VESICULAR-ARBUSCULAR MYCORRHIZAE AND APPLES (*Malus domestica* Borkh.) IN THE NURSERY AND IN APPLE REPLANT DISEASE

by

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ABSTRACT

The purpose of this study was to determine if different clonal rootstock from apple (Malus domestica Borkh.) formed different vesicular arbuscular mycorrhizal (VAM) associations. Different fertilizers and VAM fungi were tested to determine their effects on apple seedling growth in apple replant diseased (ARD) soils. VAM associations in apple stoolbed nursery were low. Over 80% of all samples had less than 10% mycorrhizal colonization. This reduction in VAM colonization amongst various rootstock clones is a result of detrimental management practices in the stoolbed nursery. Apples grown in a budded nursery had high mycorrhizal colonization, the lowest colonization rate was 30%. Different rootstocks from the budded nursery do not show any significant differences in VAM colonization, whereas clonal rootstocks from the stoolbed nursery do. From the stoolbed nursery, Malling (M) 2 consistantly showed higher VAM colonization rates, compared to M 4, M 7, M 9, M 26, Malling Merton (MM) 106, MM 111, Alnarp 2 and Ottawa 3.

Apple replant disease (ARD) is identified as the reason for poor growth of apple seddlings in 5 soils from the Okanagan Valley of British Columbia. Sterilization by autoclaving, pasteurization and formalin increased test seedling height. Air-drying test soil does not affect ARD pot bioassays. However, air-drying the soil and pasteurizing or adding formalin increased plant height significantly more than these treatments in nonair-dried soils.

The fertilizer monoammonium phosphate (11-55-0) increased plant height more than ammonium nitrate (34-0-0) while triple superphosphate (0-45-0) did not increase plant height. Root growth was increased by 0-45-0 only. VAM fungi were drastically reduced or eliminated by sterilization and 11-55-0, but not by the other fertilizers.

VAM fungi in 2 ARD soils do not overcome ARD. Test seedlings grown in sterilized ARD soils inoculated with 4 species of VAM fungi do not show as great an increase in shoot height compared to the addition of 11-55-0 fertilizer. Root growth shows the inverse response. Glomus intraradices Schenck and Smith, was the best colonizer but inoculation with G. versiforme (Karsten) Berch resulted in the greatest shoot and root growth. Glomus clarum Nicholson and Schenck, and G. monosporum Gerdemann and Trappe, did not result in increases in plant growth in ARD soils.

In sterilized ARD soils, VAM fungi do not increase shoot growth as expected, but do increase root growth, suggesting the initial growth of inoculated apple seedlings is root mass. Seedlings given 11-55-0 fertilizers show the reverse pattern of growth.

In nonsterilized ARD soils, the growth of seedlings appears to be inversely proportional to VAM colonization.

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INTRODUCTION

Apples (Malus domestica Borkh) are initially propagated in stoolbeds. Prior to establishing the stoolbed, the soil is fumigated and fungicides are applied for disease control throughout the growing season.

A mother plant is established and allowed to grow for one season (Figure 1.1). In the fall, after leaf drop, the mother plant is cut just above the soil line. The following spring new shoots arise from the old mother plant. Throughout the growing season soil or sawdust is hilled around the new shoots to initiate rooting (Figure 1.2). In the fall, after the buds are dormant, the rooted shoots (rootstock) are cut just above the soil line leaving the roots of the mother plant in the stoolbed. The mother plant overwinters and the following spring, the cycle begins again.

The rootstocks are placed in cold storage until the following spring when they are shipped to the orchard or to another nursery for budding (Figure 1.3). In the budded nursery the rootstocks are planted in the spring and are allowed to grow vigorously till mid summer. In the latter part of August, the shoots are budded about 15 cm above the soil line. The rootstock with its new bud overwinters in the nursery. In the spring, the original shoots of the rootstock are cut just above the bud union. The buds are allowed to grow for 1 season and the rootstock with its new scion is ready for harvest in the fall.

After 2 growing seasons in the budded nursery, the rootstock

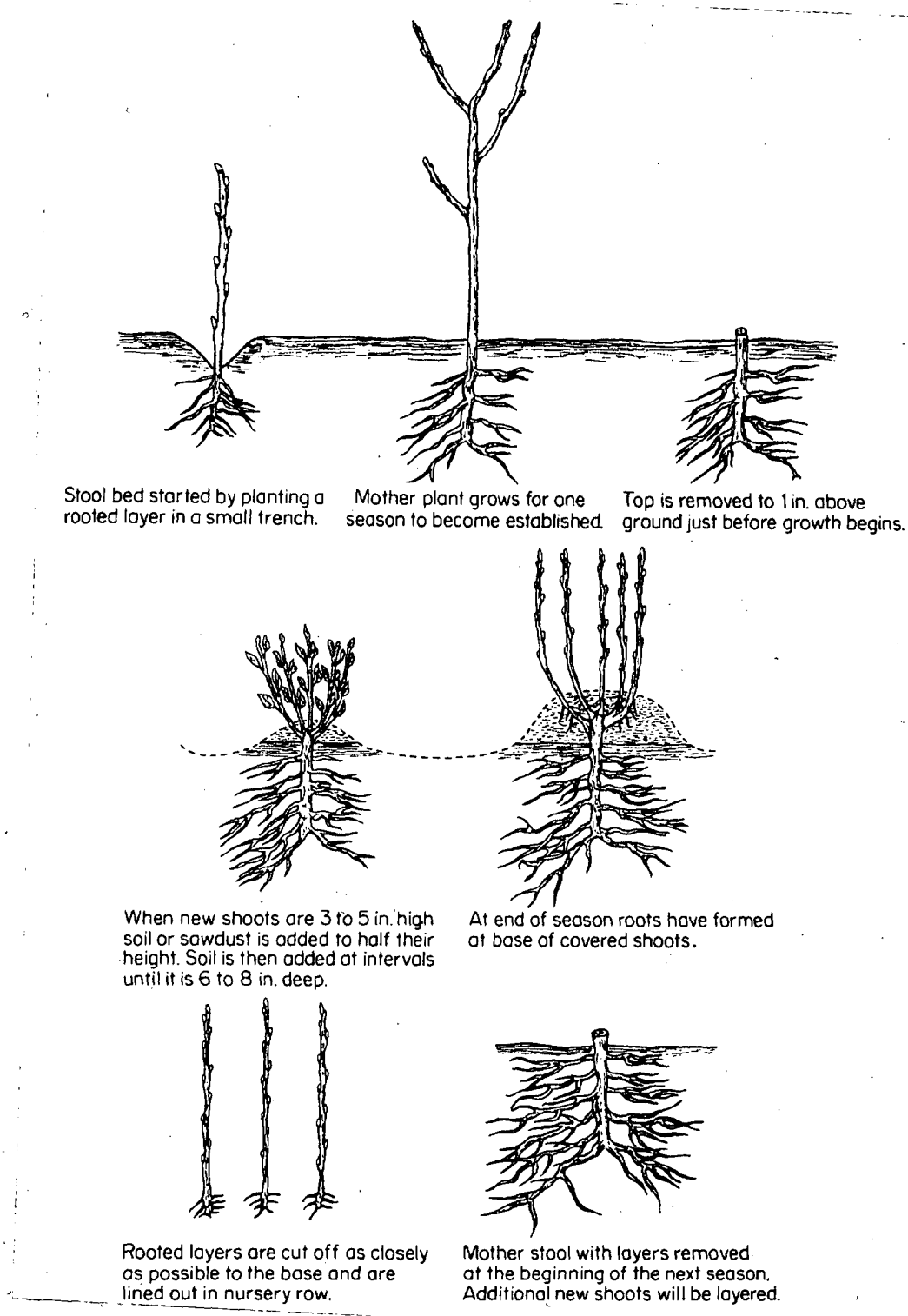

Figure 1.1: Apple propagation in Stoolbeds
(Hartman, H.T. and D.E. Kester 1975)

Figure 1.2: Apple Stoolbeds in Soil

Figure 1.3: Apples in Budded Nursery

and its scion are lifted, placed in cold storage and shipped to the orchard.

Cultivation of apples in the orchard is changing. Growers are moving away from the low density, vigorous trees, to the high density, dwarfing trees (Figures 1.4 and 1.5). The initial cost of establishing these intensive orchards is high, and the grower must be able to ensure a return on investments as soon as possible. The orchards must therefore be in full production within 3 - 4 years. Any factors which prevent healthy, early producing orchards must be avoided.

Apple rootstock transplanted into old apple orchard soil will often not grow very well. In the first year in the orchard the scion does not elongate, there is little internode growth and there is no fibrous root growth (Figure 1.6). No one pathogen is always associated with this disorder. However, if the orchard soil is fumigated prior to transplanting the scion will often grow more vigorously. This disorder is known as a 'replant disease'. This disease is prefixed by the host it is associated with. Thus this disease associated with apples is referred to as apple replant disease (ARD).

ARD has been reported for over 300 years and is found in all apple growing areas of the world (Buszard and Jensen 1986, Hoestra 1968, Ryan 1975, Mai and Abawi 1981, Ross et al. 1984, Benson et al. 1978, Savory 1967, Slykhuis and Li 1985). Replant diseases are found in numerous plants, especially fruit and plantation crops (Deal et al. 1971, Havis et al. 1958, Hwang

Figure 1.4: Low Density Orchard

Figure 1. 5: High Density Orchard

a

b

c

d

Figure 1.6: Apple Transplant Showing Apple Replant Disease: a) Stunted Shoot Growth b) Healthy Tree c) Shortened Internodes d) Reduced Root System

1988, Mountain and Boyce 1958, Pepin et al. 1975, Trudgill 1984).

No single pathogen has been identified with all reported cases of ARD. In Czechoslavakia, the fluorescent pseudomonads (Catska et al. 1982) and Penicillium claviforme (Catska et al. 1988) have been identified as the causal agents of ARD. The fungus Pythium spp. in Great Britain, (Sewell 1981) and nematodes in Holland (Hoestra and Oostenbrink 1962) and New York (Jaffe et al. 1982) have also been identified as the causal agents of ARD. It therefore appears that though a contributing agent is biological, it is not a simple disease.

Slykhuis and Li (1985) and Sewell and Roberts (1985) reported that in addition to fumigation, ARD is overcome by the addition of high nitrogen and phosphorus fertilizers. The importance of phosphorus in apple growth is well documented (Miller et al. 1985b, Hoepfner et al. 1983). The biological nature of the disease and the phosphorus factor suggest that mycorrhizae may play a role in ARD.

Apples form a mutualistic association with vesicular-arbuscular mycorrhizal (VAM) fungi. The fungus acts as root extensions exploring soil that is inaccessible to the plant, moving phosphorus from the soil matrix through the hyphae and into the plant. At sites within the cell, the fungus exchanges phosphorus for simple sugars from its host plant.

The fungus forms inter- and intracellular hyphae in the plant root. After penetration of the root epidermis, the hyphae coil and spread throughout the cortex of the root but never enter

the meristem tissue (Figure 1.7a). Hyphae penetrate the cortical cells then repeatedly branch dichotomously. These branches take on the form of 'little trees' and are known as arbuscules (Figure 1.7b). Nutrient transfer between the host and root occur within these arbuscules. During the later stages of development, vesicles form intercellularly and are filled with oil droplets (Figure 1.7c). Hyphae grow outside the root and may terminate in chlamydospores, though these spores may also be found within the root. Identification of the fungus is based on spore morphology. Both the vesicles and spores are resistant to harsh environmental conditions.

Nurseries are irrigated regularly, given adequate amounts of fertilizers and heavily sprayed with pesticides. If VAM fungi are found in nursery beds, they will be adapted to that environment. If rootstocks are mycorrhizal upon lifting from the nursery, the mycorrhizae may not survive cold storage.

Old apple orchards have populations of VAM fungi that have stabilized over the years. New apple rootstock transplanted into these old apple soils may not benefit from mycorrhizae indigenous to old apple soils.

The purpose of this study was to: 1) determine the mycorrhizal status of apple rootstock grown in stoolbeds; 2) establish the distribution of mycorrhizal fungi in stoolbed and budding nurseries; 3) determine the effects of cold storage on the mycorrhizae of different rootstocks; 4) identify different ARD soils from the Okanagan Valley of British Columbia; 5)

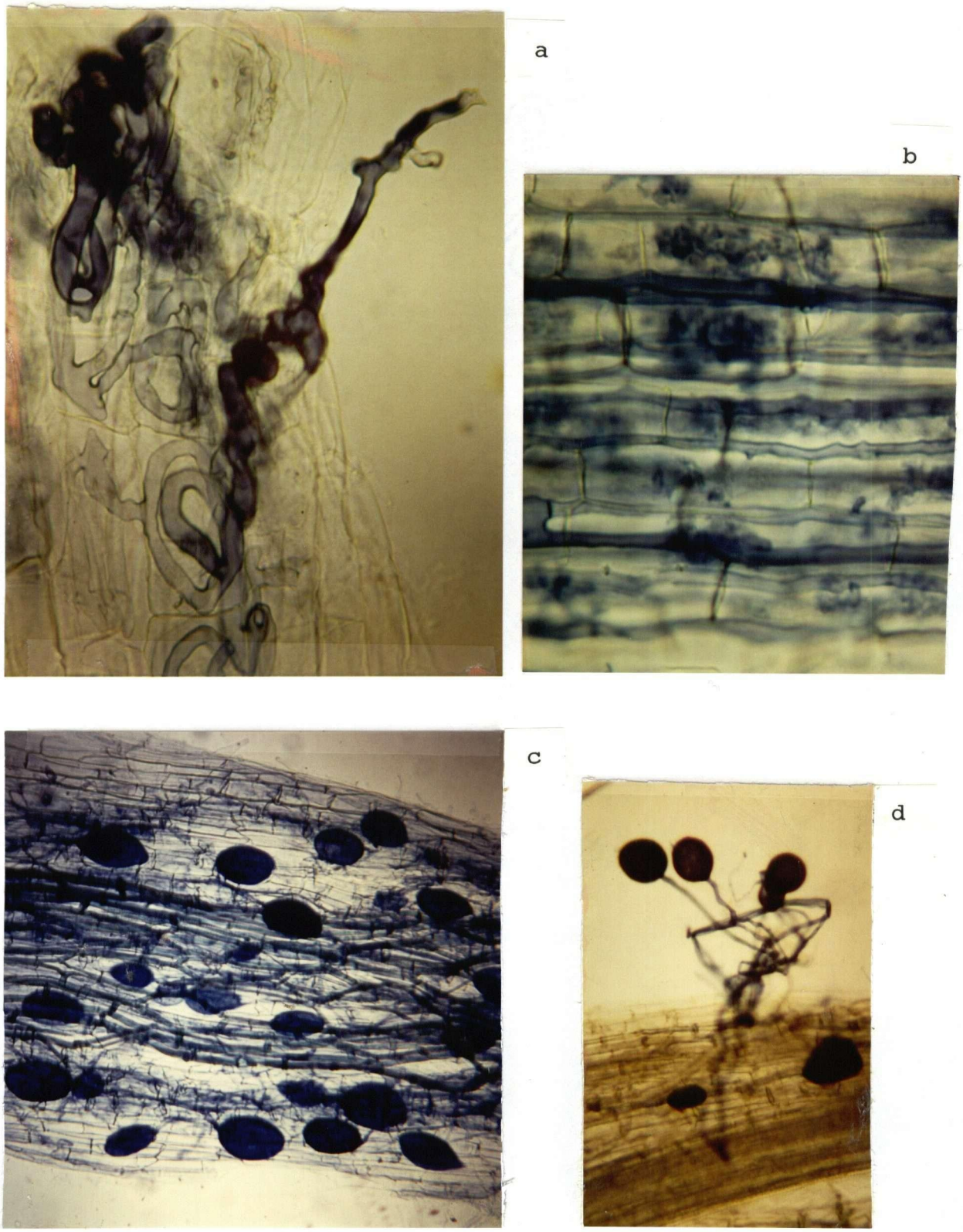

Figure 1.7: Mycorrhizae in Apples a) Root penetration and hyphal spread b) Arbuscules within cortical cells c) Vesicles d) Stained external spores

determine the effects of various fertilizers on growth of apple seedlings in ARD pot bioassays; 6) determine if air-drying ARD soils affects ARD pot bioassays; and 7) determine the effects of inoculating ARD soils with various VAM fungi on the growth of apple seedlings in pot tests.

VA MYCORRHIZAE AND APPLES (*Malus domestica*) IN THE NURSERY

INTRODUCTION

There is increasing interest in the role of mycorrhizae in many orchard problems. Apples that have problems becoming established in orchards and that respond to the additions of phosphorus fertilizers may have no VAM fungi, or may have VAM fungi that are not adapted to the orchard environment. Different clonal apple rootstock in the orchard have been shown to have different mycorrhizal fungi (Miller *et al*. 1985a), though this difference was thought to be a result of the geographic distribution of the orchard rather than the rootstock type. The mycorrhizal status of apple rootstock prior to transplanting is important and should be established.

Management practices in the nursery may adversely affect VAM fungi. Certified strawberry plants grown in nursery fields fumigated prior to establishment showed low VAM colonization intensities during the first year of growth but in the second year showed a wide range of colonization (Robertson *et al*. 1988). Apples are propagated in nursery stoolbeds, harvested, placed in cold storage and then transplanted into another nursery where they are budded. Prior to establishing the stoolbed, the soil is fumigated. In citrus and peach nurseries, plants grown in fumigated soil showed nutrient deficiencies linked to the absence of VAM fungi (Kleinschmidt and Gerdemann 1978, La Rue *et al*. 1975). Inoculating these nurseries with VAM fungi overcame this problem.

While apples in stoolbeds may not show nutrient deficiencies in fumigated soils, they are heavily fertilized and given numerous applications of fungicide throughout the season. These practices are most likely to be detrimental to VAM fungi in stoolbeds. It is well documented that in the presence of high P, VAM fungi are suppressed (Hoepfner et al. 1983, Miller et al. 1985b). If mycorrhizal fungi do become established in nursery stoolbeds, it is possible that different clonal rootstocks may form different VAM associations. There is no information available on the mycorrhizae present in clonal rootstock from nurseries. If mycorrhizae are present, the distribution of the fungi throughout the nursery would be a major factor on the probability of a rootstock becoming colonized before leaving the nursery.

The objectives of this study were to: 1) establish the mycorrhizal status of different clonal rootstock from stoolbeds from the Fraser Valley of B.C.; 2) determine the distribution of VAM fungi in stoolbed and budded nurseries in the Fraser Valley of B.C.; 3) to determine the effects of cold storage on the mycorrhizae in different rootstock from a stoolbed nursery from the Fraser Valley of B.C.

MATERIALS AND METHODS

STOOLBED NURSERY

Mycorrhizal Status of Apple Rootstock

Rootstock sampling occurred at Traas Nursery, 24355 - 48th Avenue, Langley, B.C. The soil belongs to Marble Hill series

which has over 50 cm of medium textured aeolian deposits over gravelly glacial outwash deposits. It is a well drained ortho humo-ferric podzol with 0.5 - 2.0% slope (Luttmerding 1980).

In April 1987, 9 different apple rootstocks, propagated in stool beds in soil, were examined for the presence of VAM fungi. Two sampling strategies were used for this study. Malling (M) 4, M 7a, and Malling Merton (MM) 111, from the entire nursery had already been lifted and were either in cold storage or being processed for cold storage. Ten trees from each rootstock were taken randomly from the storage pile and then placed in cold storage, separate from the commercial stock.

MM 106, M 2, M 7, M 26, Alnarp 2 and Ottawa 3 were still in the field. From each rootstock block, 10 trees were tagged randomly with fluorescent surveyor tape. Through the fall and winter, 1986/87, as each rootstock went dormant and was lifted, tagged trees were placed in cold storage separate from the commercial stock.

Throughout the winter, tagged trees were watered occasionally to prevent dessication of the roots. In April 1987, all the rootstock had been lifted and the roots from each sampled tree were placed in FAA (Formaldehyde (90): Acetic Acid (5): Ethanol (5)), (vol:vol:vol:) and examined for the presence of VAM.

All samples taken showed a binomial distribution and were therefore analysed using the Mann-Whitney U non-parametric test for significant differences in percent colonization.

