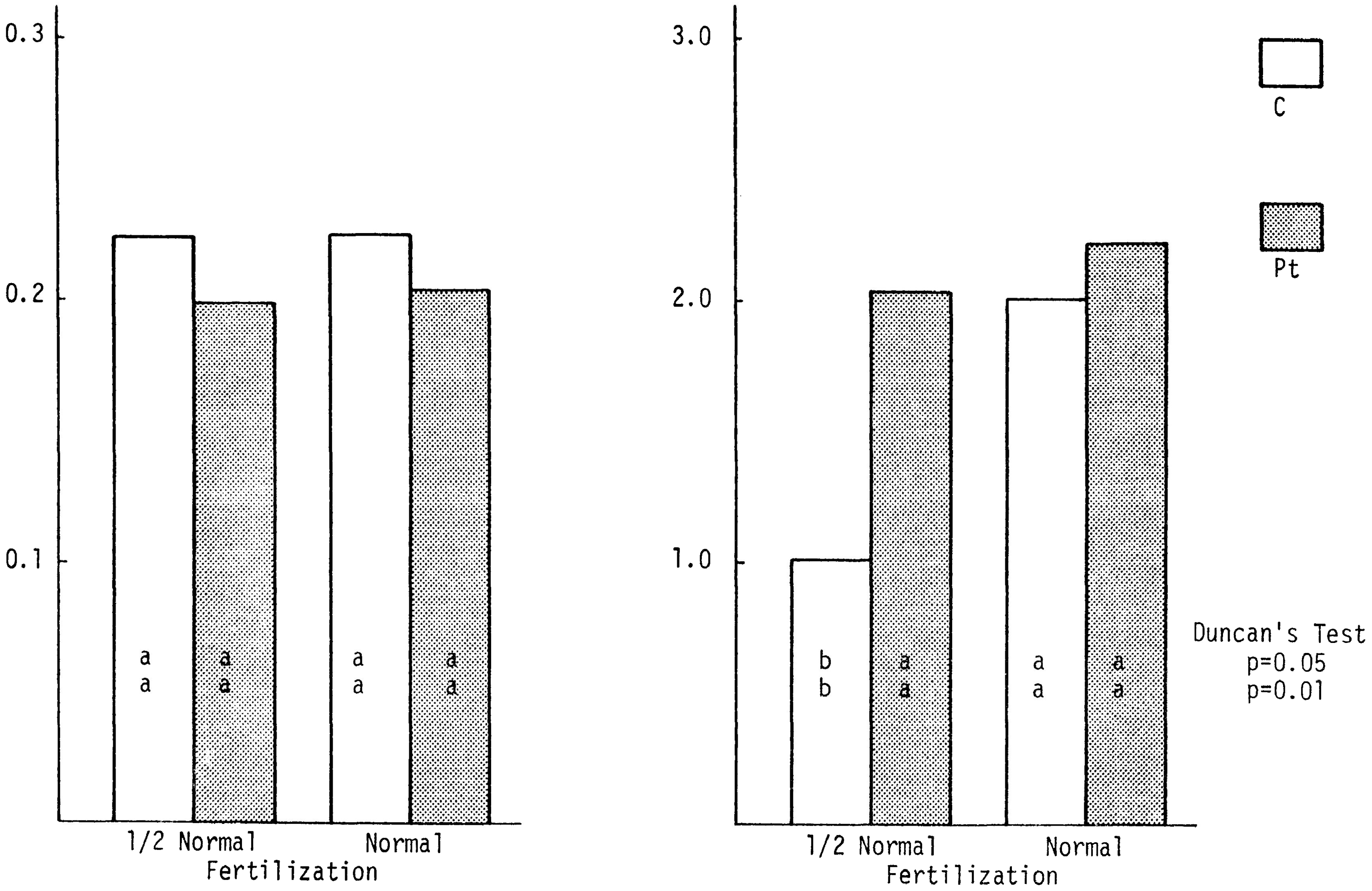
Figure 5.23: Outplanted seedlings - Concentration and Content of N in the root tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

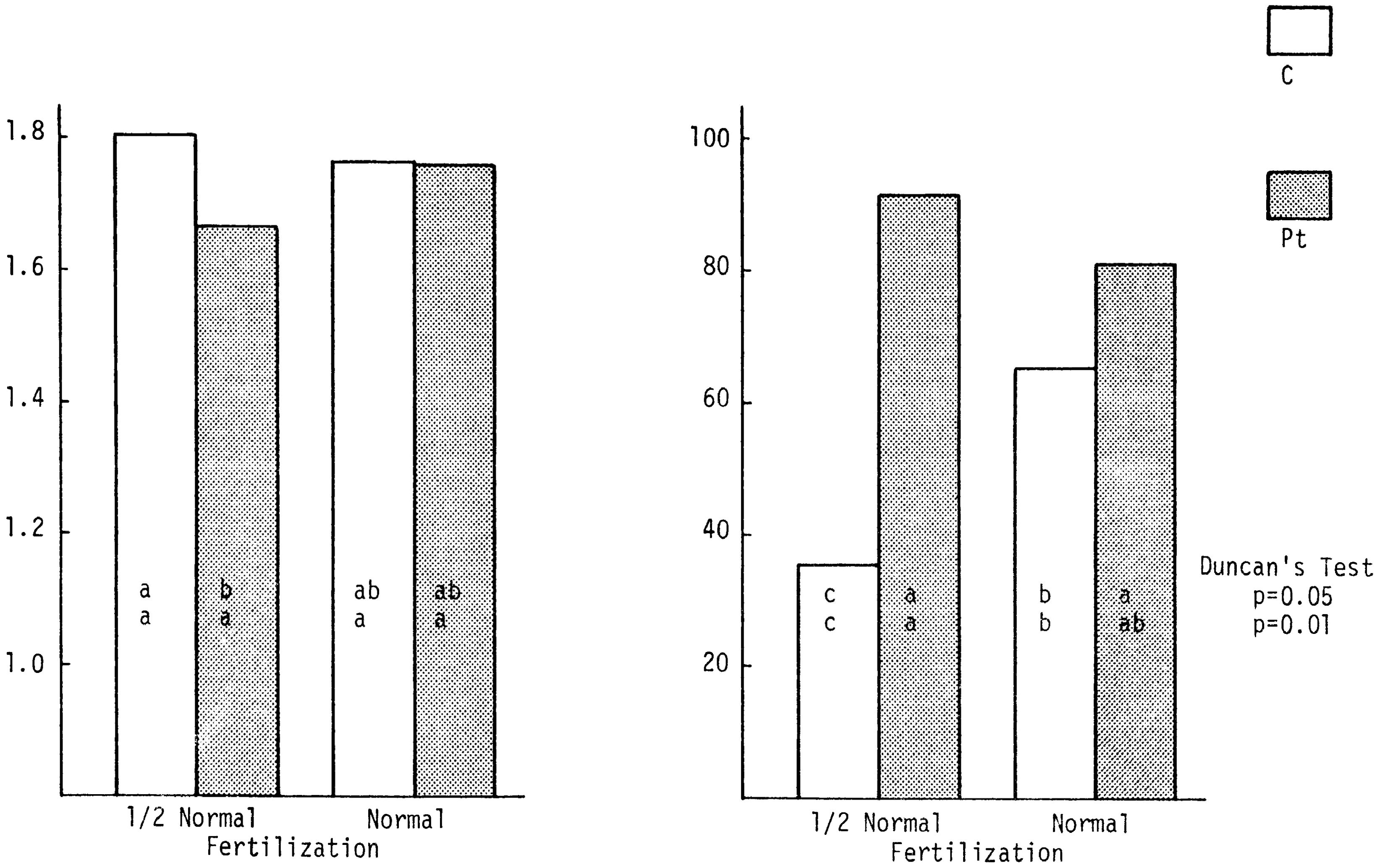
Figure 5.24: Outplanted seedlings - Concentration and Content of K in the root tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

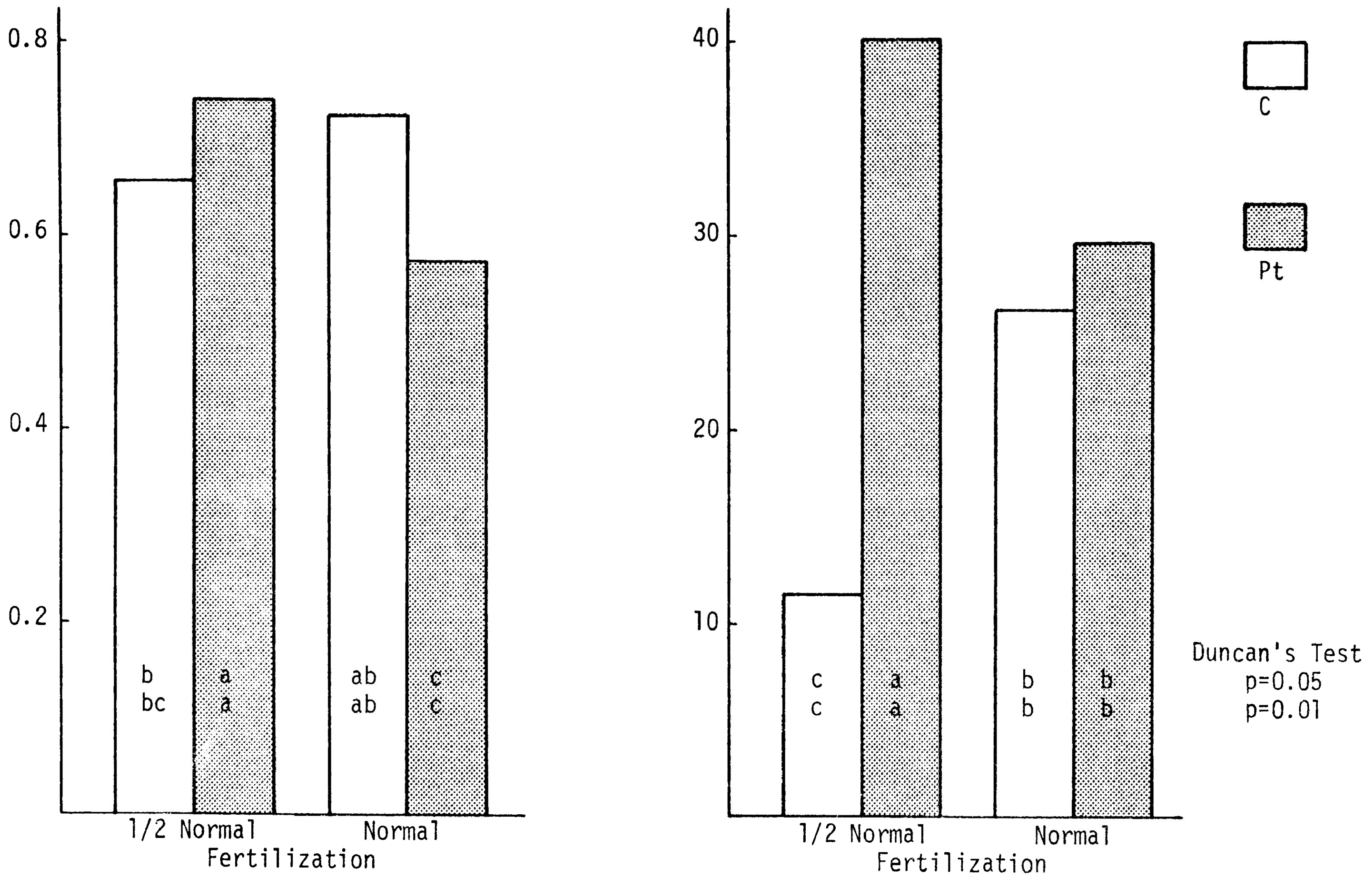
Figure 5.25: Outplanted seedlings - Concentration and Content of P in the root tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

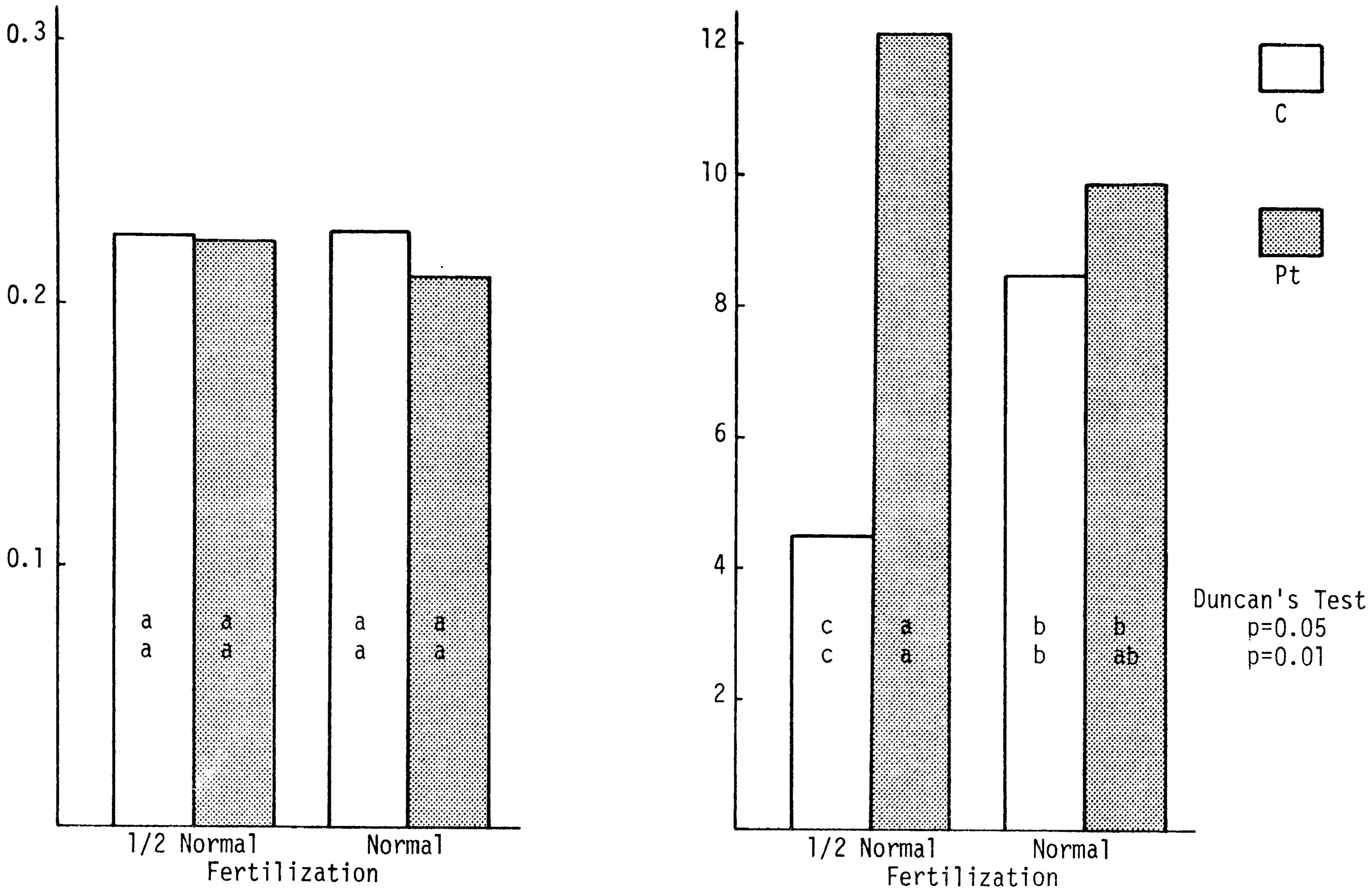
Figure 5.26: Outplanted seedlings - Concentration and Content of N in the second season tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