Distribution of VAM Fungi

In the fall of 1987, Traas Nursery was sampled in greater detail, in order to determine the distribution of VAM fungi in the fields. The rootstocks sampled were identical to those used above but M 9 was included in this study. Four fields, each containing different combinations of rootstock were sampled. The maturity of the stoolbeds varied, the oldest one is in field 4 and is 18 years old, the youngest is an M 9 block in field 3 which is only 2 years old. All rootstocks were not necessarily found in each field.

Each stoolbed was fumigated prior to establishment, and then fertilized and given pesticides throughout the year as necessary. To help initiate rooting, pine bark mulch was spread on the rows and then soil was hilled around the growing shoots.

Each field was blocked according to rootstock (Figure 2.1, 2.2 and 2.3). Number of blocks per field varied depending on the position and number of rows in each block. The number of samples taken from each block varied according to the size of the block. The minimum number of samples taken from a block was 1 and the maximum was 12 (Figures 2.1, 2.2, 2.3).

The area of each of the larger blocks was divided into 100 equal sections. Lotus 123 software computer package was used to generate 2 random numbers each used to indicate a position on the vertical and horizontal axis. The sample position within a block was located at the intersect of these 2 axes.

Each sample was composed of of 3 sub-samples from which

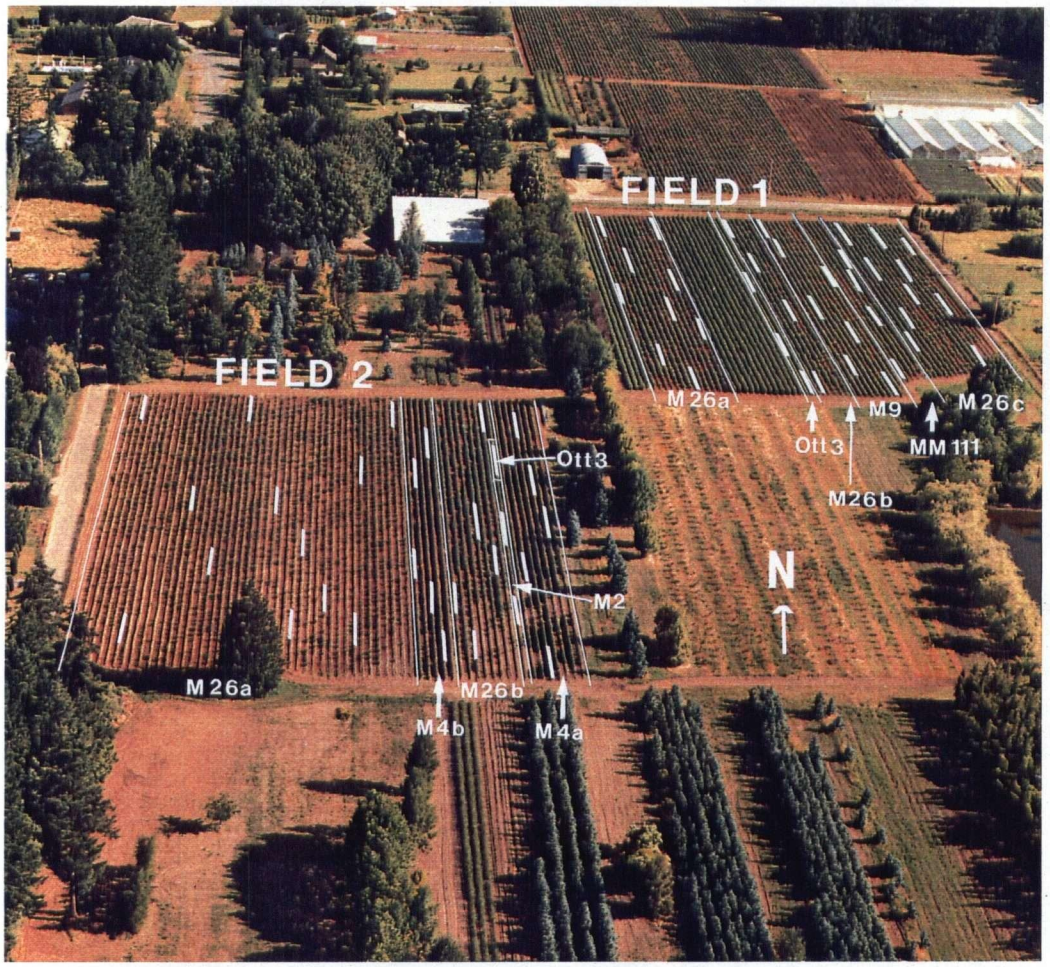

Figure 2.1: Sampling Strategies for Traas Nursery: Fields 1 and 2

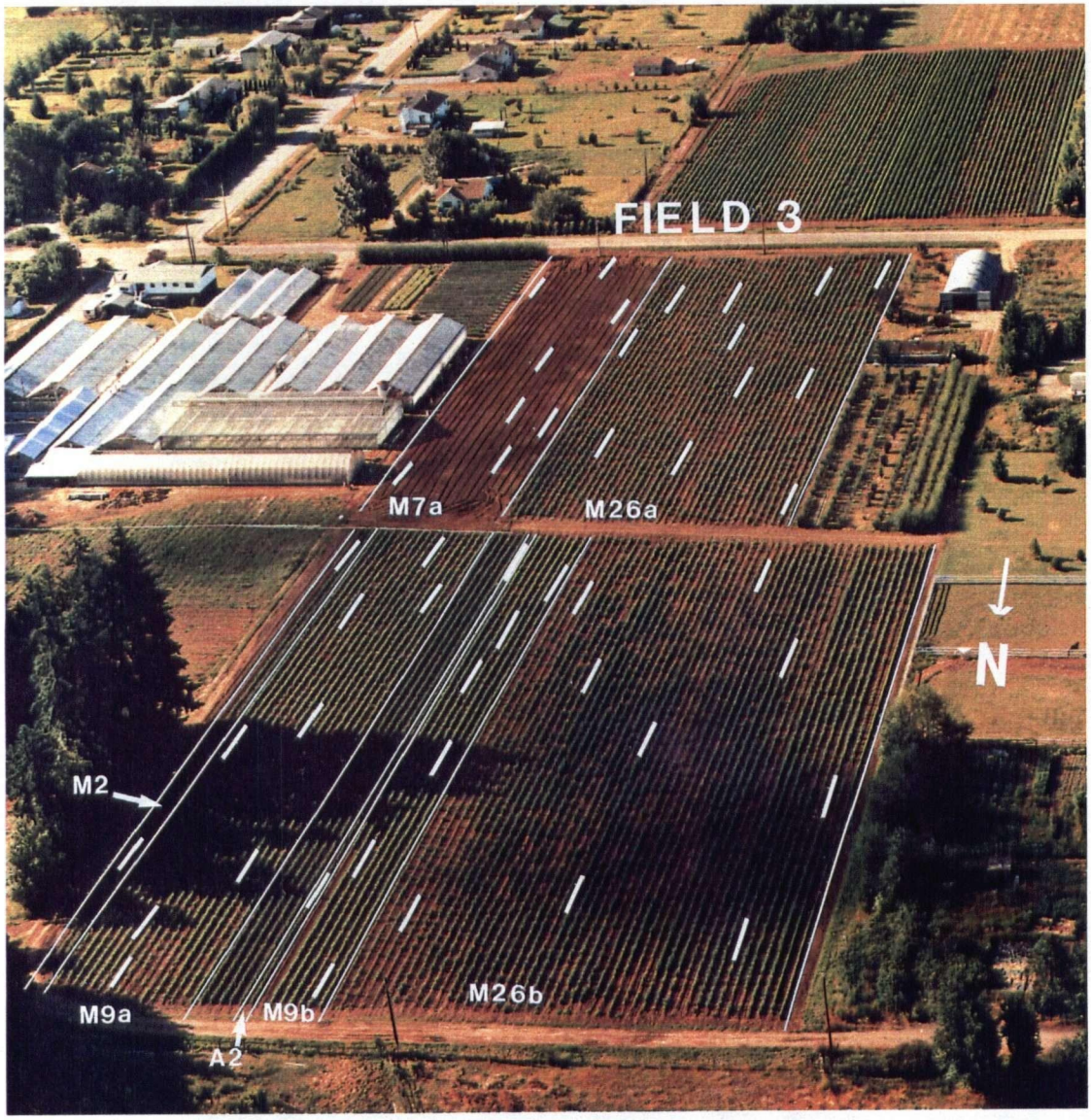

Figure 2.2: Sampling Strategies for Traas Nursery: Field 3

Figure 2.3: Sampling Strategies for Traas Nursery: Field 4

roots were collected. A sample was 20 meters long and was divided into 3 sub-samples of which two, (sub-samples 2 and 3) were located 8.3 m from a central sub-sample (sub-sample 1) (Figure 2.4).

At each sub-sample site the soil was brushed aside to expose the roots of each rootstock. The beds had been kept weed-free throughout the growing season and the roots of the young trees were suberized already and were therefore easy to identify (Figure 2.5b). The roots were shallow, so it was not necessary to dig deeper than 15 cm to expose them (Figure 2.5a).

Three roots, at least 3 mm - 6 mm in diameter with approximately 50 laterals, were each removed from the apical portion of the root system and bulked. The excised roots were placed in a polyethylene bag with about 300 mls of soil. Those roots still attached to the young trees were then re-covered with soil. All bags containing roots and soil were brought to the laboratory where the roots were placed in labelled bottles and fixed in FAA. The soil was air-dried and then stored. All roots were examined for VAM fungi.

Sampling occurred field by field over 5 days. Field 2 was sampled first, followed by Fields 1, 3, and 4.

The data collected for all four fields were arcsine transformed (Zar 1984) prior to statistical analysis. A modified Nested Two Way Analysis of Variance was used to analyze the data. Tukey's multiple range test was used to test for any differences amongst the fields, rootstocks, blocks and any interactions

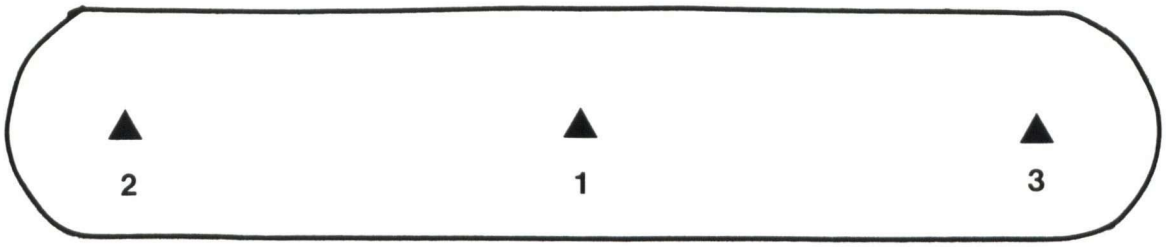

Figure 2.4: Schematic Diagram of a Sample

a b

Figure 2.5: a) Collecting Roots from Sub-Sample Site; b). Apple Roots from Stoolbed

amongst these variables.

BUDDED NURSERY

Distribution of VAM Fungi

During the first and second week of November, 1987, two fields from Cannor Nurseries at the Froese site, 10020 Gillanders Road, Chilliwack, B.C. were sampled to determine the distribution of VAM fungi in a budded nursery. This soil belongs to the Monroe Series which is medium-textured with a silty loam texture and has laterally accreted floodplain deposits. It is moderately well to well drained. This soil has a shallow phase which means that there are areas of the soil that are similar in all respects to the named soil, except that the depth of the soil profile is shallower than the modal. It has a 3% slope and is classified as an eluviated eutric brunisol (Luttmerding 1981). The soil in this nursery is not fumigated prior to transplanting rootstock, and only sprayed 1 - 2 times with a copper chloride fungicide per season for disease control.

All rootstocks had spent 2 seasons in the field and were to be shipped in the spring of 1988. A number of the rootstocks examined from this nursery originated from Traas Nursery. Each rootstock had been budded with different scion varieties. Malling 27 was the only rootstock found in this nursery that was not also found in the stoolbed nursery. Rootstocks examined were M 4, M 7, M 9, M 26, M 27 and Alnarp 2.

Sampling strategies for this nursery were identical to those of the stoolbed nursery (Figures 2.6 and 2.7).

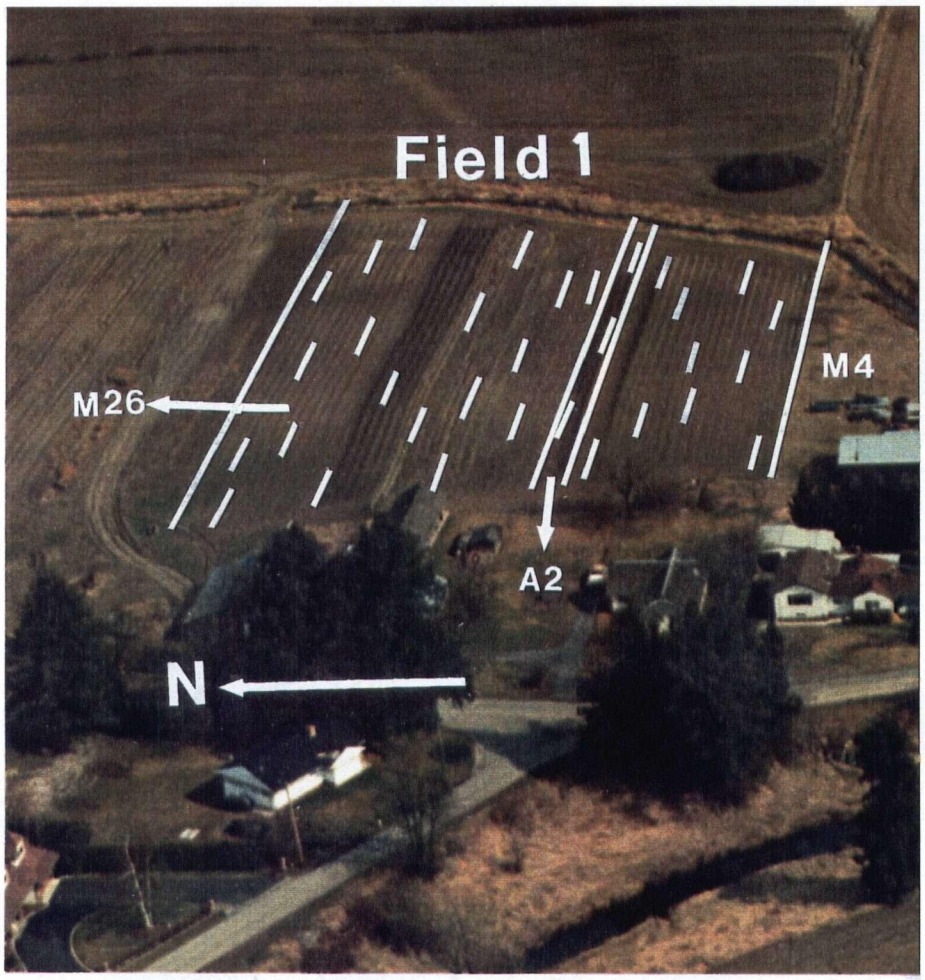

Figure 2.6: Sampling Strategies for Cannor Nursery: Field 1

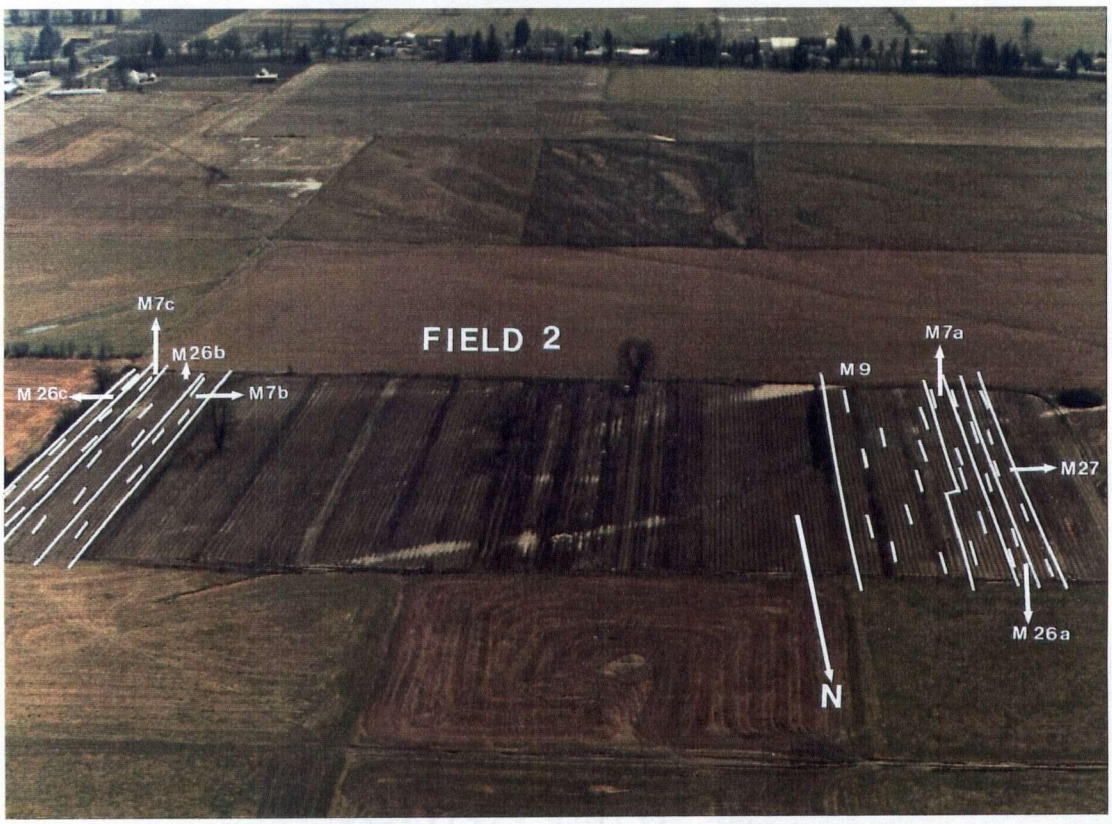

Figure 2.7: Sampling Strategies for Cannor Nursery: Field 2

A modified nested ANOVA was used to test for significant differences between the 2 fields and rootstocks. No interaction between the fields and rootstocks was possible as M 26 was the only rootstock of the 6 examined that occurred in both fields.

COLD STORAGE AND ROOTSTOCK MYCORRHIZAE

Rootstocks MM 111, MM 106, M 26, M 4 and M 7 from Traas Nursery were used in this study. MM 111 and M 7 were lifted from the field over the period of 15 November to December, 1 1987 and were placed in cold storage (2-4^{o}C and 75-80% humidity) on 4th of January, 1988. On January 5, 1988, 10 plants were taken randomly from each of the storage piles of these 2 rootstocks. The entire root system from each plant were fixed in FAA.

By January 9, 1988, MM 106, 1 field of M 26, and M 4 had also been lifted though they were not processed for cold storage yet. Ten plants from these 3 rootstocks were taken from the storage pile and their roots fixed in FAA, for VAM counts.

After spending 15 weeks in cold storage at 2 - 4^{o}C and relative humidity of 75 - 80% they were again sampled. M 4 had not been placed in cold storage, but rather in a dry, dark, cool shed. Ten plants from each of the rootstock piles were taken. The roots from each of the trees were fixed in FAA for VAM counts.

VAM ANALYSIS

All roots were examined for the presence of mycorrhizae using a modified version of Kormanik and McGraw's (1982) technique. All roots were darkly stained, so it was necessary to bleach roots for 20 - 30 minutes in 30% alkaline H_2O_2. Percent

mycorrhizal colonization was measured using the gridline intersect method (Giovanetti and Mosse 1980).

Surfer, a computer software package was used to prepare all VAM fungal distribution graphs.

RESULTS

STOOLBEDS

Mycorrhizal Status of Apple Rootstocks

Vesicles and hyphae were the only VAM fungal structures observed in the roots of all samples examined. Significant differences in percent colonization amongst the nine rootstocks examined were obtained at 0.05 confidence limits (Table 2.1).