Figure 5.27: Outplanted seedlings - Concentration and Content of K in the second season tissues



a. Concentration (% o.d.w.)

b. Content (mg/seedling part)

Figure 5.28: Outplanted seedlings - Concentration and Content of P in the second season tissues

6.0 RESULTS OF THE MYCORRHIZAL ASSESSMENT

6.1 Visual assessment

Exposure of the seedling roots to natural soil, either in the greenhouse or at the planting site, resulted in changes to the ectomycorrhizal associations. New infections were established and the initial level of P. tinctorius colonization modified. Based on visual appearance, three types of ectomycorrhizae were distinguished on the roots of seedlings from both study locations. These three types were labelled "Pt-like", "Black" and "Brown" for the purposes of this report.

"Pt-like" refers to ectomycorrhizae typical of those produced on the roots of seedlings inoculated with P. tinctorius in the original I.M.R.D. study and subsequently verified as P. tinctorius by D. Marx (Table 3.1). The structures were white to yellow in colour, noticeably swollen and exhibited simple forking.

"Black" denotes a group of ectomycorrhizae which were black to very dark brown in colour, not swollen except very slightly at the tips and showing simple forking. The gross appearance of these structures suggested that the mycobiont responsible may have been Cenococcum graniforme.

"Brown" describes the type which were reddish-brown to orange in colour with lighter, almost yellow, tips. The tip and much of the rootlet was swollen and simple to complex branching was noted. These ectomycorrhizae resembled the structures often associated with Thelephora terrestris.

No attempts were made to verify the identities of the fungi causing the "Black" and "Brown" types of ectomycorrhizae. Publications describing the mycorrhizae of Boreal tree species are scarce and therefore identification is very difficult. Furthermore, under the experimental conditions, local Basidiomycetes may have produced structures unlike those produced naturally. Likewise, the classic appearance of P. tinctorius (Coker and Couch 1928) may also been altered. The scope of this assessment is therefore limited. It is sufficient, however, to indicate some trends in the mycobionts' responses to the experimental variables. Tables 6.1 and 6.2 present the pertinent data for the greenhouse and outplanted seedlings.

Table 6.1: Visual assessment of "Pt-like" ectomycorrhizae on roots of Greenhouse Study and Outplanted seedlings

Location and Treatment	"Pt-like" Ectomycorrhizae Before Transplanting %trees	%roots	After Lifting* %trees	%roots
Greenhouse Study				
Pt-1/2F-S	100*	50+	55	8
Pt-1/2F-NS	100	50+	65	11
Pt-F-S	100	50+	55	14
Pt-F-NS	100	50+	70	13
Outplanting				
Pt-1/2F	85**	36	15	7
Pt-F	60	8	10	5

* as determined by the author
**as determined by I.M.R.D.

Table 6.2: Visual assessment of "Other Species" ectomycorrhizae on the roots of Greenhouse Study and Outplanted seedlings

Location and Treatment	"Other Species" Ectomycorrhizae				
	B.T.*	"Black"**		"Brown"**	
	% roots	%trees	%roots	%trees	%roots
<u>Greenhouse Study</u>					
C-1/2F-S	6.4	75	14	95	17
C-1/2F-NS		35	12	100	21
C-F-S	2.2	55	11	100	24
C-F-NS		35	16	100	38
Pt-1/2F-S	2.9	50	8	95	30
Pt-1/2F-NS		65	10	95	29
Pt-F-S	2.2	50	13	100	33
Pt-F-NS		55	11	100	29
<u>Outplanting</u>					
C-1/2F	6.4	45	21	90	45
C-F	2.2	35	23	95	35
Pt-1/2F	2.9	40	29	100	45
Pt-F	2.2	50	34	70	50

* Before Transplanting - as determined by I.M.R.D.
**After Lifting - as determined by the author

The following trends were exhibited by the seedlings:

1) The number of seedlings displaying "Pt-like" ectomycorrhizae decreased over the second growing season. Table 3.1 states that for Pt-inoculated (Outplanted) groups the percentage of mycorrhizal seedlings before transplanting were 60 and 85 for the Normal and 1/2 Normal fertilizer regimes. The data in Table 6.1 shows that these levels dropped to 10% and 15% respectively at the time of lifting (one year later). Lesser decreases occurred among the Pt-colonized (Greenhouse) seedlings. At the time of potting, all Pt-colonized seedlings displayed "Pt-like" ectomycorrhizae according to the sorting criterium. After a four month growing period, the percentage of seedlings with the "Pt-like" ectomycorrhizae had decreased to between 55 and 70.

2) The percentage of roots supporting "Pt-like" ectomycorrhizae also decreased from the time of transplanting. Pt-inoculated seedlings exhibited average initial Pt incidences of 8% and 36% for Normal and 1/2 Normal fertility as assessed by I.M.R.D. (Table 3.1). The second year assessment found colonization levels of 5% and 7% respectively (Table 6.1). Pt-colonized seedlings also recorded a reduction in "Pt-like" ectomycorrhizae from a level greater than 50% (visual estimation) to an average of 12%.

The above observations indicate that while P. tinctorius ectomycorrhizae are capable of surviving Boreal forest conditions, the species is not an aggressive competitor against the local mycobionts.

3) On the average, 50% of the mycorrhizal seedlings displayed "Black" ectomycorrhizae (Table 6.2). The percentage of roots colonized varied from 10% among the greenhouse seedlings to 30% among the planted seedlings; an increase from the 2-3% "other species" pre-transplanting level as assessed by I.M.R.D. (Table 3.1)

outplanted seedlings; an increase from the 2-3% "other species" pre-transplanting level assessed by I.M.R.D. (Table 3.1). The difference between infection levels of the greenhouse and outplanted seedlings indicates that mycobiont populations and/or soil conditions were more favourable towards colonization at the latter site. The results for Control seedlings (Table 6.2) were very similar indicating a lack of either enhancement or inhibition caused by the initial _P. tinctorius_ inoculations.

4) The number of trees which displayed ectomycorrhizae of the "Brown" type was uniformly high, averaging between 90% and 100% for both the greenhouse and outplanted seedlings. The levels of colonization by this type were not markedly affected by any of the experimental variables. Site, however, enhanced slightly the percent colonization of the outplanted seedlings. Results of the visual assessment for "Brown" ectomycorrhizae on Control seedlings were again similar to those for the mycorrhizal seedlings.

The preceding observations concerning "other species" of mycobionts indicate that 1) initial _P. tinctorius_ inoculation neither hindered nor helped colonization of the roots by other mycorrhiza-formers, 2) that competition from other symbionts was probably more intense at the outplanting site than under the artificial conditions of the greenhouse, and 3) that neither initial fertilization nor soil moisture appear to have affected the reductions in _P. tinctorius_, nor the increase in "other species", ectomycorrhizae.

6.2 Isolations

The primary objective of the isolation attempts was the re-isolation of P. tinctorius from the roots of tranplanted seedlings. Success would prove that the fungus had survived the winter conditions of the Boreal forest in Northwestern Ontario. A second objective, based on the results of the visual survey, was to verify that P. tinctorius was associated with the "Pt-like" ectomycorrhizae. Table 6.3 summarizes the results of 300 isolation attempts made in three sessions over the course of one week.

The data show that large numbers of the original plates were contaminated by other organisms present on the seedling roots. Contaminants most often noted were species of Penicillium, Pythium and an unidentified bacteria. Due to the relatively fast rate of growth of the contaminants compared to the slower rate of the Basidiomycetes, it was difficult to obtain pure cultures. As evidenced by the increase in numbers of sterile plates (Table 6.3, Series #3), an attempt was made to reduce contamination by lengthening (30 seconds) the time of dipping in the surface sterilant.

In general, the isolation of mycorrhizal fungi is difficult. In addition to balancing the sterilization procedures to favour the Basidiomycetes, the growing media must also be appropriate. The modified Melin-Norkrans media (MMN) used in this study has proven successful with many mycobionts, including P. tinctorius (Marx 1969). Even under these conditions the success rate of isolations from P. tinctorius ectomycorrhizae is commonly less than 1% (W. Daniels, I.M.R.D., pers. comm.). Therefore, the relatively low number of cultures produced by the author corresponds well to those obtained by other workers.

Results of the isolation attempts were as follows:

1) P. tinctorius was re-isolated 8 times; seven of those from structures assessed as "Pt-like" by the author. Identification of the fungus was made on the basis of comparison to stock cultures maintained on MMN media. This result satisfied our two objectives, 1) that Pt was able to survive the winter conditions and 2) that the fungus was associated with the "Pt-like" ectomycorrhizae.

2) The production of a P. tinctorius culture from a "Brown" type ectomycorrhizae was not unexpected given the nature of mycorrhizal associations and the original levels of Pt ectomycorrhizae. It is not uncommon for more than one mycobiont to occupy a single root at any given time (Marks and Foster 1967; Wilcox 1968). Furthermore, mycelial strands can live for more than one season and may be in very close contact with ectomycorrhizae formed by other fungi (Bowen and Theodorou 1973). The lack of Pt cultures in the third series of isolations may indicate greater sensitivity of the fungus to the sterilizing procedures used.

3) Other Basidiomycetes were cultured from the roots of the experimental seedlings. A "white" and a "black" fungus were repeatedly isolated from both the greenhouse and outplanted seedlings. No pattern was apparent between the type of ectomycorrhizae ("Black" or "Brown") used for the isolation and the incidence of the two unknown fungi. "Pt-like" structures produced very few Basidiomycetes other than P. tinctorius. This suggests that the "Black" and "Brown" structures were the result of new infections, not modifications of the original Pt ectomycorrhizae.

Table 6.3: Results of the isolation attempts with ectomycorrhizae from Greenhouse Study and Outplanted seedlings

Series No.	Mycorrhizal Type	No. Isolations	No. Sterile	Basidiomycetes		No. Contaminated
				Pt	Other	
1	<u>Outplanted Seedlings</u>					
	Pt-like	20	4	4	4	8
	Black	40	6	-	9	25
	Brown	40	3	-	6	31
2	<u>Greenhouse Seedlings</u>					
	Pt-like	30	9	3	5	13
	Black	35	6	-	12	17
	Brown	35	11	1	15	9
3	<u>Greenhouse Seedlings*</u>					
	Pt-like	30	15		6	9
	Black	35	20		10	5
	Brown	35	19	-	12	4
	Total	300	93	8	78	121

*increased sterilization procedures

7.0 DISCUSSION AND CONCLUSIONS

Jack pine seedlings in the original I.M.R.D. study were grown at two fertility levels in order to determine the effect of fertilization on ectomycorrhizae development by P. tinctorius. Many researchers report that excessive or unbalanced amounts of N, P, and K are not conducive to ectomycorrhizae formation (Harley 1969; Mikola 1973). Bjorkmann (1942) suggested that excess carbohydrates required by the symbiont were unavailable due to redirection to the more rapidly growing aboveground parts. Marx, Hatch and Mendicino (1977) confirmed that a decrease in sucrose in the roots accompanied heavier fertilization and reduced the incidence of ectomycorrhizae. Wilcox (1968) reported that fertilization stimulates the rate of root growth which places the unsuberized root tip (the ideal infection site) beyond the "reach" of the slower growing fungal mycelium. Fertilization has also been shown to strengthen apical dominance thus restricting lateral root development and subsequently limiting the number of suitable infection sites (Marks and Foster 1967). Any factor (irrigation, light intensity, chemical applications, etc.) which affects the root or the habit of root growth also affects the ability of potential mycobionts to establish beneficial associations (Bowen and Theodorou 1973).

Pt-ectomycorrhizal development on the inoculated seedlings (I.M.R.D. study) was reduced at the higher fertility level thus corresponding to the previously reported observations. Regardless of inoculum origin or intensity of application, heavier fertilization resulted in: 1) fewer seedlings establishing P. tinctorius associations and 2) fewer roots exhibiting P. tinctorius ectomycorrhizae (Table 3.1). This reduction was not due to differences in moisture levels of the growing medium as seedlings on both fertilizer schedules received equal irrigation in the Thunder Bay Forest Station greenhouse.

The difference in ectomycorrhizal incidence, or quantity, having been confirmed, we wished to ascertain if the efficiency, or quality, of the associations formed under the two fertilizer schedules had also varied. In addition to Boreal forest environmental and soil conditions, moisture stress was included as a second season variable. Growth, nutrient composition and mycorrhizal colonization after this second season were the criteria for assessing the effect of Pt-ectomycorrhizae on the experimental jack pine seedlings. Each set of measurements was considered in absolute and relative terms; both contributing to our understanding of seedling response to the experimental variables.