Results obtained in this study show a large variation in percent colonization within and amongst the different rootstocks (Figure 2.8). Only M 2 had mycorrhizae present in all 10 samples examined. Malling 4, M 7 and MM 111 had 2 of the 10 samples examined with 0% colonization. Alnarp 2, and M 7a had 3 plants of the 10 examined with 0 % colonization while Ottawa 3, M 26 and MM 106 had 4, 5, and 6 samples respectively with 0% colonization. Malling 2 had a low of 10% VAM colonization and a high of 80%. The remaining rootstocks had highs of under 50%.

Malling 2 had the highest mean at 37%, followed by M 7 at 32%. Malling Merton 106 and Alnarp 2 had the lowest mean percent colonization at 2% and 9% respectively (Table 2.1).

Distribution of VAM Fungi

Mycorrhizae were found in plants from all 4 fields. However, the colonization rate in all fields was generally low. Fifty

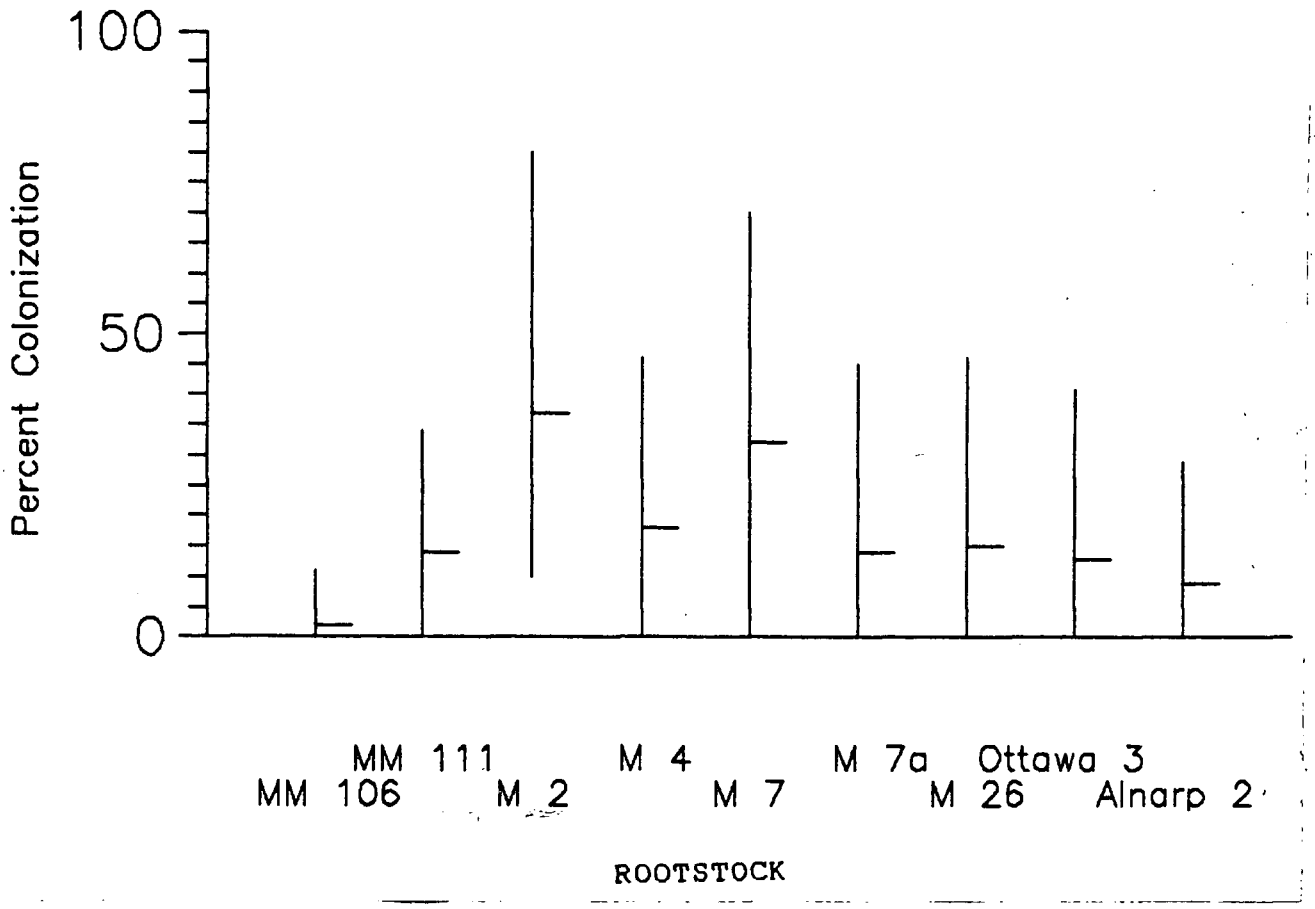

Figure 2.8: Mean and Range for Colonization of Nine Different Rootstocks from Stoolbeds: April 1987

Rootstock	% Mean Colonization
MM 106	2 a*
Alnarp 2	9 a,b
Ottawa 3	13 a,c
M 7a	14 a,d
MM 111	14 b,c,d,e
M 26	15 a,e,f
M 4	18 b,c,d,f
M 7	32 c,d,f,g
M 2	37 g

Table 2.1: Rootstock Mycorrhizal Colonization: April 1987

* same letter indicates no significance difference (α = 0.05 Mann-Whitney U test)

percent of all samples from this nursery were found to have 0% mycorrhizal colonization and 89% had less than 10% colonization in Fields 1, 2 and 3. Field 4 had 85% at less than 10 % colonization (Figure 2.9). The highest colonization rate for this nursery was 37% found in field 1 in a M 26 block.

The nature of the experimental design was highly imbalanced, so statistical conclusions should be treated with caution. There were no difference in VAM colonization amongst the four fields, the blocks within each field and interactions amongst field, rootstock and blocks (Table 2.2a). There was however, a significant difference in colonization amongst the different rootstocks. M 2 differed significantly from all other rootstocks (Figure 2.10). The remaining rootstocks, showed no significant differences amongst each other.

The pattern of mycorrhizal colonization were generally the same for Fields 1 and 2 (Figures 2.11 and 2.12). Colonization was low except for 2 to 3 isolated peaks, located along the perimeter of the field. Field 1 showed 3 peaks: 37% in the M 26c block, and 19% and 17% in the M 26a block (Figure 2.11). There was a minor peak of 8% in the center of the field in the MM 111 block.

Field 2 also showed high peaks of 23% in the M 26b block and 19% in the M 26a block, both on the field's perimeter. Toward the center of the field a peak of 17% was found in the M 2 block (Figure 2.12).

The distribution of mycorrhizae in Fields 3 and 4 were similar (Figures 2.13 and 2.14). Like Field 1 and 2, the highest

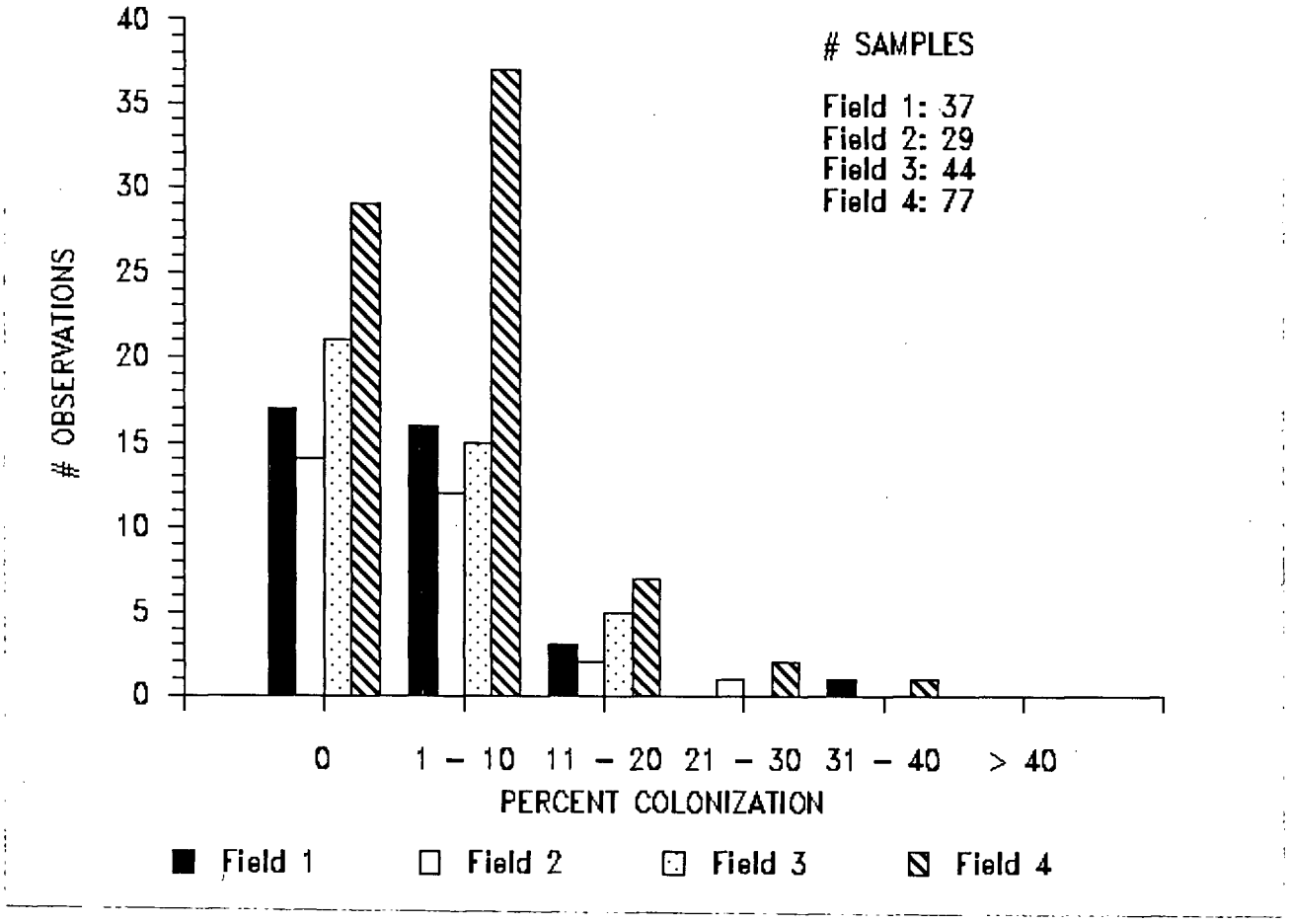

Figure 2.9: VAM Distribution in Stoolbeds by Field: Fall 1987

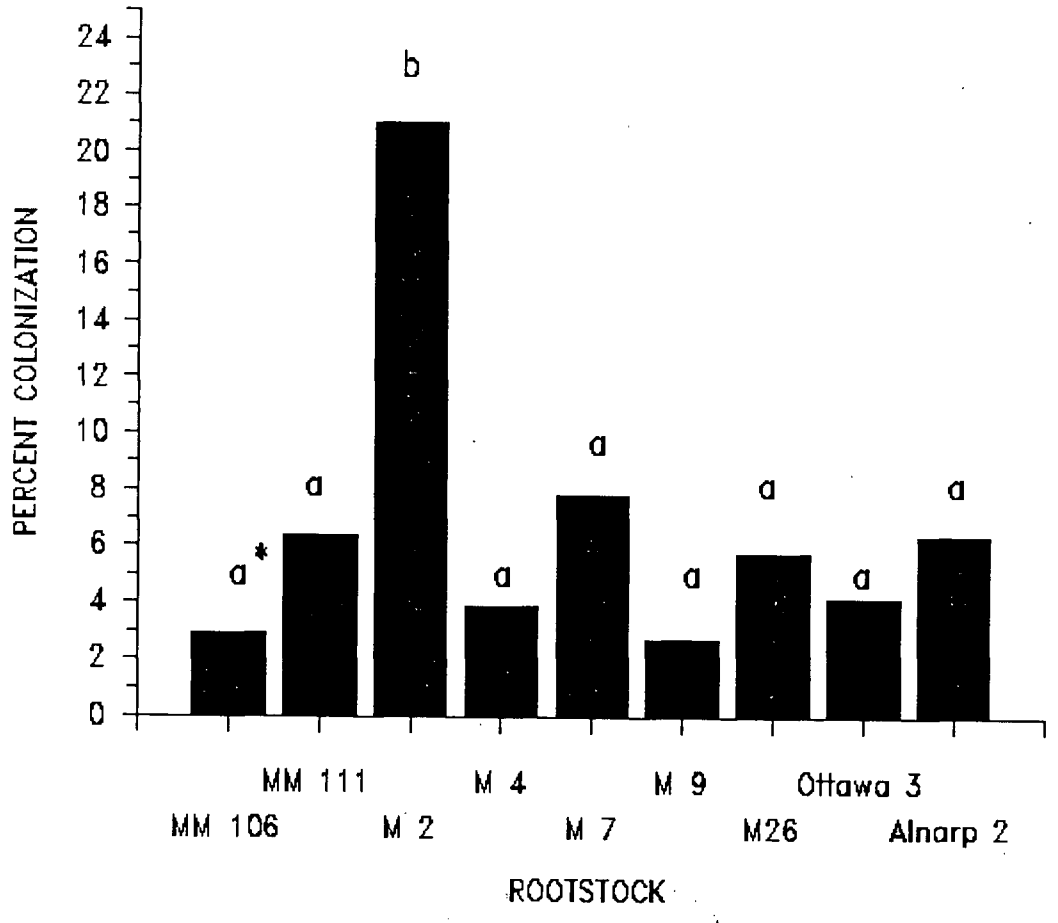

Figure 2.10: Rootstock Colonization, Traas Nursery: Fall 1987

* same letter indicates no significant differences (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Field	3	222.360	74.120	0.923	0.460
Rootstock	8	1215.200	151.910	5.753	0.008
Field*Rstock	9	237.660	26.407	0.329	0.949
Block(Field, Roostock)	12	964.380	80.365	1.506	0.128
Error	149	7952.700	53.374		
Total	181	10468.000			

Table 2.2: Analysis of Variance for VAM in Traas Nursery

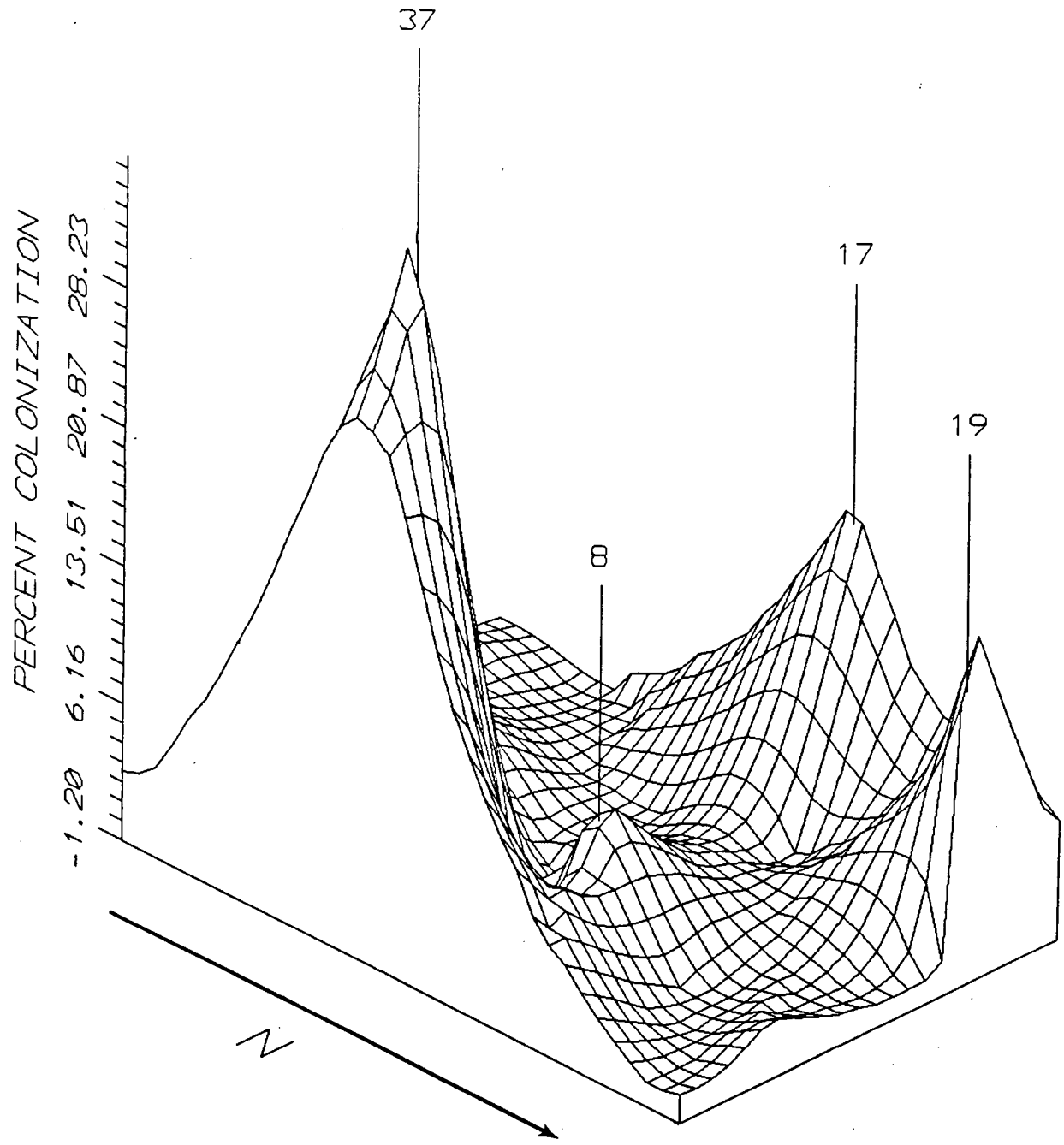

Figure 2.11: Patterns of VA Mycorrhizae in Stoolbeds: Traas Nursery Field 1

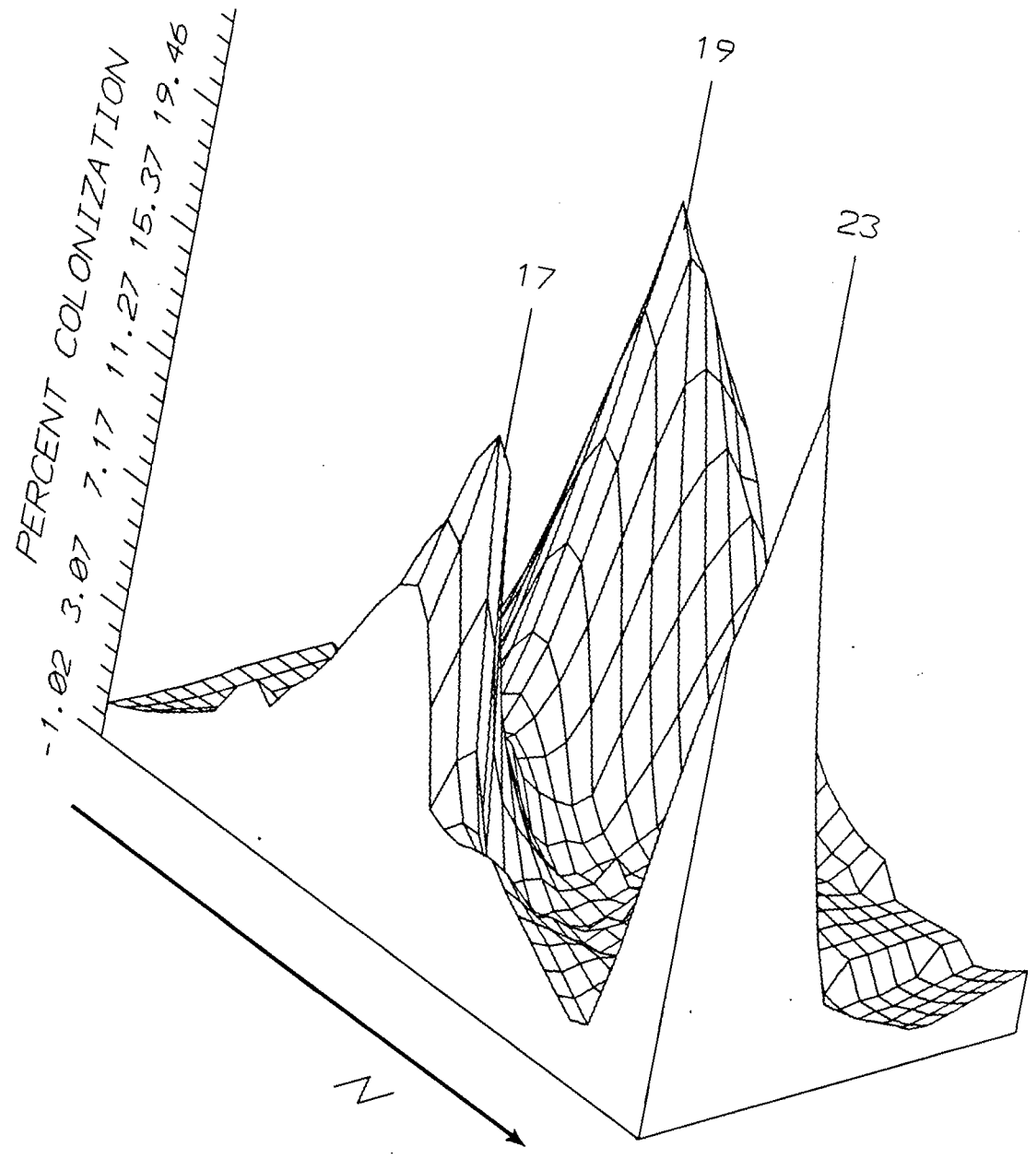

Figure 2.12: Patterns of VA Mycorrhizae Stoolbeds: Traas Nursery Field 2

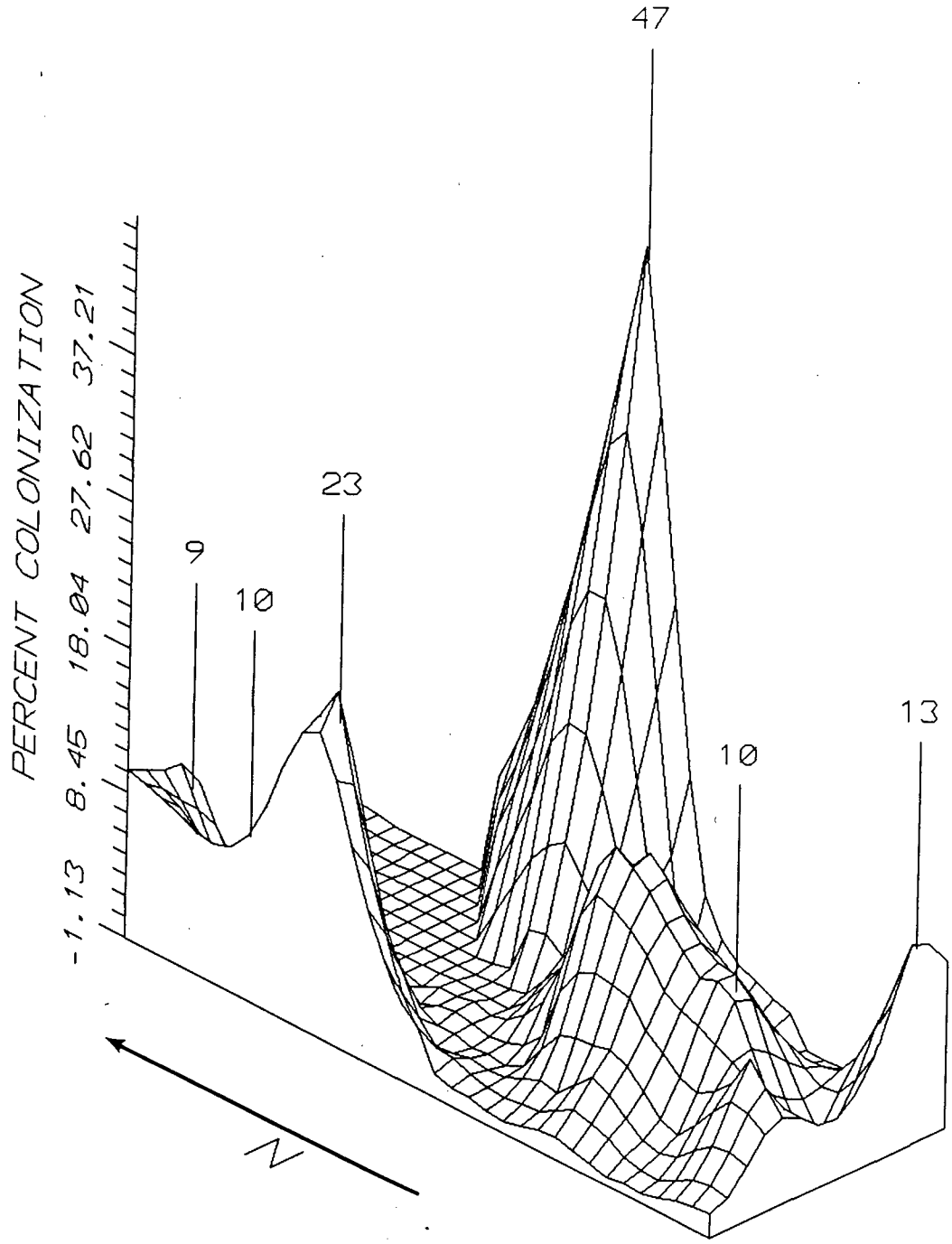

Figure 2.13: Patterns of VA Mycorrhizae in Stoolbeds: Traas Nursery Field 3

percent colonization was generally located along the periphery of the fields. In Field 3, a peak of 47% was found in the M 2 block (Figure 2.13). In the M 7 block there were peaks of 13% located along the periphery of the field. Both M 26 blocks had higher than 10% colonization; block had 10% and 18% peaks found in the center of the field and block b had 10% and 23% colonization.

Field 4 showed the most variation in mycorrhizal colonization of all 4 fields (Figure 2.14). There were 12 peaks of 10 % or greater found in this field. The highest peaks of 25%, 28% and 32% were found along the perimeter of the field, in blocks M 7b, Ottawa 3a and MM 111b respectively.

BUDDED NURSERY

Mycorrhizal Status of Apple Rootstocks

While there was a significant difference in mycorrhizal population between the two nurseries, there were no significant differences in VAM colonization of rootstocks within the budded nursery (Figure 2.15).

Distribution of VAM Fungi

There was a significant difference in mycorrhizal colonization between the stoolbed and budded nursery. In the budded nursery there were no samples collected that had 0% colonization. The lowest percent colonization found in this nursery was 30% while the highest was 73%. There was no significant difference in mycorrhizae between the 2 fields, or amongst the 6 rootstocks used in this study (Table 2.3a and 2.3b).

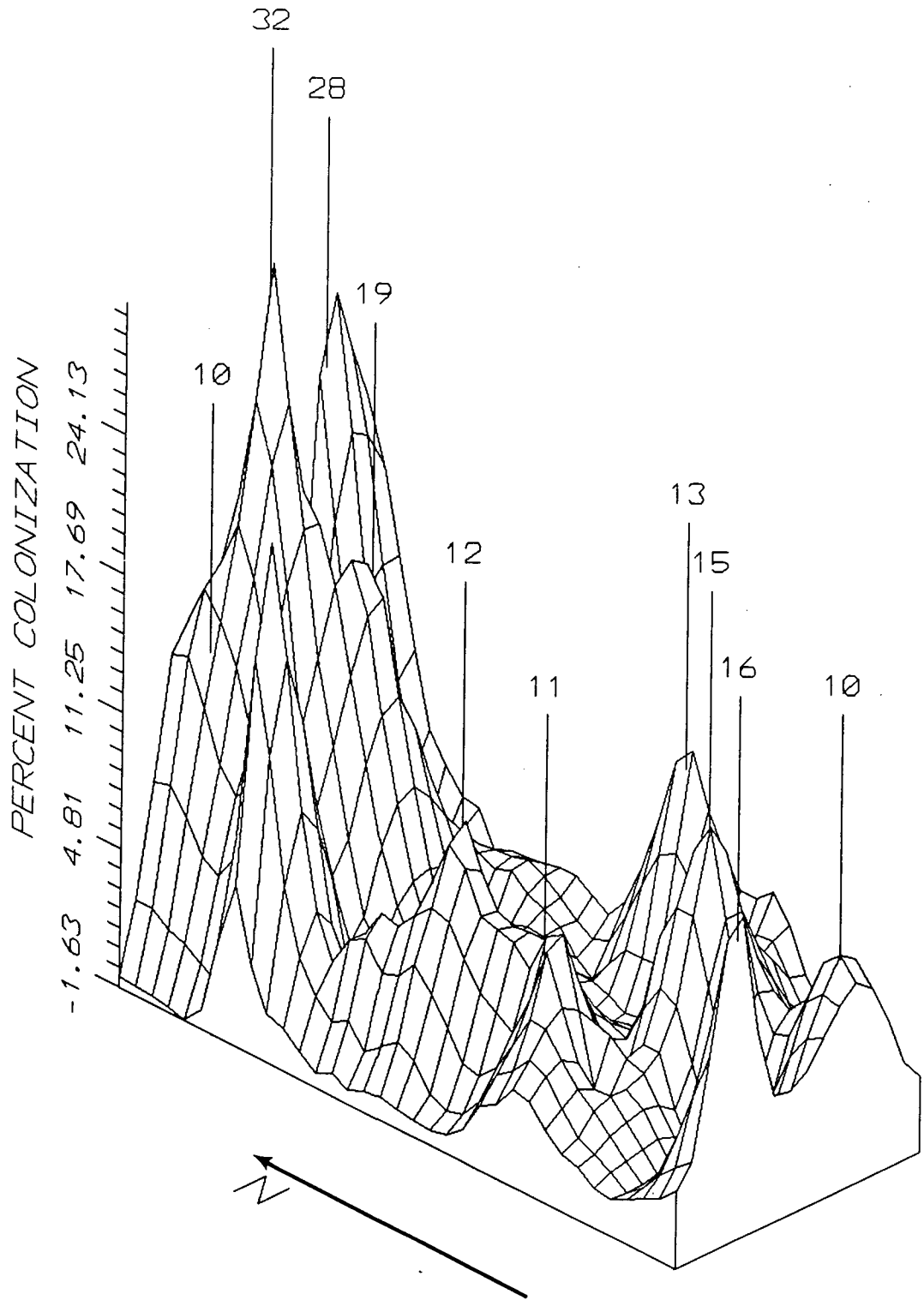

Figure 2.14: Patterns of VA Mycorrhizae in Stoolbeds: Traas Nursery Field 4

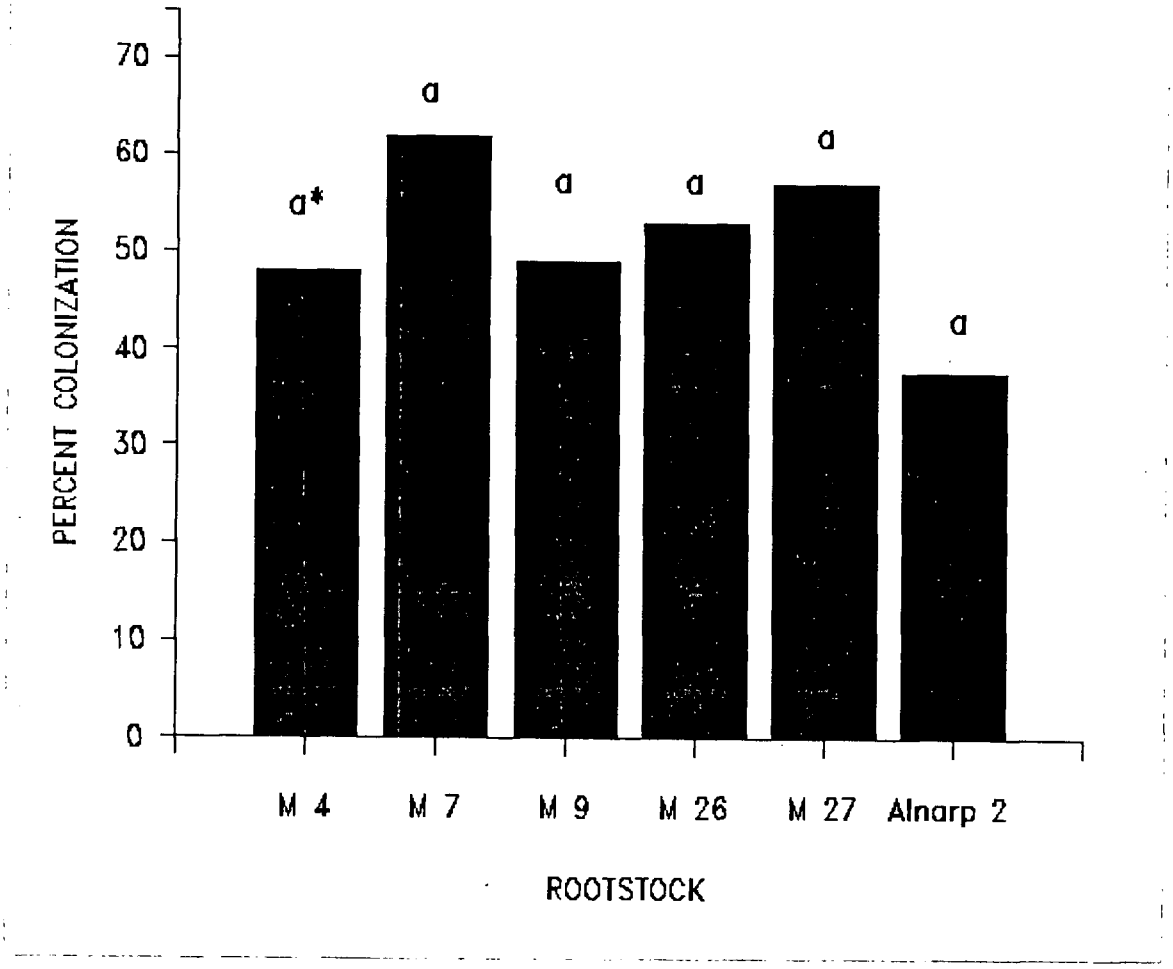

Figure 2.15: Mycorrhizal Colonization in Cannor Nursery

* same letter indicates no significant difference ($\alpha = 0.05$ Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Field	1	143.770	143.770	1.217	0.331
Rootstock	5	1611.100	322.210	2.727	0.176
Block(Field, Rootstock)	4	472.530	118.130	1.406	0.242
Error	65	5460.000	84.000		
Total	75	8665.400			

Table 2.3: Analysis of Variance for Mycorrhizae: Cannor Nursery

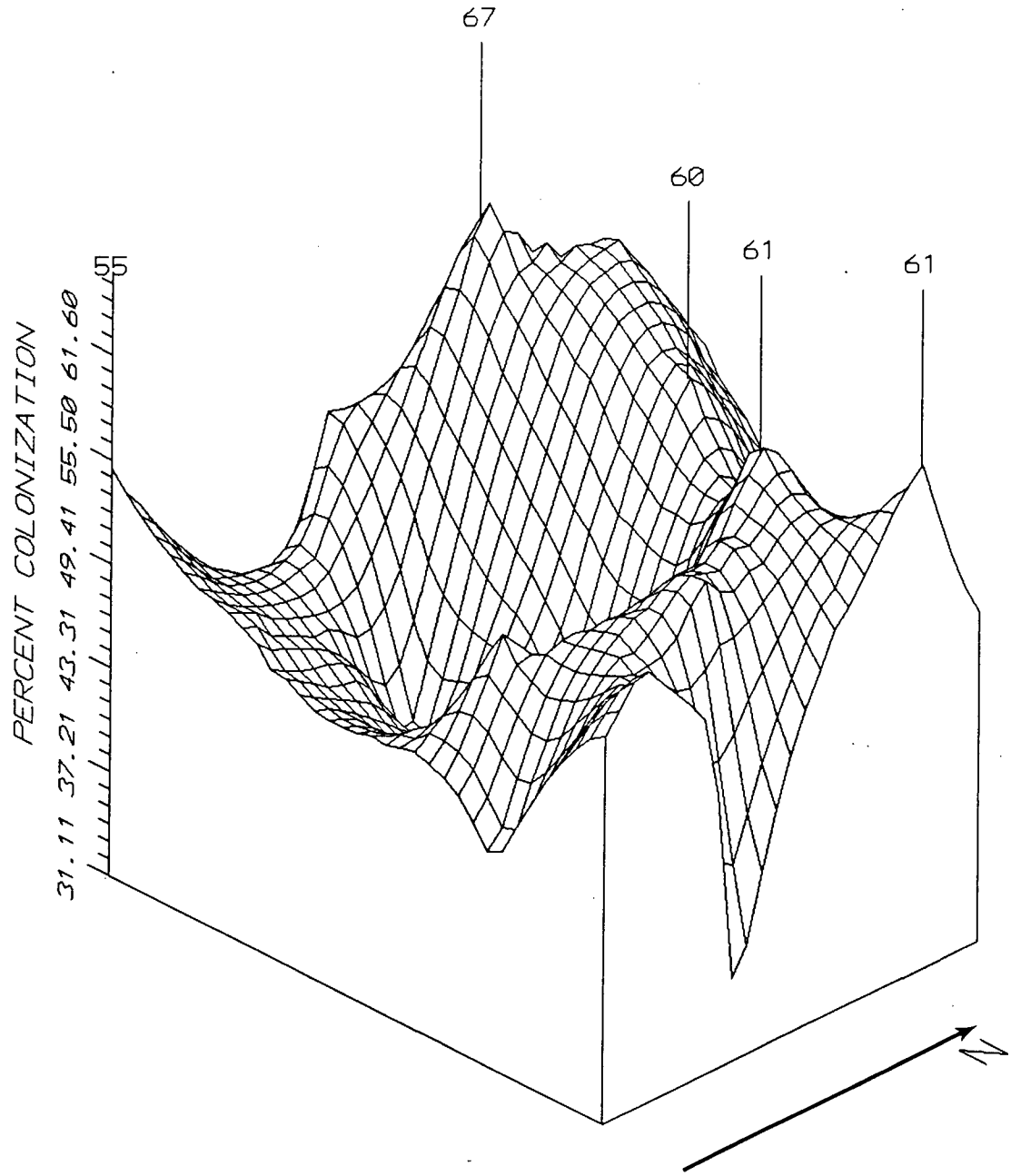

Figure 2.16: Patterns of VA Mycorrhizae: Cannor Nursery Field 1

The pattern of mycorrhizae in Field 1 did not follow that of any field from the stoolbed nursery. While the peak of 67% was found on the western perimeter of the field, there were other peaks of 55% or higher towards the eastern part of the field (Figure 2.16). Towards the central part of the field, the colonization rate dropped off though not below 30% (Figure 2.16).

As in other fields, the highest mycorrhizal population in Field 2 of the budded nursery was found along the perimeter of the field. Peaks of 73% and 75% were found in this field along the north eastern and south western parts of the field (Figure 2.17). Colonization did drop off towards the central portion of the field though there appeared to be a plateau of lesser colonization rates on the eastern side of the field. As in Field 1 the colonization rate did not drop below 30%, the lowest percent colonization in the field was 36%.

Cold Storage and Rootstock Mycorrhizae

Before cold storage M 4 and MM 111 had mycorrhizae present in all 10 samples examined. Malling 26, M 7 and MM 106 had at least 2 samples with 0% mycorrhizal colonization. After 15 weeks of cold storage, all rootstock examined had at least 1 sample with 0% mycorrhizal colonization. Malling 4 and MM 111 were the only 2 rootstocks of the 5 examined that showed a significant decrease in percent mycorrhizae due to cold storage (Table 2.4).