7.1 Physical Attributes of the Seedlings

Many papers report the beneficial effects of P. tinctorius ectomycorrhizae on the growth of forest tree seedlings in the first season (Cordell and Marx 1977) and in later seasons (Marx and Artman 1979). Molina (1979), however, states that although inoculation resulted in increased P. tinctorius ectomycorrhizae formation on lodgepole pine and Douglas-fir seedlings, it did not affect growth of these seedlings in the first season.

First season growth measurements of the experimental jack pine seedlings reflect the impact of initial fertilization. For both volume and height increment (Figures 4.1 and 4.3), fertilization at the higher level increased seedling growth and eliminated variation due to mycorrhizal colonization. Similar results were obtained by Bowen and Theodorou (1967) who found that the extent of seedling response to mycorrhizal inoculation depended on the nutrient level of the soil. When nutrient levels were reduced, the contribution of the mycobiont to seedling growth became evident. Measurements of the physical attributes of 1/2 Normally fertilized jack pine seedlings confirmed this observation.

Mean first season volume, dry weight and height increment of Pt-colonized seedlings raised on the lower fertility regime (Pt-1/2F) significantly (p=0.01) exceeded those of similarily fertilized Controls (C-1/2F) as shown in Table 4.2. Furthermore, for first season volume and dry weight, these mycorrhizal means were not significantly less than those of seedlings raised on the higher fertilizer schedule (Pt-F, C-F).

The effect of mycorrhizal colonization on first season growth was most striking with respect to dry weight (Figure 4.2). At either fertilizer level, the mean of Pt-colonized seedlings was significantly (p=0.01) larger than that of either Control group. The dissimilarity noted between dry weight and volume means (Table 4.4) suggests that seedling tissue density was also affected by the experimental variables. This aspect is discussed further when relative growth values are considered.

Results of first season growth measurements of the Outplanted seedlings were very similar to those of the Greenhouse study, though differences between treatments were less significant (0.05 level as compared to 0.01). This difference may have been due to microsite or to the variation in original Pt-ectomycorrhizae incidence. All Pt-colonized seedlings in the Greenhouse study were selected to have at least half of their feeder roots mycorrhizal prior to potting, while Pt-inoculated seedlings in the Outplanting trial were assumed to be colonized at the levels reported by I.M.R.D. (Table 3.1).

Measurements of the physical attributes of roots of the Greenhouse study seedlings exemplified further the beneficial influence of Pt-ectomycorrhizae. At the 95% confidence level, all mycorrhizal seedling means were significantly larger than those of their Control counterparts (Figures 4.4, 4.5, and 4.6). If, as it has often been suggested, the ability of a seedling to produce new roots after planting is a key factor in establishment (Day et al 1976), then the advantage of a _P. tinctorius_ association has been clearly shown.

With respect to the root systems, the pattern of seedling response to the experimental variables as exhibited by the Greenhouse and Outplanted seedlings differed in magnitude and uniformity only (Tables 4.3 and 4.9). Volumes and dry weights of the former group were approximately half those of the latter, while root collar diameter was slightly larger. This is a good example of the difficulties inherent in extrapolating greenhouse results to field conditions without appropriate modifications. Nevertheless, as was the case with first season data, the results of the analysis of the root system data of the Outplanted seedlings corresponds well to that of the Greenhouse study seedlings.

The assessment by I.M.R.D. (Table 3.1) confirmed that the incidence of Pt-ectomycorrhizae on inoculated seedlings raised under 1/2 Normal fertilization was higher than that found on the Normally fertilized seedlings. This difference in mycorrhizal quantity may explain the greater root size of Pt-1/2F Outplanted seedlings as noted above. No such quantitative difference, however, existed among the Pt-colonized Greenhouse study seedlings where the mean volume, dry weight and root collar diameter of Pt-1/2F-NS seedlings were larger than those of any other treatment group. The uniformity of this response suggests that the quality, or efficiency, rather than the quantity, of Pt-ectomycorrhizae formed under the lower fertilizer regime may have been one factor responsible for the increase in growth.

Results of second season growth of the Greenhouse study seedlings varied with respect to the physical attribute being considered (Figures 4.7, 4.8, and 4.9). Three aspects which remained consistent, however, were: 1) that the means of Pt-colonized seedling groups (in all but two cases) were significantly (p=0.01) larger than those of their Control counterparts, 2) that soil moisture Stress reduced growth in the second season, and 3) that lower initial fertilization enhanced the beneficial effects of Pt-colonization.

The lack of differences betweeen Control and mycorrhizal seedlings raised on Normal fertilization and the consistent superiority of Pt-colonized seedlings raised on the 1/2 Normal fertilizer program (Pt-1/2F-NS) exemplifies the suspected difference in Pt-ectomycorrhizae efficiency due to initial fertilization. Especially under conditions of soil moisture Stress (not uncommon in the field) the quality of ectomycorrhizae produced on the lower fertilizer regime was of greater benefit to the seedlings than that produced at the higher nutrient level.

Second season growth of Outplanted seedlings indicates similar responses to the experimental variables (Figure 4.15). Conditions at the Outplanting site must have been favourable as measurements in the second season were double and triple those of the Greenhouse study potted seedlings.

Total growth over two seasons confirms the benefits of *P. tinctorius* inoculation to both the Greenhouse and Outplanted seedlings.

From the second season growth data of the Greenhouse study seedlings it became apparent that nursery practices had affected Control and Pt-colonized seedlings differently. Higher initial fertilization of Control seedlings was associated with larger volume and dry weight production in comparison to those Controls raised on the lower schedule. Controls raised on Normal fertilization were also less affected by soil moisture Stress. In contrast, lower initial fertilization enhanced Pt-ectomycorrhizae formation which subsequently benefitted growth. When total volume and dry weight are considered, the presence of Pt-ectomycorrhizae has compensated for initial differences which were due to fertilization. Only total height remains significantly affected by fertilization and here, Pt-ectomycorrhizae have reduced or eliminated the effects of soil moisture which are evident among the Control seedlings. Within the bounds of soil fertility and soil moisture imposed by the experimental conditions, both at the Outplanting and in the Greenhouse, seedlings with Pt-ectomycorrhizae have more fully utilized their potential for growth.

The absolute values of the physical assessment as discussed above are proof that inoculation with *P. tinctorius* did enhance jack pine seedling growth over two seasons. Consideration of the relative increases in growth (second season as a percentage of first season) provides further insight into the the mechanisms of this enhancement.

Tables 4.5 and 4.10 present second season growth as a percentage of first season growth. In the majority of treatment comparisons, the increase in size exhibited by mycorrhizal seedlings was at least 10 percent larger than that shown by comparable Control groups. These results clearly indicate the beneficial influence of P. tinctorius inoculation at the time of seeding.

In contrast, volume increases among 1/2 Normally fertilized seedlings appear to favour the Control treatments. In order to explain this trend, volume and dry weight must be considered jointly. Table 7.1 presents density (mg DW per 1 cm^3 Volume) data for the first and second seasons.