DISCUSSION

This is the first report of different clonal rootstocks forming different percent mycorrhizal colonizations in apple

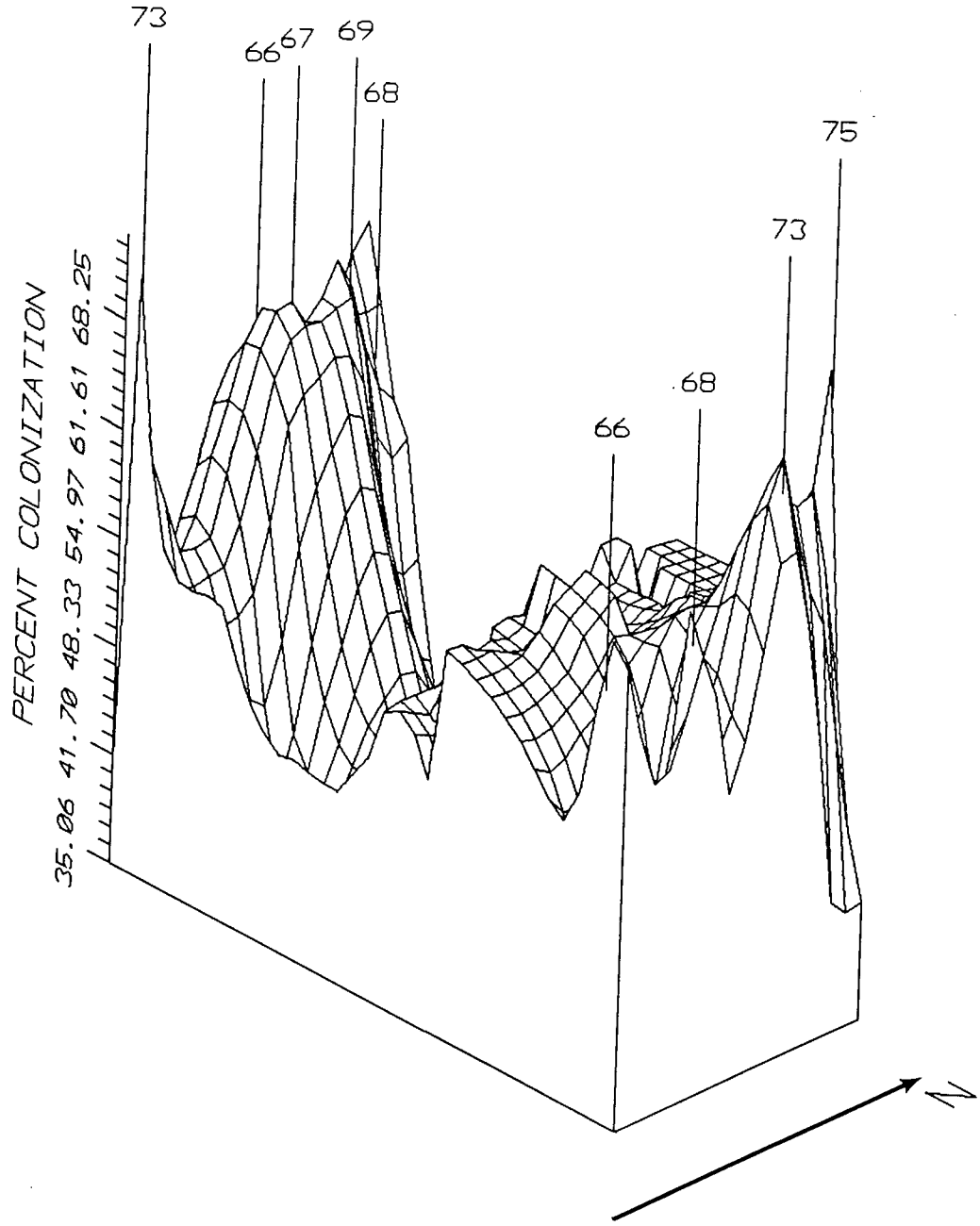

Figure 2.17: Patterns of VA Mycorrhizae: Cannor Nursery Field 2

	Before	After
M 4	23 a*	9 b
M 7	15 a	19 a
M 26	22 a	23 a
MM 106	13 a	11 a
MM 111	18 a	8 b

Table 2.4: VAM Colonization Before and After Cold Storage in Rootstock from Stoolbeds

* same letter indicates no significant difference (α = 0.05 Mann-Whitney U test)

rootstock from a stoolbed nursery. Traquair and Berch (1988) and Miller et al. (1985a) reported that there was no difference in VAM colonization amongst different clonal peach and apple rootstock. The clonal rootstock that these authors examined came from plants already established in the orchard and not from nursery beds. In stable environments such as orchards, the indigenous population of VAM fungi is diverse and well established. Under these situations, there may be no difference in clonal receptiveness to VAM fungi.

Different cultivars of clonal strawberries in the nursery also showed no difference in mycorrhizal colonization (Robertson et al. 1988). However, the root systems of the various clonal strawberries do not differ as much as the root systems amongst clonal apples. Granger et al. (1983) proposed that MM 111 and M 7 may differ in growth response to VAM inoculation, but they do not differ in receptiveness to VAM fungi. However, these tests were done in vitro. In field analysis such as this study, there may be a different receptiveness of the clones to VAM fungi. Granger et al. (1983) attribute the different growth responses to the different root systems of these two apple clones.

While the root morphology of each rootstock was not examined in this study, it is possible that the different root systems found in clonal stock may influence the receptiveness of the host to the fungus. There are many factors that influence root development of apples in the nursery. In stoolbeds, high humidity, deepness of hilled soil and low light favours the

production of roots and burrknots (Rom and Brown 1979, Rom and Motichek 1987).

One of the major factors influencing root development is the production of burrknots. Burrknots are nonpathogenic clusters of root intials capable of forming roots under favourable conditions. Different apple clones produce different amounts of burrknots, M 2 consistently producing fewer than other rootstocks (Rom and Brown 1973).

Different apple clones also produce different levels of auxin and gibberellin, low vigour clones showed higher levels of auxins and giberellins than high vigour clones (Grochowska et al. 1984). Inoculation of lemon and tamarillo with VAM fungi induced root initiation and development in the absence of rooting hormones (Cooper 1983), indicating that these fungi influence levels of growth hormones in roots of trees.

Malling 2 consistently showed higher VAM colonization than the other rootstocks. The high incidence of the VAM fungus in this clone may influence the amounts of growth hormones produced therefore influencing the amounts of burrknots and its root development. Malling 2 spread over a larger soil area in 5 different soils than any other rootstock (Coker 1958). It is therefore possible that the VAM fungus influences the size of the root system of the clone. The larger the root system, the more soil area the clone is able to reach and therefore the probability of encountering live VAM propagules is increased.

In both nurseries, the highest percent mycorrhizal

colonization is found along or towards the perimeter of each field. Traffic and therefore live sources of inoculum, is greatest along the perimeters. It would appear as if the VAM fungi are moving inwards in each field.

In the budded nursery, the fields were never fumigated allowing the indigenous VAM fungi to become well established. The fungi may be set back through the summer after an application of fungicide, but indications are that the fungi are either not affected, or if they are, they are able to reinvade the seedlings. It has been hypothesized that the additions of certain fungicides may increase root exudates, thus increasing the VAM colonization (Menge 1982). The rooting depth of the budding nursery is also deeper than the stoolbed nursery. The fungi in the budding nursery may therefore be physically protected from soil applications of nonsystemic fungicides and may even be stimulated by releases of root exudates after fungicide application.

Fumigation of the soil eliminates VAM fungi and this is probably the primary reason for the low colonization rate in the stoolbed nursery. Two to six months after fumigation, VAM fungi are able to reestablish in soils (Menge 1982). Many of the stoolbeds in the nursery used in this study were older than 5 years. Mycorrhizae should have become established in the field by this time. The stoolbeds are sprayed for fungal pathogens 5 - 7 times during the season. It is most likely that spraying the stoolbeds throughout the season may be adversely affecting the

VAM fungi. While there may be VAM fungi present in the stoolbed nursery they are never allowed to proliferate and spread throughout the field. Management practices in harvesting and processing of rootocks from stoolbeds may also be detrimental to the VAM fungi.

Nemec (1987) reported that storing VAM plants at 5 - 10°C did not affect the ability of the fungi to spread through citrus roots. The temperature under which rootstocks used in this study were stored should not be affecting VAM colonization. However, high moisture during prolonged storage appears to be detrimental to viability of VAM fungi as this leads to detrimental reductions of oxygen tension (Nemec 1987). The humidity in the cold storage rooms is maintained at over 75%. Such high humidities could be deleterious to VAM colonization in those rootstocks that are stored beyond a specific threshold time period.

Malling Merton 111 was the first rootstock to be placed in cold storage and was therefore under the most stress. Reduced O_2 concentration in this part of the cold room could have had detrimental effects on the VAM fungi. Either the temperature, the humidity, O_2 level, or duration of storage may have reduced the VAM fungi in this rootstock.

Malling 4 was not placed in cold storage, but simply stored in a cool dry shed. Reid and Bowen (1979) found that under very dry situations, VAM colonization dropped drastically. In this instance the M 4 roots may have been too dry during storage, causing a reduction in root colonization.

The mycorrhizal status of apple rootstock clones in and leaving stoolbed nurseries varies. However, the variation in mycorrhizae found in this study is more likely to be a result of management practices and position of the rootstock in the field in relation to sources of inoculum than to actual genotype. In areas where there is high inoculum such as along the perimeter of the fields, the roots are more likely to come in contact with the inoculum. As rootstocks are planted by block, rather than randomly the mycorrhizal colonization of each clone depends on its position in relation to inoculum source.

Management practices in this stoolbed nursery including high rates of fertilization, use of pesticides, harvesting time and storage may be detrimental to VAM in certain clonal rootstocks. The environment in this stoolbed nursery does not favour indigenous VAM fungi, while in budded nurseries it does. It may be necessary to alter management practices, specifically to reduce the amounts of pesticides used, to ensure that VAM fungi are not detrimentally affected. Alternatively, inoculating stoolbeds with VAM fungi will aid in the propagation of VAM fungi and thereby increasing the probability of the clones becoming mycorrhizal.

If VAM fungi do play a role in the establishment of healthy orchards, rootstock that spends time in the budding nursery should perform better in the orchard than rootstock directly from stoolbed nurseries. If orchard soils are fumigated, VAM inoculations in the nursery should be carried out.

STERILIZING OLD APPLE SOILS INCREASES PLANT HEIGHT IN POT TESTS: CONFIRMATION OF THE PRESENCE OF APPLE REPLANT DISEASE

INTRODUCTION

Crops planted into soil that previously supported the same crop will often show a 'sickness' that is easily overcome by sterilizing the soil (Deal et al. 1971, Hwang 1988, Pepin et al. 1975, Trudgill 1984). This disorder is especially common in old apple soils (Covey et al. 1984, Hoestra 1968, Ross et al. 1984, Savory 1967, Slykhuis and Li 1985). In the absence of an identifiable pathogen, this sickness in old apple soils has been termed 'Apple Replant Disease' (ARD). The increase in shoot height due to sterilization indicates that a biological agent may be involved with this problem. Slykhuis and Li's study (1985), indicating that phosphorus fertilizers may also alleviate this problem suggests that vesicular-arbuscular mycorrhizal (VAM) fungi may play a role in ARD.

To eliminate the speculation on when ARD appears, pot bioassays are being used to predict if a soil has ARD. Hoestra (1968) developed the basic bioassay and variations are used universally (Ryan 1975, Sewell and White 1981, Slykhuis and Li 1985, Upstone 1974). Half of the test soil is fumigated. Apple seedlings, in the 1 - 2 leaf stage are transplanted into both fumigated and nonfumigated soil. If there is a significant increase in shoot height in the fumigated pots compared to the nonfumigated, in the absence of an identifiable pathogen, the

soil is identified as being ARD. Very specific soil handling and testing methods are used in pot bioassays. For instance, soils should be collected from under the orchard canopy, preferably in the fall and must be kept cool and moist until use. Soil from one orchard in this study was air dried, to determine if air-drying would interfere with the outcome of ARD pot bioassays.

Slykhuis and Li (1985) found that increases in test shoot height occurred after a number of biocides and combinations of biocides and fertilizers were used. While the change in shoot height due to soil treatment is well documented, little is known of how these treatments affect soil nutrients, VAM colonization of seedling roots, and root dry weight.

If sterilization is to be used in bioassays, it is important to understand its effects on soil pH and nutrient availability. The effects of sterilization on soil nutrients varies depending on the soil and the type of sterilant used. Soils high in organic matter will have a flush of nitrogen (N) and phosphorus (P) after sterilization (Eno and Popenoe 1963, Skipper and Westermann 1973, Warcup 1957). Autoclaving and steam sterilizing increase soil P more than other methods of sterilization, but decrease the pH (Skipper and Westermann 1973). In other soils, sterilization by autoclaving does not affect soil pH or P but only Mn (Gennari et al. 1987, Williams-Linera and Ewel 1984). Air-drying the soils for extended periods increases the rate of organic matter decomposition upon rewetting and the subsequent release of soil nutrients especially N and P (Liegel 1983, Mack 1963). However,

sterilization effects on N and pH are more pronounced in moist soils than in dry soils (Salonius et al. 1967).

This study was therefore undertaken to confirm Slykhuis and Li's (1985) findings that soil sterilziation and added phosphorus will increase plant height in old apple soils; to determine how sterilization affects soil pH and available P; to determine how air-drying affects seedling growth in pot bioassays; and to determine the effects of soil treatments on VAM colonization of seedling roots.

MATERIAL AND METHODS

COLLECTING SOILS FROM ORCHARDS

Soils 1 - 4

Soils 1 - 4 were identified as having ARD after being tested by Dr. J. Slykhuis at his Apple Replant Testing Service in Summerland, B.C.

During the first week in May 1987, the soils were collected from the following orchards: Soil 1, from Clark Brothers, Upper Bench Rd., RR# 1, Keremeos, B.C.; Soil 2 from Bahnson Brothers, Black Sage Rd., RR# 1, Oliver B.C.; Soil 3 from Harlequin Farms, Naramata Rd., Naramata B.C.; Soil 4 from Lammers Orchard, 10th Ave., RR# 1, Keremeos, B.C. (Table 3.1).

At the time of soil collection, old trees had been removed from all orchards. Soil 1 had been replanted with M 26 apple rootstock. The new rows of trees had been planted directly into the old rows. It was therefore easy to identify the rows and alleyways from the previous crop. Soils 2 and 4 had been

	Longiti.	Latitude	Soil Series	Soil Description
Soil 1	49° 13'	119° 49'	Rutland Gravelly Sandy Loam	Surface soils are dark brown with varing amounts of stones in the low-parts. Underlying materials stratif-ied sand & gravel.
Soil 2	49° 9'	119° 32'	Osoyoos Loamy Sand	Surface soils are brown; progressive reduction of silt & clay content to bottom of solum. Lime accumulates in lower B horizon
Soil 3	49° 34'	119° 30'	Penticton Silty Loam	Solum to depth of of about 100 cm is greysish silt loam Substratum compos-ed of deep beds of stratified silty, clay & fine sand
Soil 4	49° 12'	119° 50'	Nisconlith Loam	There is a decid-uous leaf layer at soil surface. Deep A horizon, follow-ed by a structure-less G horizon.
Soil 5	49° 57'	119° 22'	Rutland Gravelly Sand Loam	Surface soil dark brown shading to brown in lower layer, with vary-ing amounts of stones and gravel.

Table 3.1: Site Location and Description of Orchards (Kelly and Spilsbury 1949)

ploughed, disced and summer fallowed. Soil 2 had a dense cover of broadleaf and grass weeds, while Soil 4 had been seeded with barley. At site 3, the soil had been ploughed and disced the previous day so there was no cover crop.

An attempt was made to keep the soils collected from old rows and alleyways separate, in case the pH differed significantly between these two areas in the orchard. Only at site 1 was this possible. For the remaining orchards, old rows had to be estimated by aligning rows from adjacent blocks. Soil was collected from fifteen sites from the alleged rows and 15 sites from the alleged alleyways, for a total of 30 sites per orchard.

At each collection site polyethylene bags were filled with approximately 1 litre of soil from the top 15 cm. All plants and large roots were removed. All bags were labelled and brought to the laboratory at the University of British Columbia (UBC) where they were stored in a cool dry area. A subsample of about 500 gm of soil was taken from each bag, air-dried, and the pH determined. Since no difference in soil pH for the rows and alleyways was evident (Appendix), a composite sample for each orchard was made. The soil from each bag was passed through a 2 cm sieve to remove the larger pieces of debris. All composite samples were then air-dried at 22^{o}C over a period of 7 days. Once all soils were air-dried they were stored in large containers until further use.

Soil 5

Soil 5 had been identified as having ARD by pot and field tests done by Dr. G. Neilsen of Agriculture Canada, Summerland B.C. and Dr. J. Yorston of B.C. Ministry of Agriculture and Fisheries, Kelowna, B.C.

During the third week of September 1987, Soil 5 was collected from Ummard's orchard, Springer Ave., Kelowna, B.C. (Table 3.1).

The soil was collected from a row of trees that had been used in ARD field trials. Not all of the old trees from the row had been removed. Soil was collected from under the canopy, about 1 - 1.5 meters from the trunk of the old tree avoiding treated areas. The top 7 - 8 cm of soil including the grass cover crop was discarded. Soil to a depth of 30 cm, including roots from the apple tree, was placed in 20 litre containers.

All filled containers were brought back to the laboratory at UBC. A composite sample was made from all soil collected from this orchard. Soil was passed through a 2 cm sieve. Half of the composite sample was air-dried at 22^{o}C over a period of 7 days, and then stored. The other half was kept moist in a cool dark container until further use.

POT BIOASSAY TESTS FOR ARD

Test Seedlings

Open-pollinated Red Delicious or MacIntosh apple seeds treated with Captan were used in all pot tests. Seeds were obtained from the virus-free orchard of Agriculture Canada Research Station in Summerland, B.C. The seeds were wrapped in

moist paper towels, placed in polyethylene bags and vernalized in the refrigerator for 10 - 14 weeks. Once radicles had emerged from 80% of the seeds, they were planted in trays containing moist calcined montmorillonite clay (Turface, Applied Industrial Materials Corp., Deerfield Il.). The seedlings grew for about 1 week to the 1 -2 leaf stage and were then used for pot experiments.

Soils 1 - 4

All soils were treated identically. One half of each composite sample was sterilized, and to both the sterilized and non sterilized soil, 3 fertilizer treatments were added. The fertilizers used were monoammonium phosphate (11-55-0), ammonium nitrate (34-0-0) and triple superphosphate (0-45-0).

Four autoclavable and 4 large polyethylene bags were each filled with 4 litre of air-dried soil from each orchard. Enough water (500 ml) was added to each bag to moisten the soil. The 4 autoclavable bags were autoclaved for 1 hour at 94 kPa at 120^{o}C. The bags were kept at 22^{o}C for 24 hours and then autoclaved again as above. They were removed from the autoclave, opened and allowed to aerate for 3 days allowing any volatile phytotoxic substances to escape. The polyethylene bags were stored until pot tests were begun.

Three hundred mls of sterilized or nonsterilized soil were placed in 8 cm pots. One apple seedling was transplanted into each pot. There were 6 replicates per treatment. The various fertilizers at rates used in standard ARD pot bioassays were

Figure 3.1: Plant Growth Bench

added to the test pots: 11-55-0 at 1.5 gm/l (0.165 gm N and 0.36 gm P), 34-0-0 at 0.45 gm/l (0.15 gm N) and 0-45-0 at 1.8 gm/l (0.35 gm P) (Slykhuis and Li 1985).

The fertilizers were incorporated into the soil without disturbing the seedling. Each pot was watered thoroughly and placed on a plant growth bench (Figure 3.1). The seedlings were grown under 215 μmol/m^2/sec light at 25^{o}C with 16 hour daylight.

During the first 2 weeks after transplanting, seedlings that had died were replaced. After 2 weeks, dead seedlings were considered to be a result of treatment. Ten weeks after transplanting, shoot height was measured for each treatment.

Roots from Soils 1 and 2 were fixed in FAA for mycorrhizal counts and root dry weights. After mycorrhizal counts were made, root fragments for each sample were placed on labelled filter

papers, washed thoroughly with tap water and oven-dried at 60°C for 5 days. Analysis of Variance (ANOVA) was done for all test soils.

Soil 5

Soil from this orchard was used to determine if air-drying the soil may affect pot test results. Ten autoclavable bags were each filled with 4 litres of air-dried (AD) and nonair-dried (NAD) soil. Both the AD and NAD soils were given the following 5 treatments: 1) Formalin (FM) at 25 mls 14.8% formalin/l soil (vol/vol); (Slykhuis and Li 1985); 2) Autoclaving twice (A1) at 94 KPa at 120°C for 1 hour each 24 hours apart; 3) Autoclaving (A2) at 94 KPa at 120°C for 3 hours (Buszard and Jensen 1986); 4) Pasteurizing (PA) at 70°C for 1 hour in a hot water bath (Slykhuis and Li 1985); and 5) Untreated control (CT).

Bags treated with formalin were taped shut and incubated for 14 days. The bags were aerated for 21 days before use in pot tests. With both autoclaved treatments the bags were allowed to aerate for 3 days.

One 8 cm pot replicated 8 times was filled with 300 mls of treated or non-treated soil. One 1 - 2 leaf stage apple seedling was transplanted into each pot. The pots were thoroughly watered and placed on the plant bench. After 6 weeks, the height of each seedling was measured, and the roots were fixed in FAA. Percent mycorrhizal colonization and root dry weights were measured. Analysis of Variance was conducted for all treatments.

VAM FUNGI

Examination of roots for mycorrhizae was done as previously described (see Chapter 2 page 23).

SOIL ANALYSIS

Soil pH

Soil pH in water (1:1) (vol:vol) was taken for all samples from orchards 1 - 4 prior to making composite samples. After soil sterilization the pH was taken for each of the 5 orchard soils (Lavkulich 1978 pp 1). For pH from rows and alleyways a Fisher Accumet Model 420 Digital pH/ion meter was used. For the pH from sterile and nonsterile soil an Orion Research, Analog pH metre, Model 301 was used. The electrode was placed in the supernatant and the reading was allowed to stabilize before recording.

Soil Phosphorus

The available P of each treated and nontreated orchard soil used for pot experiments was analysed using the Olsen method as the preliminary pH readings for these soils were high (Lavkulich 1978 pp 55-57).

RESULTS

BIOASSAY TEST

Seedling Mortality

Soil 5 was the only soil in which there was 100% seedling survival. In Soils 1 - 4 there was some mortality of test seedlings, though there appeared to be no pattern in death of the seedling due to treatment.

Soils 1 - 4

Shoot Height

There were significant increases in plant height due to sterilization for Soils 1 - 4 (Tables 3.2, 3.3, 3.4, and 3.5). A significant interaction between sterilization and fertilizer occurred only in Soil 3 (Table 3.4). Sterilizing this soil resulted in an additive shoot increase only in the 0-45-0 treatment (Figure 3.4)

In Soils 1, 2 and 3, fertilizers caused significant increases in shoot height (Figures 3.2, 3.3 and 3.4). The fertilizers 11-55-0 and 34-0-0 caused significant increases in shoot height in these 3 soils while 0-45-0 did not (Figures 3.2, 3.3 and 3.4). Fertilizers did not cause any significant changes in shoot height in Soil 4 (Table 3.5). There were increases in shoot growth in the 0-45-0 treatment for Soils 1 and 3 only after sterilization.

Root Dry Weight

Sterilizing Soil 1 did not change root dry weight, but adding 0-45-0 increased root dry weight significantly (Figure 3.6). Root dry weight did not change significantly due to sterilization or the addition of fertilizer in Soil 2 (Table 3.7). There was no significant interaction between soil sterilization and fertilizer for root dry weight for either Soil 1 or 2 (Tables 3.6 and 3.7).

VAM Fungi

Sterilizing the soil eliminated the VAM fungi in both Soils 1 and 2 (Figure 3.8 and 3.9). In the nonsterile treatments, 11-55-0 decreased the mycorrhizae significantly in both soils

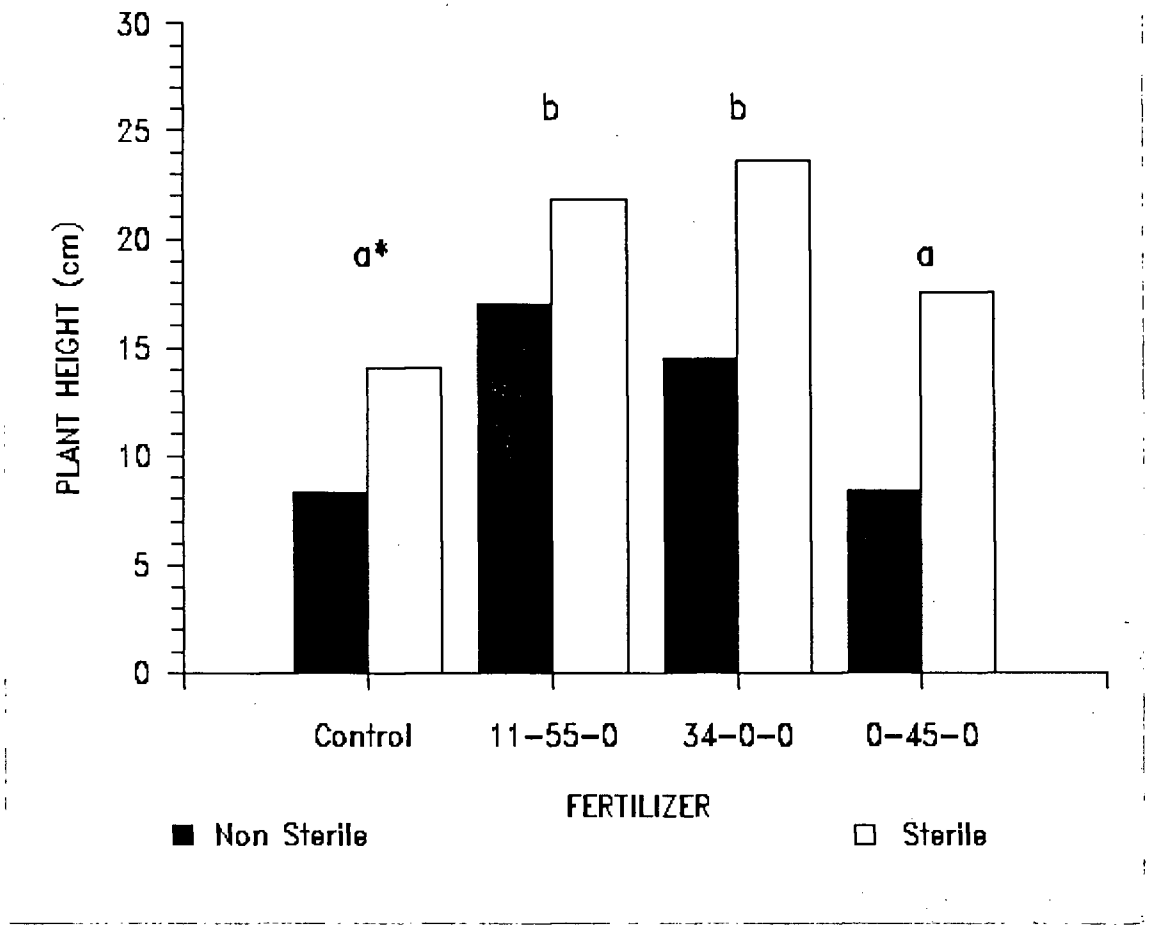

Figure 3.2: Mean Shoot Height at 10 weeks: Soil 1

* same letter indicates no significant difference for mean of each fertilizer treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	495.49	495.49	34.89	0.000
Fertilizer	3	521.15	173.72	12.23	0.000
Steriliz.* Fertilizer	3	34.38	11.46	0.81	0.500
Error	31	440.30	14.20		
Total	38	1448.00			

Table 3.2: Analysis of Variance for Shoot height: Soil 1

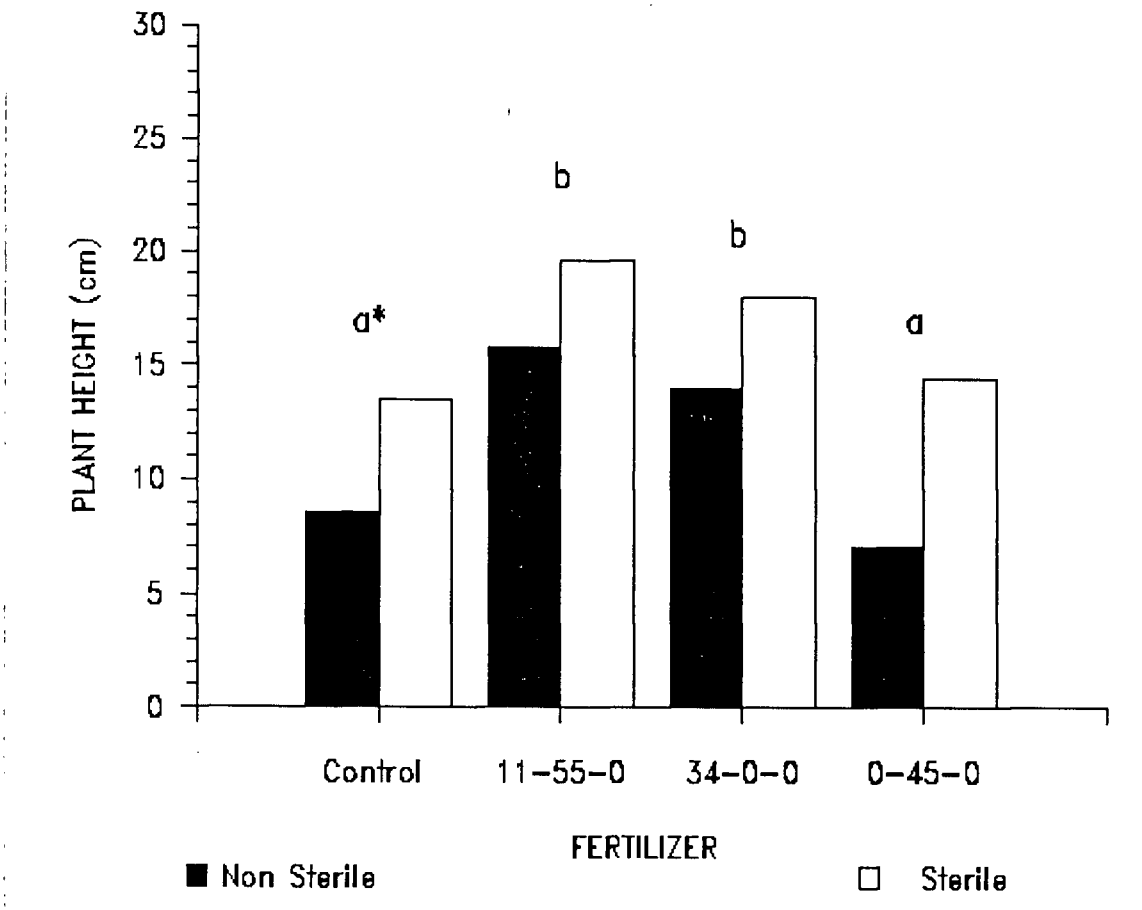

Figure 3.3: Mean Shoot Height at 10 weeks: Soil 2

* same letter indicates no significant difference for mean of each fertilizer treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	300.55	300.55	13.58	0.000
Fertilizer	3	438.65	146.22	6.61	0.000
Steriliz.* Fertilizer	3	23.37	7.79	0.35	0.788
Error	39	863.30	22.14		
Total	46	1641.20			

Table 3.3: Analysis of Variance for Shoot Height: Soil 2

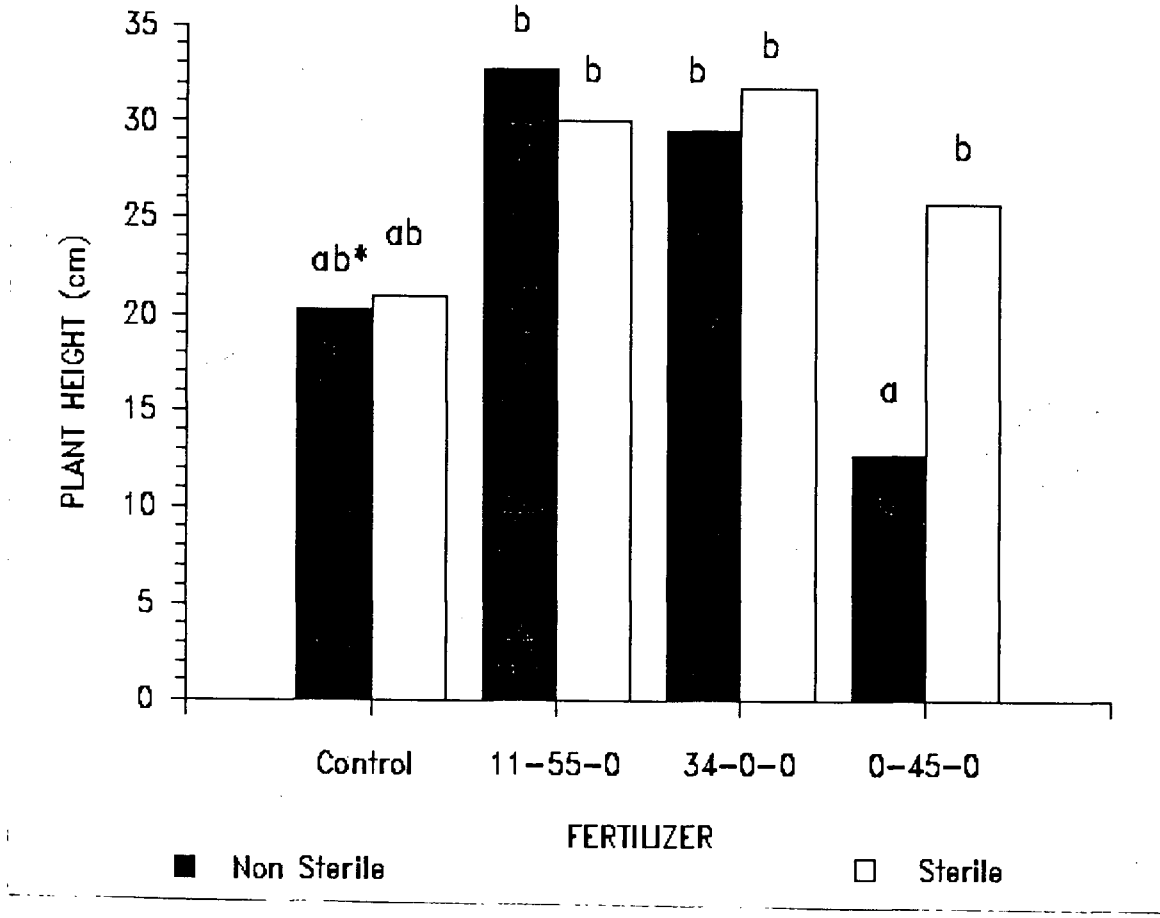

Figure 3.4: Mean Shoot Height at 10 weeks: Soil 3

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	163.43	163.43	4.22	0.047
Fertilizer	3	1267.60	422.53	10.92	0.000
Steriliz.* Fertilizer	3	375.42	125.14	3.23	0.033
Error	37	1432.30	38.71		
Total	44	3310.80			

Table 3.4: Analysis of Variance for Shoot Height: Soil 3

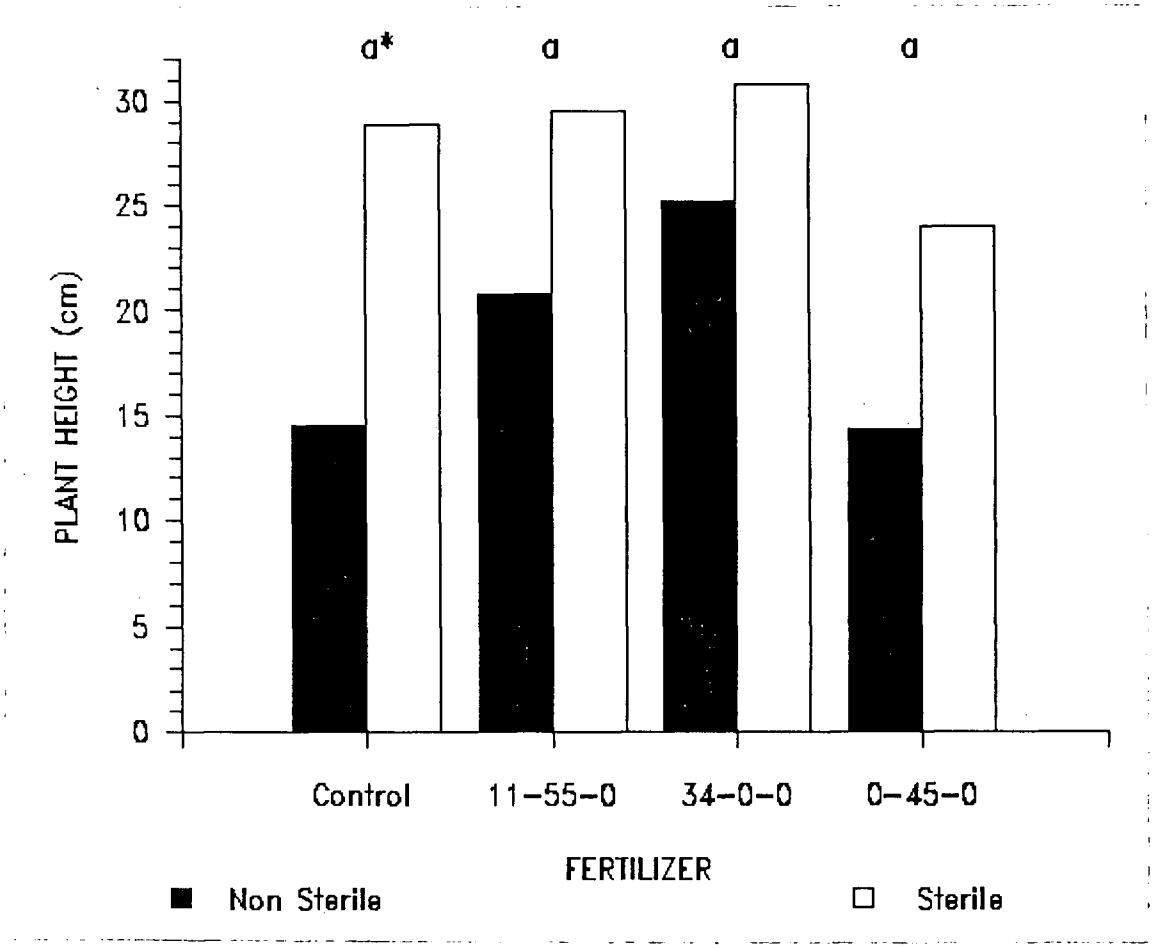

Figure 3.5: Mean Shoot Height at 10 weeks: Soil 4

* same letter indicates no significant difference for mean of each fertilizer treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	1070.50	1070.50	11.52	0.002
Fertilizer	3	531.27	177.09	1.91	0.145
Steriliz.* Fertilizer	3	121.62	40.54	0.44	0.728
Error	38	3530.10	92.90		
Total	45	5293.20			