Density values for first season tissues of Control seedlings raised on the lower fertility regime were much higher than those of any other treatment groups. The presence of this denser tissue suggests that some factor was limiting cell expansion. Because the density of Pt-colonized seedlings on the 1/2 Normal regime and those of all seedlings raised on the Normal regime were very similar, it appears that the presence of Pt-ectomycorrhizae compensated for this factor at the lower fertility level. Since the concentrations of N, P, and K in the 1/2 Normally fertilized Control seedlings were as high or higher than those in the Pt-colonized seedlings (Section 5.2.1), we may assume that the deficiency was not in any of these elements. Some other element or biological factor must have been lacking during the first season.

Table 7.1: Greenhouse Study seedlings - Density of the seedling parts (mg of dry weight per cubic cm of volume)

Myc Colonization	Control				Pt-Colonized			
Fertilization	1/2 F		F		1/2 F		F	
Soil Moisture	S	NS	S	NS	S	NS	S	NS
First Season	79.1	95.2	52.4	52.5	58.5	66.2	60.0	54.3
Second Season	16.3	12.4	25.7	22.6	18.7	17.7	22.6	23.1

The uniformity of density values within fertilizer groups for the second season tissues indicates that the "first season deficiency" referred to in the previous paragraph has been overcome. While it is possible that the natural soil was able to supply this element or biological factor directly, it is more likely that the formation of new mycorrhizal associations was responsible for alleviating the deficit. The uniformity of density also suggests that Pt-ectomycorrhizae were not highly effective during the second growing season when both inoculated and Control seedlings developed similar levels of "Black" and "Brown" ectomycorrhizae from the local mycobionts.

Returning to Table 4.5, we note that even though Control seedling volume may have increased by 200-300 percent, in absolute terms these seedlings are still significantly (p=0.05) smaller than their Pt-colonized counterparts. Formation of "other species" ectomycorrhizae has undoubtedly improved the growth of non-inoculated seedlings, however, it is questionable whether the initial difference between inoculated and Control seedlings will ever be overcome. Theodorou and Bowen (1970) found that this was the case with Pinus radiata seedlings when natural infections occurred in the controls 28 months after outplanting.

Among the Outplanted seedlings, increases in growth of 1/2 Normally fertilized, Pt-inoculated seedlings were consistently the highest. If we assume that second season ectomycorrhizal effects were primarily caused by "other species" mycobionts (as the density data implies), then the influence of P.tinctorius must have occurred prior to soil freeze-up and/or at the very beginning of the second growing season. It is known that the roots of woody plants continue to expand after bud set (Meyer 1973) and that fertilizer applied in nurseries at this time enhances seedling growth in the following season. It is very possible that the Pt-ectomycorrhizae present on roots at the time of transplanting were responsible for increased nutrient and water uptake in the fall and, subsequently, better growth the following spring. The difference between growth of Pt-inoculated seedlings (Table 4.10) raised on the two fertilizer regimes supports the theory that ectomycorrhizae formed on the lower fertilizer schedule were more beneficial or more effective than those formed on the higher one.

The results of the physical assessment indicate five conclusions:

1) The addition of P. tinctorius vegetative inoculum to the growing medium of containerized jack pine seedlings can result in substantial levels of ectomycorrhizae development.

2) The presence of Pt-ectomycorrhizae on the roots of jack pine

seedlings enhances growth after transplanting.

3) The nature of Pt-ectomycorrhizae formed at the 1/2 Normal fertilizer level was more beneficial to seedling growth than that developed at the Normal level. This difference may have been the result of enlarged root absorptive area or it may indicate some variation in hormonal and/or metabolic balances.

4) P. tinctorius need not form the dominant mycorrhizal association in order to affect and enhance seedling growth in the second season.

5) Local species of mycorrhizal fungi, which rapidly colonized the roots of both inoculated and Control seedlings, were also effective growth promoters.

7.2 Nutrient Assessment of the Seedlings

Nutrient content and concentration were affected by experimental treatment (including study location) and the effects differed with the nutrient element involved and the morphological part being considered. Mean concentration exhibited more variation than did mean content, as illustrated by the results of linear regression with dry weight (Table 5.7). As noted previously, concentration alone is not a consistent nor easily understood indicator of seedling condition, nor of seedling response to experimental variables (Timmer and Stone 1978). This discussion will therefore deal primarily with the nutrient content data which, when combined with the appropriate dry weight and concentration values, allows a better understanding of seedling efficiency in the uptake and utilization of nutrients.

Why is improved growth repeatedly associated with mycorrhizal colonization? Some authors report that mycorrhizal fungi produce growth stimulating hormones such as cytokinins (Miller 1971). Others have found that mycorrhizal seedlings are less susceptible to pathogens (Marx 1969) and soil toxicities (Zak 1971). Still others argue that mycorrhizae impart drought resistance to infected seedlings (Goss 1960; Theodorou and Bowen 1970). The majority of authors maintain, however, that enhanced growth is primarily due to increased uptake of nutrients (Bowen 1973). Just as this aspect is not yet fully understood, so the mechanisms of increased uptake remain uncertain.

In 1894, Frank suggested that mycorrhizal fungi could obtain nitrogen more easily from forest humus than could the higher plants. Hatch (1937) reported that increased uptake was the result of physical expansion of the root system. Hatch suggested, and others have confirmed, that the volume of soil exploited by mycorrhizal roots is larger due to increased longevity of the mycorrhizae; swelling and branching of the feeder roots; hyphal extensions and rhizomorphs. Refinements to Hatch's theory include greater absorbing power of mycorrhizae (Bowen 1973) and easier movement of ions through hyphae than through root tissues (Skinner and Bowen 1974; Smith 1974). Harley (1969) has investigated selectivity of ion absorption by mycorrhizae while others have reported the importance of mycorrhizosphere organisms on the movement and capture of ions (Davey 1971; Katznelson et al 1962). It has also been shown that mycorrhizae act as nutrient sinks which release stored nutirents to the plant when external sources are depleted (Harley and McCready 1952). There is, as well, a school of thought which maintains that mycobionts function by increasing the availability of ions through the production of chelating compounds (Wilde 1968).

Whatever the exact mechanism, or combination of mechanisms, may be, increased uptake of many elements, including N, P, and K, by mycorrhizae has been confirmed both in solutions and in soil (Bowen 1973).

One objective of our study was to determine if increased growth of the jack pine seedlings was associated with increased nutrient uptake. Nutrient analysis was not performed prior to transplanting, therefore we do not know the effect of the two fertilizer regimes in the first season. The results from first season tissue samples as measured after the second season are indications of nutrient "reserves" which may have been the result of late season storage or the action of sinks. The experimental seedlings were lifted at a time of heightened nutrient demand by the growing shoots (van Den Dreissche 1974) and frozen immediately to prevent any movement of ions. Since ions within a plant are mobile and tend to move toward areas of new growth, it is reasonable to suggest that nutrient composition should therefore reflect any differences in previous nutrient uptake due to P. tinctorius ectomycorrhizae.

Hatch's (1937) early work with Pinus strobus reported increases of N, P, and K in mycorrhizal seedlings. His figures were obtained by calculating the total seedling uptake per gram of root dry weight. Hatch beleived that this data was an indication of efficiency in nutrient uptake. Table 7.2 on the following page presents the results of similar calculations for our experiment.