Table 3.5: Analysis of Variance for Shoot Height: Soil 4

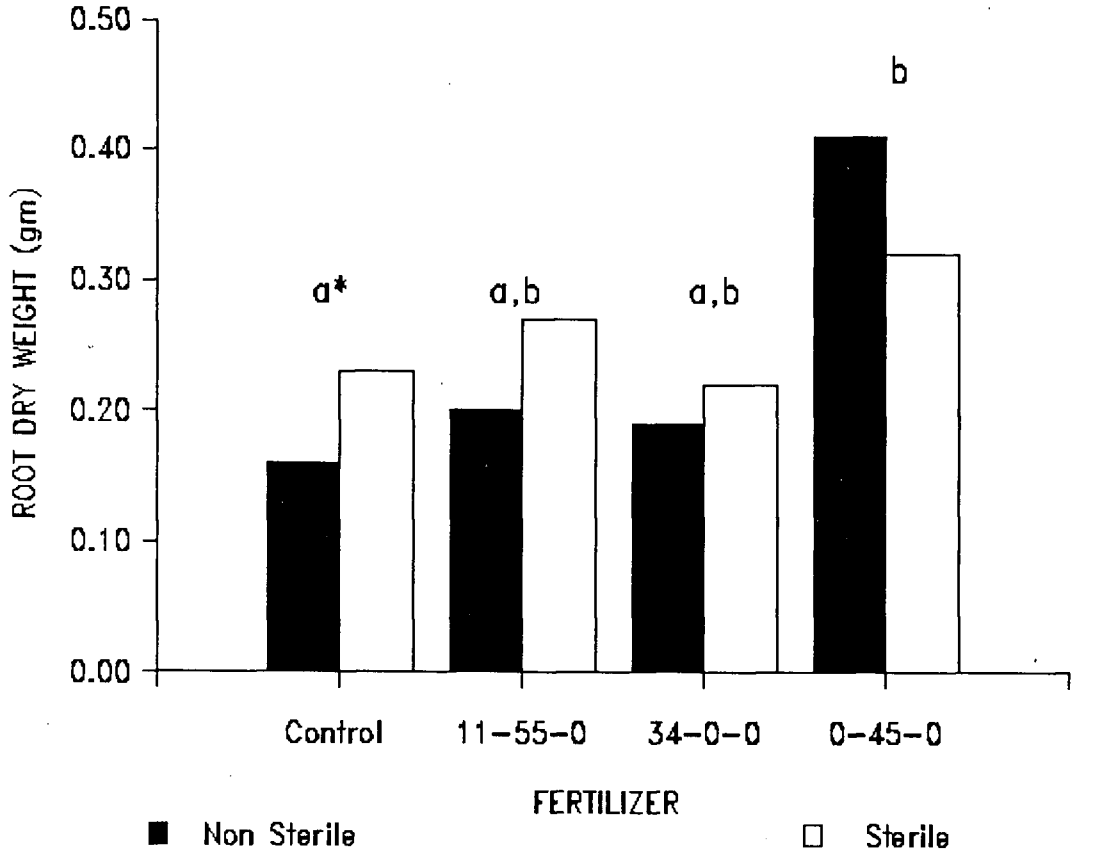

Figure 3.6: Mean Root Dry Weight at 10 weeks: Soil 1

* same letter indicates no significant difference for mean of each fertilizer treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.0029	0.0029	0.1226	0.729
Fertilizer	3	0.2244	0.0748	3.1584	0.039
Steriliz.* Fertilizer	3	0.0439	0.0146	0.6184	0.608
Error	31	0.7342	0.0236		
Total	38	1.0048			

Table 3.6: Analysis of Variance for Root Dry Weight: Soil 1

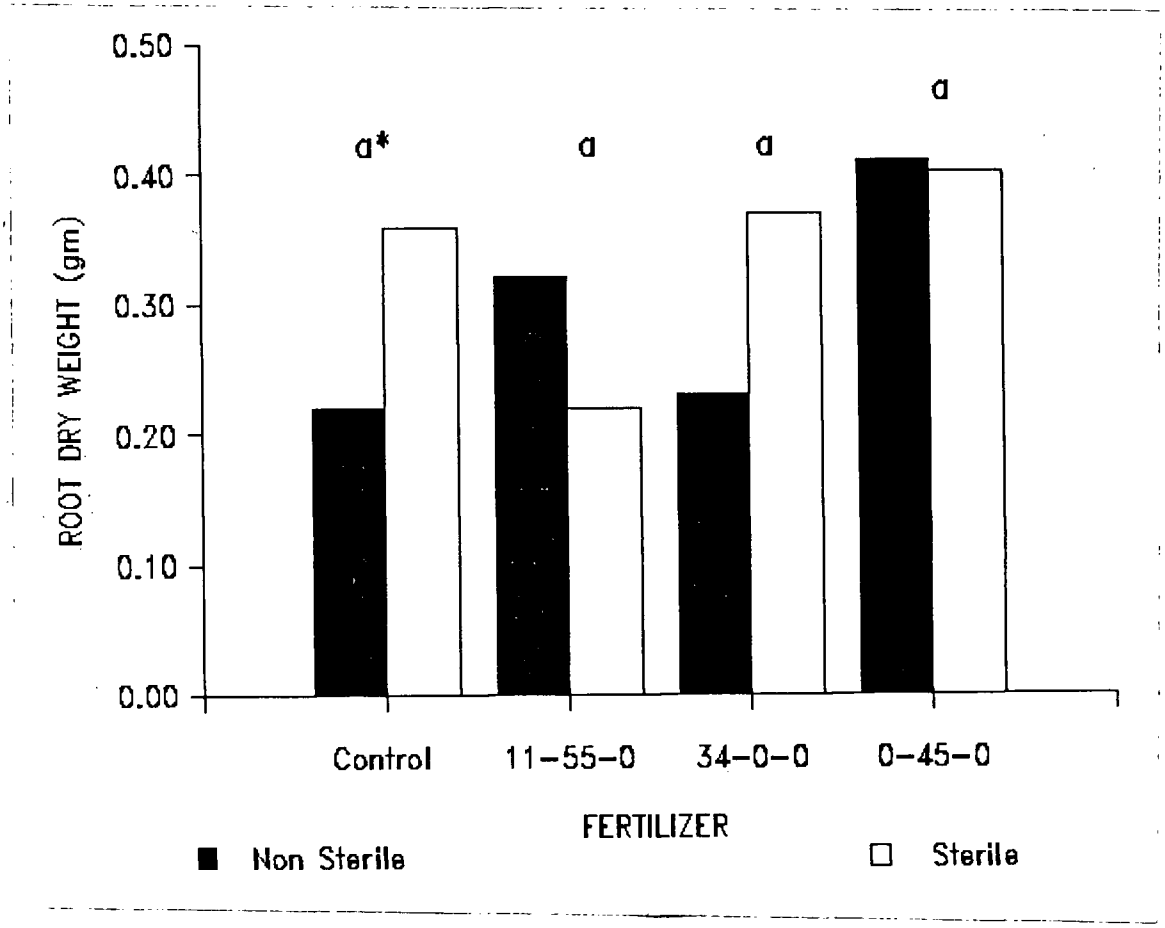

Figure 3.7: Mean Root Dry Weight at 10 weeks: Soil 2

* same letter indicates no significant difference for mean of fertilizer treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.0196	0.0197	0.6050	0.441
Fertilizer	3	0.1415	0.0472	1.4494	0.243
Steriliz.* Fertilizer	3	0.1166	0.0388	1.1940	0.325
Error	39	1.2689	0.0324		
Total	46	1.5479			

Table 3.7: Analysis of Variance for Root Dry Weight: Soil 2

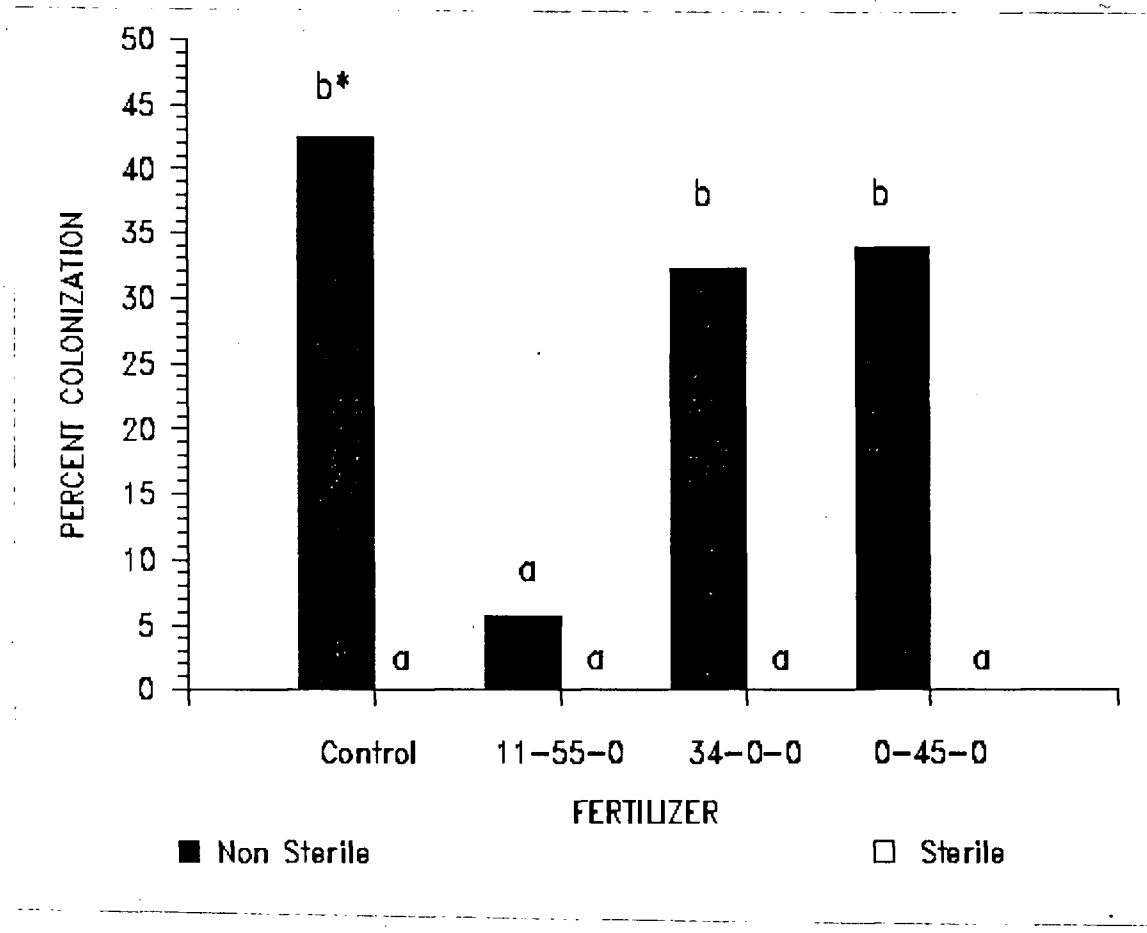

Figure 3.8: Percent Mycorrhizal Colonization: Soil 1

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	8535.40	8535.30	88.33	0.000
Fertilizer	3	2154.50	718.20	7.43	0.001
Steriliz.* Fertilizer	3	1824.80	608.25	6.30	0.002
Error	31	2995.70	96.64		
Total	38	15271.0			

Table 3.8: Analysis of Variance for VAM Colonization: Soil 1

(Figure 3.8 and 3.9). The mycorrhizae in the 34-0-0 and 0-45-0 treatments did not differ significantly from each other in either Soils 1 or 2, but were significantly reduced from the controls in Soil 2 only (Figure 3.9).

Soil Analysis

No significant differences were found in soil pH and soil P due to sterilization in Soils 1, 2, and 4 (Table 3.10c, 3.11c and 3.13c). There was a significant decrease in soil pH but not soil P after sterilization in Soil 3 (Table 3.12c).

AIR-DRYING/ARD POT BIOASSAY

Soil 5

Shoot Height

Air drying alone did not cause any significant changes in plant height (Table 3.14). However, pasteurizing and adding formalin to the air-dried soil increased plant height significantly (Figure 3.10). Neither of the autoclaving treatments increased plant height significantly. A significant interaction between air-drying and soil sterilization occurred (Table 3.14). Air-drying the soil increased the pasteurization and formalin effects (Figure 3.10). However, air-drying the soil negated any increases in plant height that may have occurred due to either autoclaving treatment (Figure 3.10).

Root Dry Weight

Air-drying the soil did not result in any significant changes in root dry weight (Table 3.15). However, pasteurizing and adding formalin to the soil significantly increased root dry

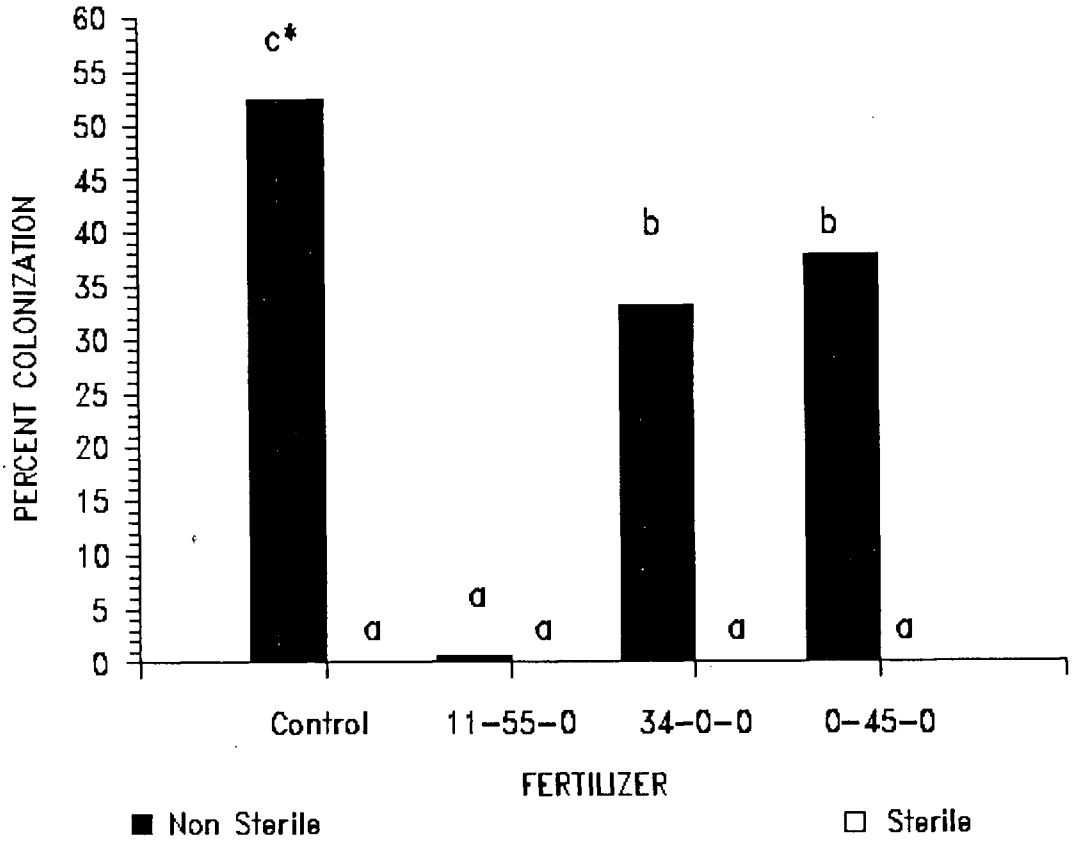

Figure 3.9: Percent Mycorrhizal Colonization: Soil 2

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	10945.00	10945.00	319.14	0.000
Fertilizer	3	4460.40	1486.80	43.35	0.000
Steriliz. Fertilizer	3	4202.80	1400.90	40.85	0.000
Error	39	1337.60	34.30		
Total	46	21324.00			

Table 3.9: Analysis of Variance for VAM Colonization: Soil 2

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.027	0.027	0.229	0.658
Error	4	0.467	0.117		
Total	5	0.494			

Table 3.10a: Analysis of Variance for Soil pH: Soil 1

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	31.510	31.510	0.190	0.685
Error	4	663.542	165.885		
Total	5	695.052			

Table 3.10b: Analysis of Variance for Soil P: Soil 1

	Non Sterile	Sterile
pH	6.1 a*	6.2 a
P (ppm)	100.0 a	95.0 a

* same letter within in category indicates no significant differences (α = 0.05 Tukey's (HSD) (test)

Table 3.10c: Soil pH and P (Olsen Method) before and after Sterilization: Soil 1

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.027	0.027	0.165	0.705
Error	4	0.647	0.162		
Total	5	0.674			

Table 3.11a: Analysis of Variance for Soil pH: Soil 2

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	3.662	3.662	0.017	0.902
Error	4	851.107	212.777		
Total	5	854.769			

Table 3.11b: Analysis of Variance for Soil P: Soil 2

	Non Sterile	Sterile
pH	5.7 a*	5.6 a
P (ppm)	43.0 a	42.0 a

* same letter within in category indicates no significant differences (α = 0.05 Tukey's (HSD) test)

Table 3.11c: Soil pH and P (Olsen Method) before and after Sterilization: Soil 2

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.167	0.167	25.000	0.007
Error	4	0.027	0.007		
Total	5	0.194			

Table 3.12a: Analysis of Variance for Soil pH: Soil 3

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	84.375	84.375	0.301	0.612
Error	4	1120.833	280.208		
Total	5	1205.208			

Table 3.12b: Analysis of Variance for Soil P: Soil 3

	Non Sterile	Sterile
pH	6.2 a*	5.9 b
P (ppm)	87.0 a	95.0 a

* same letter within in category indicates no significant differences (α = 0.05 Tukey's (HSD) test)

Table 3.12c: Soil pH and P (Olsen Method) before and after Sterilization: Soil 3

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	0.082	0.082	1.140	0.346
Error	4	0.287	0.072		
Total	5	0.369			

Table 3.13a: Analysis of Variance for Soil pH: Soil 4

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Steriliz.	1	12.760	12.760	0.069	0.805
Error	4	735.417	183.854		
Total	5	748.177			

Table 3.13b: Analysis of Variance for Soil P: Soil 4

	Non Sterile	Sterile
pH	6.3 a*	6.5 a
P (ppm)	85.0 a	82.0 a

* same letter within in category indicates no significant differences (α = 0.05 Tukey's (HSD) test)

Table 3.13c: Soil pH and P (Olsen Method) before and after Sterilization: Soil 4

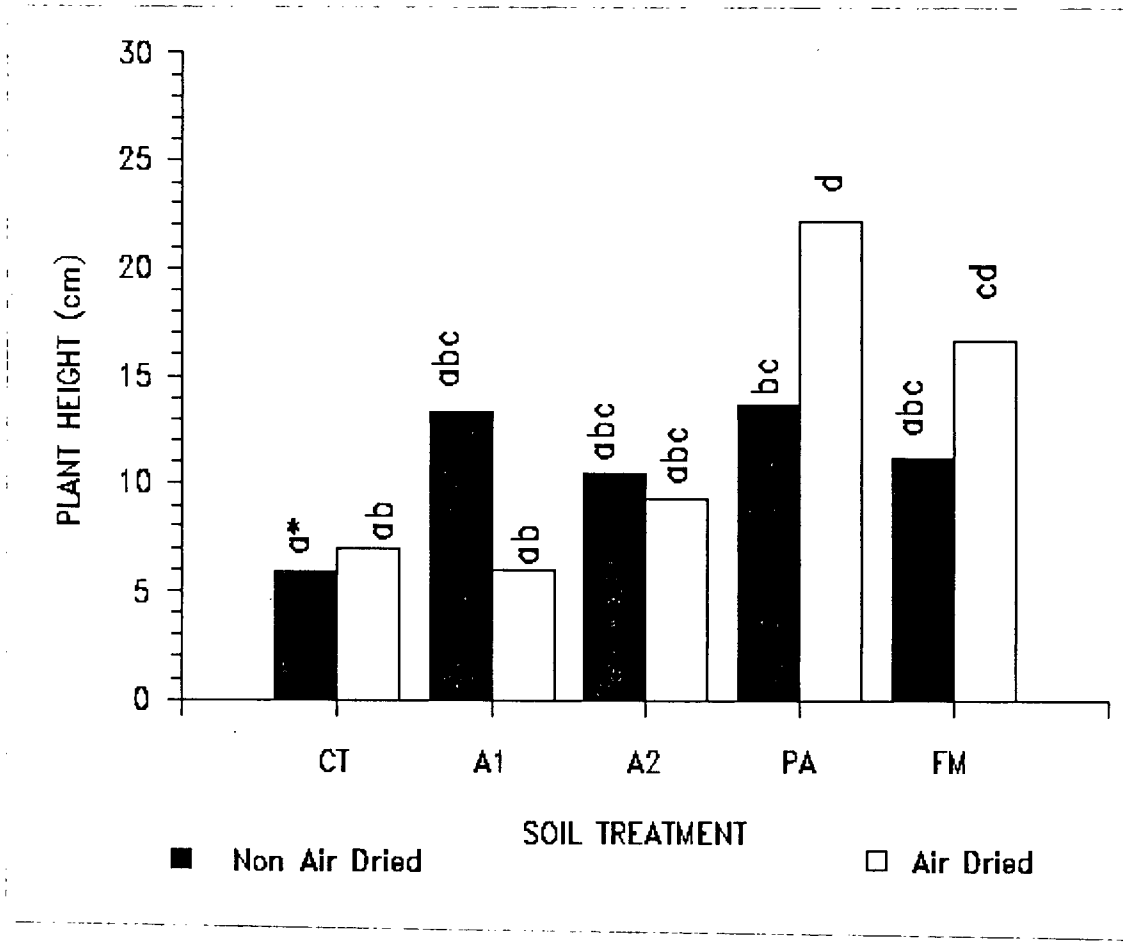

Figure 3.10: Mean Shoot Height: Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Air-Dried	1	35.112	351.120	1.508	0.224
Steriliz.	4	1280.300	320.070	13.748	0.000
Air-Dried Steriliz.	4	595.210	148.800	6.392	0.000
Error	70	1629.600	23.281		
Total	79	3540.200			

Table 3.14: Analysis of Variance for Shoot Height: Soil 5

weight compared to the 2 autoclaving treatments (Figure 3.11).