Table 7.2: Total seedling nutrient content per gram of root dry weight - Greenhouse Study and Outplanted seedlings

Mycorrhizae	Control				Pt-Inoculated			
Fertilization	1/2 Normal		Normal		1/2 Normal		Normal	
Soil Moisture	S	NS	S	NS	S	NS	S	NS
Greenhouse Study								
Root DW (g)	1.31	1.72	1.87	1.95	1.90	3.13	2.47	2.81
N (mg/g)	31.31	27.02	30.58	26.32	29.11	22.56	24.47	23.30
K (mg/g)	12.92	12.02	9.01	10.29	9.04	9.55	7.52	9.40
P (mg/g)	5.28	5.03	5.27	4.99	5.12	4.45	4.36	4.11
Outplanting								
Root DW (g)	0.45		0.89		1.08		1.07	
N (mg/g)	100.95		91.38		104.13		94.04	
K (mg/g)	40.40		39.62		45.77		35.68	
P (mg/g)	13.89		13.15		14.38		12.36	

As can readily be seen from Table 7.2, the differences in total seedling nutrient content per gram of seedling root dry weight were very small. The explanation for this trend is found in the results of the mycorrhizal assessment. Both inoculated and Control seedlings developed very similar amounts of "other species" ectomycorrhizae after transplanting to the forest soil. Given the severe reduction in Pt-ectomycorrhizae incidence over this same period, we may assume that nutrient uptake in the second season was governed more by the new associations than by the initial *P. tinctorius* infection.

The evidence, however, of *P. tinctorius*' influence on seedling size and nutrient content was clearly presented throughout the Results section of this paper. Seedlings inoculated with *P. tinctorius* were significantly larger in all physical attributes and their nutrient contents were consistently greater. Thus, the question of mycorrhizal efficiency must be investigated through the relationship of nutrient concentration, content and dry weight.

Repeatedly in this data there are examples of Pt-inoculated seedlings with low nutrient concentrations, high nutrient contents and large dry weights. From these it is clear that within each seedling part, tissue production by the inoculated seedlings required lower relative amounts (concentrations) of N, P, and K. Even if Control and inoculated seedlings had absorbed equal total amounts of these nutrients, the mycorrhizal seedlings, given their greater efficiency, would still be larger. The data show, however, that total uptake by the inoculated seedlings was consistently greater and growth was therefore further enhanced. The explanation for increased efficiency of tissue production may be hormonal or metabolic (enzymatic) and requires extensive investigation.

Greater total absorption may be a physiological characteristic of mycorrhizal fungi or it may simply be the direct result of absorption by a larger root-hyphae system. Since our investigation shows that the percentage of Pt-ectomycorrhizae was greatly reduced sometime after transplanting, it is unlikely that the latter hypothesis can explain the enhanced growth of inoculated seedlings. As mentioned previously, the effective period of the *P. tinctorius* association was probably prior to soil freeze-up and/or at the very beginning of the second growing season. Increased nutrient uptake and the production of growth stimulators must therefore have taken place at those times. Interestingly, the ratios of nutrient content in Outplanted seedlings (Table 5.11) do indicate larger amounts in the first season tissues of inoculated seedlings. Since demand by newly expanded shoots was high at the time the seedlings were lifted, one would expect reserves in the older tissues to be depleted. The presence of larger reserves in the Pt-inoculated seedlings thus supports the hypothesis of increased initial uptake.

7.3 Mycorrhizal Assessment of the Seedlings

Results of the physical and nutrient assessments have shown that the presence of Pt-ectomycorrhizae on seedling roots at the time of transplanting enhanced growth and nutrient uptake. One might expect that this induced mycorrhizal association, in order to be of benefit, must remain unchanged after exposure of the roots to natural soil.

Zak (1975) argues that this is not a realistic, nor perhaps even a desirable, goal. Mycorrhizal fungi, well adapted to each site, abound in forest soil (Meyer 1973). Disturbances, such as logging and scarification, reduce fungal populations only temporarily. The airborne basidiospores of ectomycorrhiza-formers recolonize such soils quickly (Cordell, Marx and Bryan 1974) and newly established seedlings will form mycorrhizal associations with local symbionts eventually (Tainter and Walstead 1977; Trappe 1969). The key point in the sequence is the time which elapses between seedling establishment (planting or seeding) and the development of a beneficial mycorrhizal association. In order for new infections to occur suitable infection sites and mycobionts must both be available. The ability of seedlings to regenerate roots after transplanting determines the rate at which colonization can proceed (Bowen and Theodorou 1973). New associations may therefore require as many as two or more growing seasons for establishment if root growth is restricted.

Two difficulties arise when nursery seedlings are outplanted: 1) the seedlings must live long enough to form the new mycorrhizal associations and 2) the period preceding establishment of the new mycobiont is usually characterized by poor seedling growth. The solution to both these problems is the immediate regeneration of roots after transplanting. This growth depends on adequate water and nutrient uptake (Day and MacGillivray 1975) which can be stimulated by the presence of beneficial mycorrhizae. The advantage of mycorrhizal inoculation to nursery production lies in the ability of induced ectomycorrhizae to extend or encourage root regenration.

The results of this study do not prove conclusively that root growth between the time of transplanting and soil freeze-up was enhanced by either Pt-colonization or Pt-inoculation. It does show, however, that after two growing seasons,root systems of the ectomycorrhizal seedlings were significantly larger ($p=0.01$) than those of comparable Controls. Therefore, we may conclude that between the time of transplanting (August 1979) and lifting (July 1980), P.tinctorius ectomycorrhizae did enhance the potential for root regeneration.

Within this time period, both mycorrhizal and Control seedlings developed new mycorrhizal associations of the "Black" and "Brown" types. The appearance of these two types of ectomycorrhizae were very similar to those described for Cenococcum graniforme (Trappe 1964) and Thelephora terrestris (Marx and Bryan 1970; Marx, Bryan and Cordell 1970), both of which are common mycorrhiza-formers and have been reported in the Boreal forest (Miller and Farr 1975). As previously noted, however, no attempt was made to verify the identities of the two ectomycorrhizal types.

The approximate number of seedlings infected and the percentage of roots supporting the new associations were similar for the inoculated and Control treatment groups (Table 6.2). Neither the presence nor the absence of Pt-ectomycorrhizae appears to have affected subsequent colonization of the seedling roots by other mycobionts. This observation, and the results of Table 6.1, shows that the level of Pt-colonization was greatly reduced over the second growing season and indicates that P. tinctorius, under Boreal forest conditions, was not an aggressive competitor. In contrast, many studies have shown that P. tinctorius is aggressive, especially under difficult site conditions such as extremes of pH and soil temperature (Marx 1975; Schramm 1966). This fungus, on the roots of southern pine seedlings outplanted in Florida and North Carolina, was able to maintain its position as the dominant symbiont for a minimum of four years (Marx and Artman 1979). T. terrestris, however, was found on the roots of inoculated seedlings and composed the majority of ectomycorrhizae associated with the control seedlings.

The factor most likely responsible for the reduction in Pt-ectomycorrhizae incidence in the author's study was soil temperature. Results of the isolations proved that P. tinctorius was able to survive the winter temperatures of the Boreal forest. Other researchers have shown that temperatures as low as -8° C in the soil (Marx 1979b) and -10° C under artificial conditions (France et al 1979) have neither modified nor killed this fungus. The optimum soil temperature, however, for the development of Pt-ectomycorrhizae on Pinus taeda was reported by Marx, Bryan and Davey (1970) to be between 25° C and 35° C. It is doubtful that the soils in our experiment maintained temperatures approaching the optimum for any length of time.

Given these arguments, and those presented in the discussions concerning the physical and nutrient profiles of the seedlings, it is reasonable to suggest that the Pt-ectomycorrhizae present at the time of transplanting were effective prior to the first winter and/or at the very beginning of the second growing season. Nutrient and water uptake may have been improved, root regeneration must have been enhanced and, perhaps, growth stimulating hormones were produced by the fungal associate at those times. These possibilities do not preclude the functioning of P. tinctorius in the following growth period, however, the relative mass of new roots produced and the noticeable decline in Pt-ectomycorrhizae incidence indicates that direct contributions to seedling growth by P. tinctorius had been reduced in the second growing season.

7.4 Practical Considerations

The results of this study indicate that mycorrhizal technology can be an effective tool in the containerized production of jack pine seedlings for reforestation purposes. The following six points summarize the results of this study.

1) Satisfactory ectomycorrhizae formation was induced by the addition of P. tinctorius vegetative inoculum to the growing medium at a ratio of 1:15 (inoculum to substrate).

2) P. tinctorius inoculation enhanced the growth of jack pine seedlings in both the first and second growing seasons. The qualities which contributed to this improvement will likely continue to affect performance in succeeding years.

3) Fertilizer applications reduced to approximately half the amount "normally" used resulted in initially smaller seedlings, with heavier P. tinctorius infections, which exhibited superior growth after transplanting.

4) Irrigation level in the greenhouse need not be reduced in order for seedlings to develope adequate mycorrhizae. Lower levels, however, may favour P. tinctorius which is known to be strongly aerobic.

5) P. tinctorius, reported to be a high temperature fungus, did survive the soil and climatic conditions of Northwestern Ontario. It was not an aggressive competitor; although its presence was consistenly associated with increased growth and nutrient uptake throughout two growing seasons.

6) Colonization of seedling roots by local mycobionts was abundant within one year of transplanting. Mycorrhizal fungi which occur naturally in the Boreal forest soil could be screened for the potential to benefit nursery stock and promising symbionts should be tested under nursery and greenhouse conditions according to the specifications outlined by Trappe (1977).

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APPENDIX

Appendix A.1: Fertilization of containerized jack pine seedlings at the Thunder Bay Forest Station, I.M.R.D. Study (grams of element/Spencer-Lemaire flat)

Date	Fertilizer Type	Fertilization Schedule					
		Normal			1/2 Normal		
		N	P	K	N	P	K
April 21	20-20-20	.045	.019	.037	.045	.019	.037
25	20-20-20, NH_4NO_3	.103	.028	.054	-	-	-
28	" "	.146	.048	.090	.146	.048	.090
May 1	" "	.146	.048	.090	-	-	-
4	" "	.146	.048	.090	.146	.048	.090
8	" "	.146	.048	.090	-	-	-
11	" "	.146	.048	.090	.146	.048	.090
16	" "	.146	.048	.090	-	-	-
18	" "	.146	.048	.090	.146	.048	.090
20	NH_4NO_3	.076	-	-	.076		-
24	20-20-20, NH_4NO_3	.146	.048	.090	-	-	-
26	" "	.146	.048	.090	.146	.048	.090
30	" "	.146	.048	.090	-	-	-
June 1	" "	.146	.048	.090	.146	.048	.090
3	NH_4NO_3	.076	-	-	.076	-	-
6	20-20-20, NH_4NO_3	.146	.048	.090	-	-	-
8	" "	.146	.048	.090	.146	.048	.090
13	" "	.146	.048	.090	-	-	-
16	" "	.146	.048	.090	.146	.048	.090
21	" "	.146	.048	.090	-	-	-

Appendix A.1: cont'd

Date	Fertilizer Type	Fertilization Schedule					
		Normal			1/2 Normal		
		N	P	K	N	P	K
June 22	10-52-10, MH_4NO_3	.047	.062	.022	-	-	-
23	20-20-20, NH_4NO_3	.073	.024	.045	.073	.024	.045
27	" "	.073	.024	.045	.073	.024	.045
28		.073	.024	.045	.073	.024	.045
29		.073	.024	.045	.073	.024	.045
30		.073	.024	.045	.073	.024	.045
4		.073	.024	.045	.073	.024	.045
5		.073	.024	.045	.073	.024	.045
6		.073	.024	.045	.073	.024	.045
7		.073	.024	.045	.073	.024	.045
10		.073	.024	.045	.073	.024	.045
14	" "	.073	.024	.045	.073	.024	.045
Total (g of element/S-L flat)		3.413	1.117	1.991	2.168	.667	1.252

SUMMARY

Phillips, N.J. 1981. Response of jack pine (Pinus banksiana L.)
seedlings to inoculation with Pisolithus tinctorius
(Pers.) Coker and Couch.
Major Advisor: Dr. S. Navratil.

Containerized jack pine seedlings treated at the time of seeding with P. tinctorius vegetative inoculum and raised on two nursery fertilizer regimes were transplanted after four months to one of two locations: 1) a Boreal forest cutover or 2) a greenhouse where soil moisture content was controlled. After a second growing season, the seedlings were assessed for physical attributes (volume, dry weight and height increment), nutrient composition (concentration and content of N, P, and K) and mycorrhizal colonization of the root tissues.

Inoculation with P. tinctorius, regardless of the accompanying levels of soil moisture or initial fertilization, significantly enhanced the growth of roots and aboveground parts. Soil moisture stress reduced seedling size. This reduction was most noticeable among non-inoculated (Control) seedlings raised on the lower fertilizer regime. Heavier initial fertilization increased seedling size in the first season but was associated with decreased growth in the second. It would appear that the intensity of fertilization affected both the quality and quantity of ectomycorrhizae produced as a result of the inoculation. Seedlings raised on the lower fertilizer schedule were smaller and had more abundant ectomycorrhizae at the time of transplanting. After transplanting, these seedlings exhibited superior growth in both study locations.

Nutrient uptake data support the observation that initial fertilization affected the nature of P. tinctorius ectomycorrhizae formed in the first season. In comparison to Control seedlings, the inoculated seedlings exhibited lower nutrient concentrations, higher nutrient contents and larger tissue dry weights. These results suggest a difference in the efficiency of nutrient uptake and utilization between Control and inoculated seedlings.

Results of the mycorrhizal assessment and isolations confirmed that P. tinctorius survived the Boreal winter conditions but was not an aggressive competitor. Colonization of both inoculated and Control seedlings by local mycobionts was uniform and abundant after transplanting of the seedlings to natural soil. Nonetheless, the effect of P. tinctorius inoculation (at the time of seeding) was consistently associated with improved growth throughout the second growing season.