VAM Fungi

Percent colonization in Soil 5 was very low (Table 3.16b) Mycorrhizae were present in the air-dried controls only.

Soil Analysis

Air-drying the soil did not significantly affect the pH of Soil 5 but it did increase the soil P (Table 3.17 and 3.18). Sterilizing the soil caused significant changes in both the pH and P levels (Table 3.17 and 3.18). Autoclaving the soil did not significantly affect the soil pH and P in Soil 5 (Figure 3.12 and 3.13). Pasteurizing and adding formalin to the soil significantly increased the pH especially if the soil was air-dried. Phosphorus was increased significantly in the pasteurized and formalin treatments. Though this increase was not as great in the air-dried soil.

VAM FUNGI AND PLANT GROWTH

Sterilizing the soil eliminates the mycorrhizae as expected. However, the greatest shoot height in ARD soils, are found in the non or low mycorrhizal plants (Figure 3.14, 3.15 and 3.16). In non-sterilized soil, the greater the mycorrhizal colonization, the lower the plant height.

DISCUSSION

Based on pot tests, all 5 soils used in this study were positively identified as having ARD. While ARD soils are identified by the increase in plant height after sterilization, Slykhuis and Li (1985) propose that a soil fertility factor

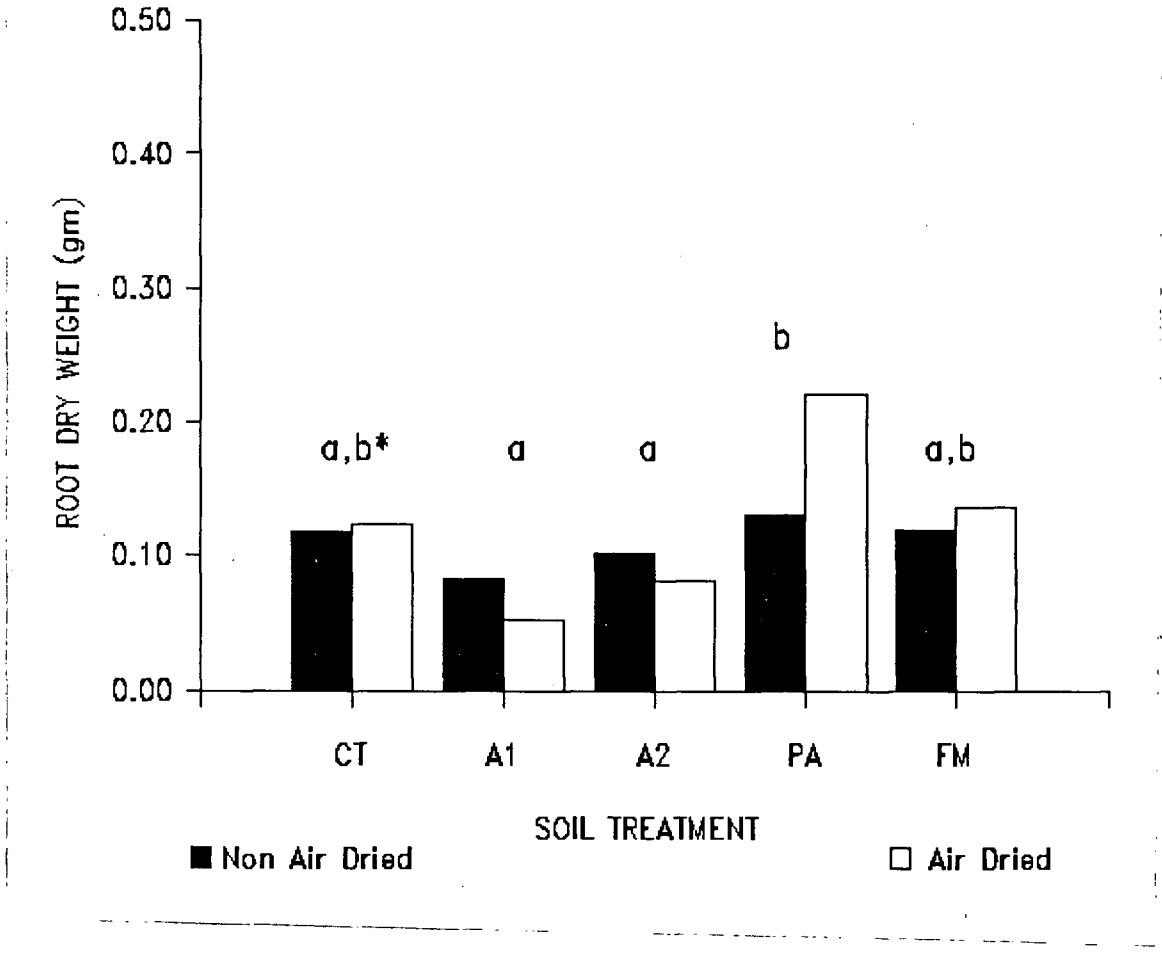

Figure 3.11: Mean Root Dry Weight: Soil 5

* same letter indicates no significant difference for mean of each sterility treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Air-Dried	1	0.003	0.003	0.598	0.442
Sterility	4	0.106	0.027	5.211	0.001
Air-Dried* Sterility	4	0.036	0.009	1.785	0.142
Error	70	0.357	0.005		
Total	79	0.503			

Table 3.15: Analysis of Variance for Root Dry Weight: Soil 5

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Air-Dried	1	68.543	68.543	2.420	0.124
Sterility	4	441.706	110.427	3.899	0.006
Air-Dried* Sterility	4	274.170	68.543	2.420	0.056
Error	70	1982.568	28.322		
Total	79	2766.987			

Table 3.16a: Analysis of Variance for Mycorrhizae: Soil 5

	Non Air Dried	Air Dried
Control	0.00 a*	10.50 b
Autoclave 1	0.00 a	0.00 a
Autoclave 2	0.00 a	0.00 a
Pasteurize	0.00 a	0.00 a
Formalin	0.00 a	0.00 a

Table 3.16b: Percent Mycorrhizal Colonization: Soil 5

* same letter indicates no significant differences (α =0.05 Tukey's (HSD) test)

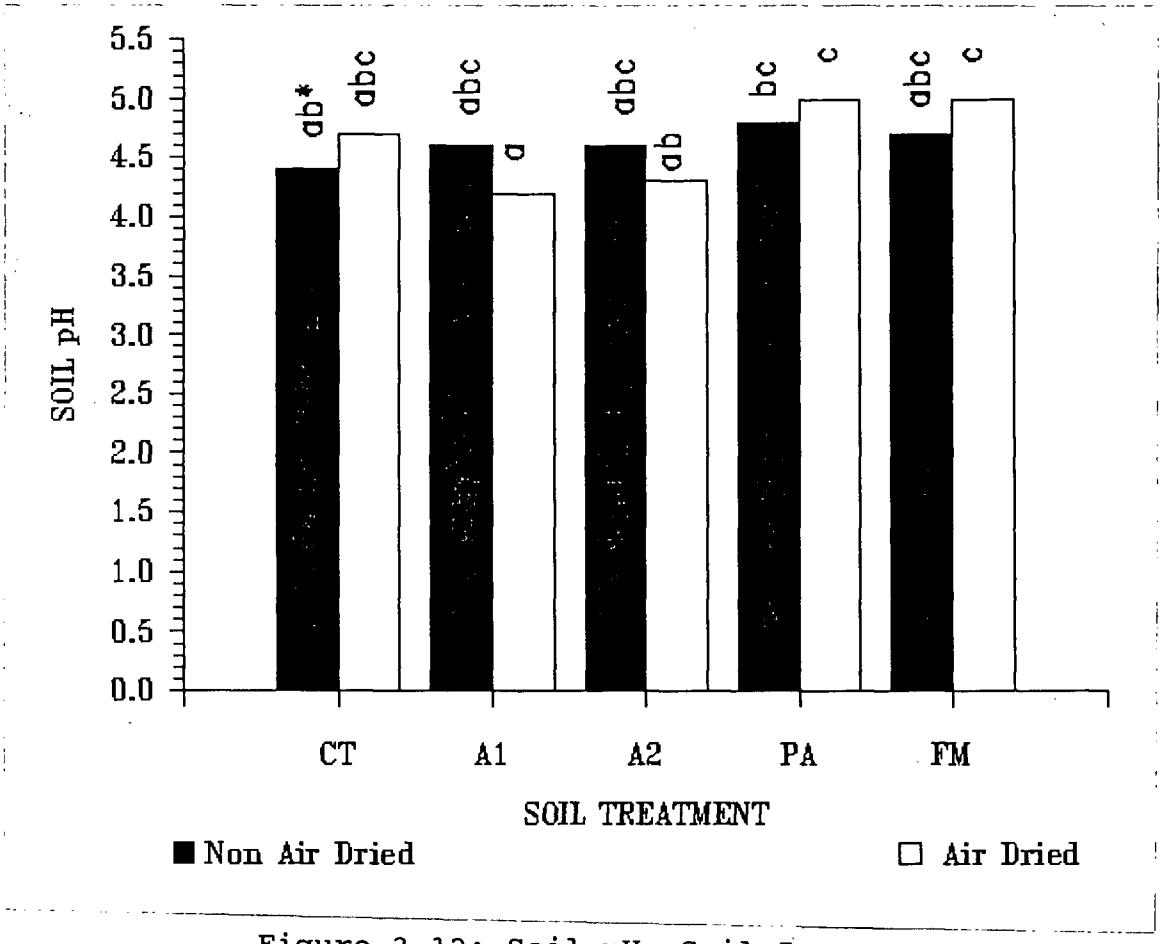

Figure 3.12: Soil pH: Soil 5

* same letter indicates no significant difference ($\alpha = 0.05$ Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Air-Dried	1	0.0003	0.0003	0.011	0.917
Sterility	4	1.2230	0.3058	10.194	0.000
Air-Dried Sterility	4	0.7180	0.1795	5.983	0.002
Error	20	0.6000	0.0300		
Total	29	2.5417			

Table 3.17: Analysis of Variance for Soil pH: Soil 5

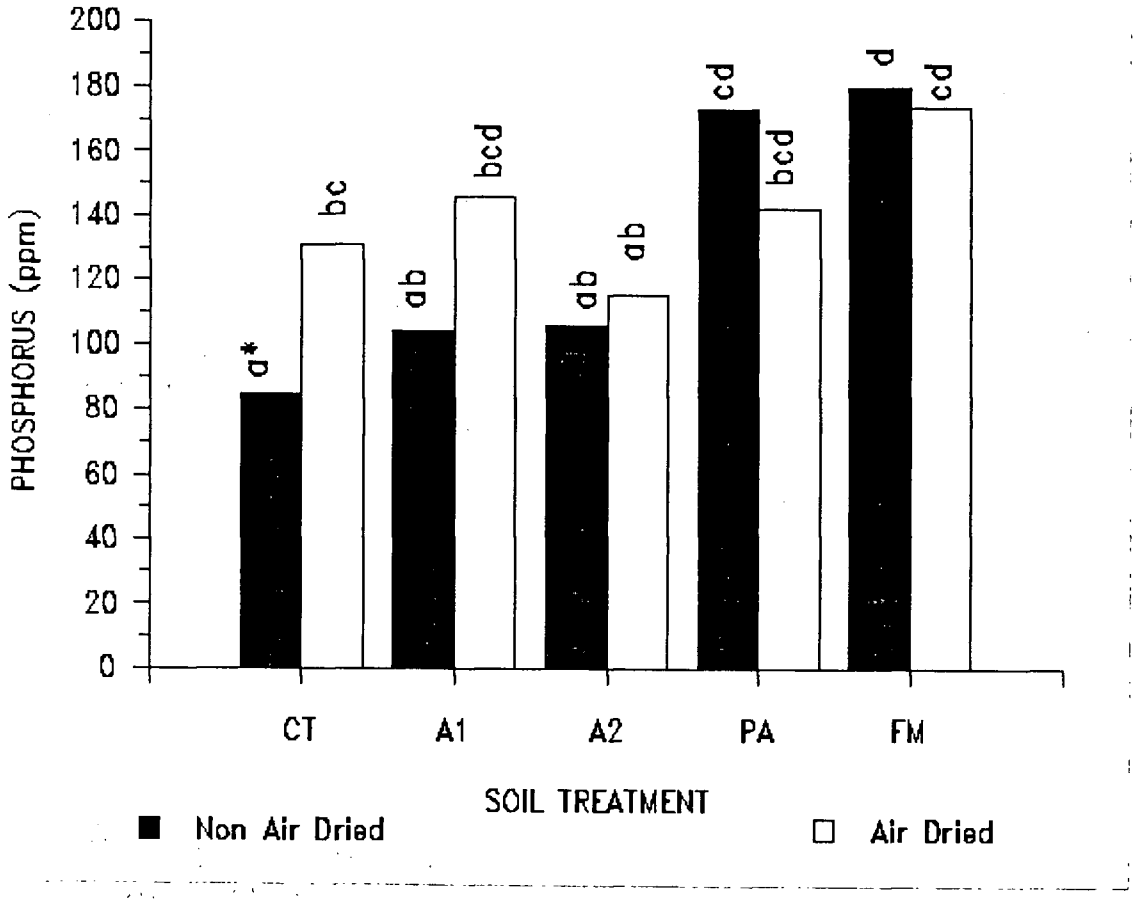

Figure 3.13: Soil Phosphorus (Olsen Method): Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Air-Dried	1	991.87	991.87	4.19	0.054
Sterility	4	22393.00	5598.30	23.66	0.000
Air-Dried* Sterility	4	6444.10	1611.00	6.80	0.001
Error	20	4732.30	236.61		
Total	29	34561.00			

Table 3.18: Analysis of Variance for Soil P: Soil 5

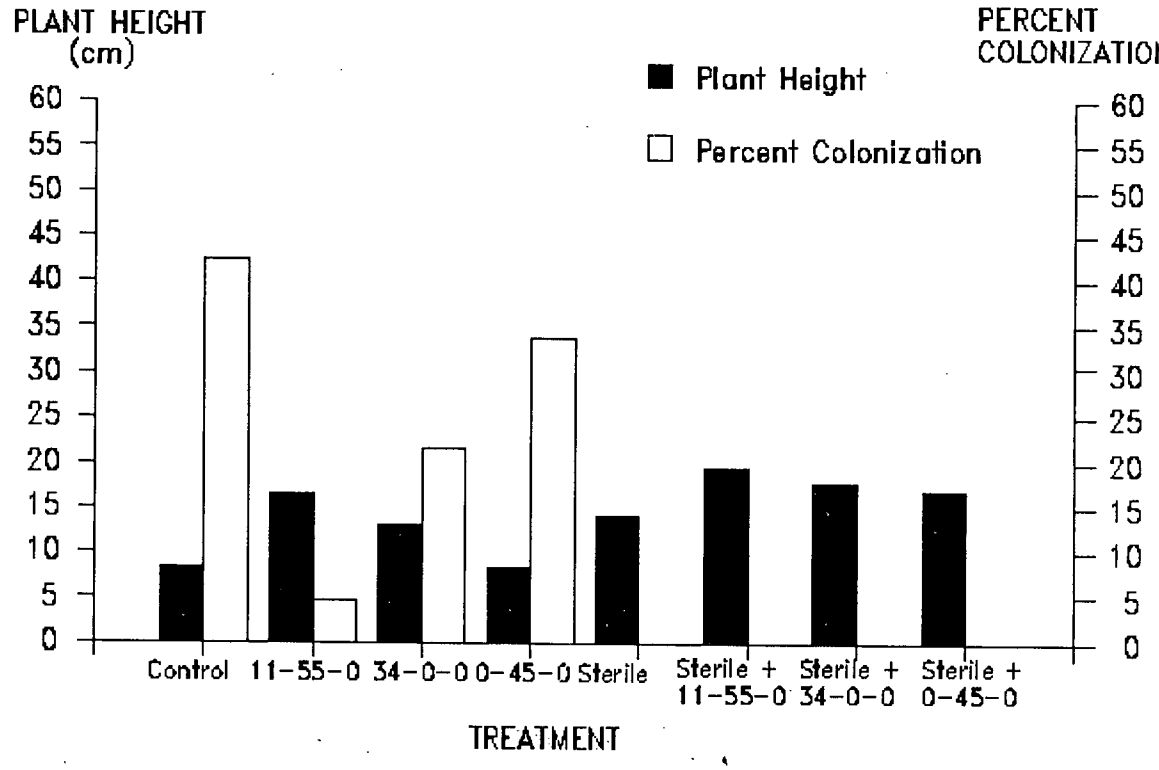

Figure 3.14: Plant Height and VA Mycorrhizal Colonization: Soil 1

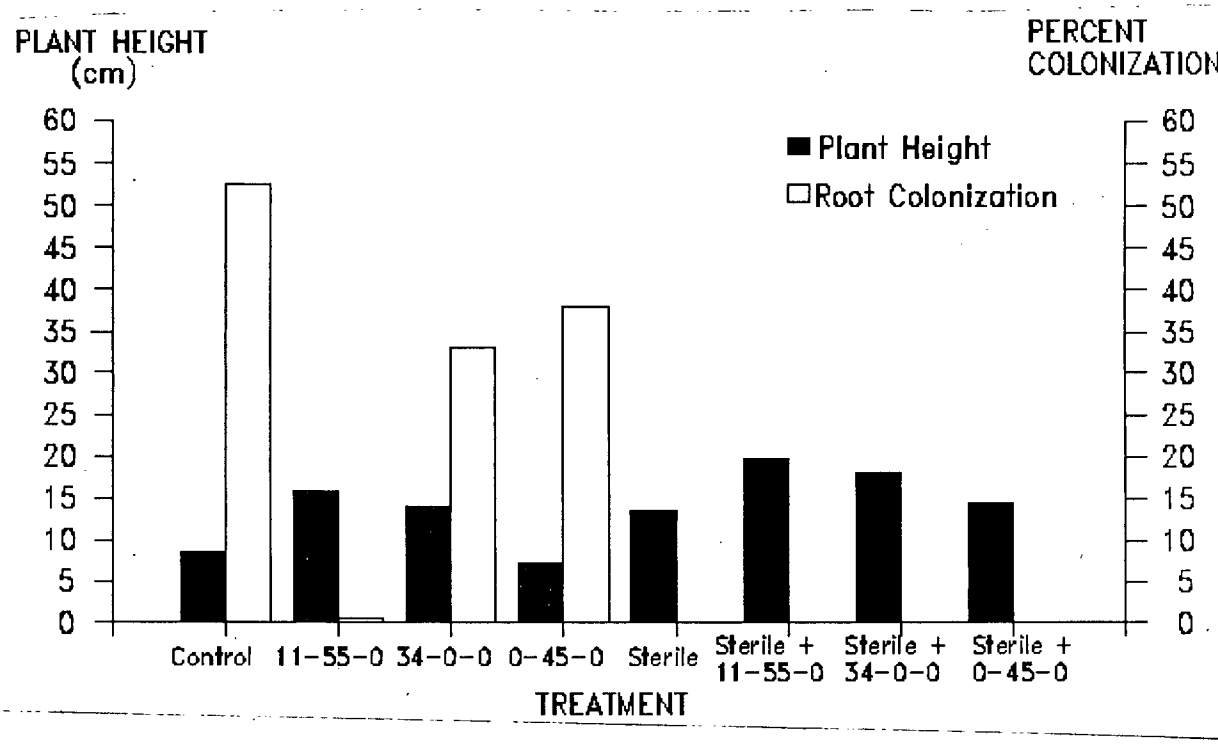

Figure 3.15: Plant Height and VA Mycorrhizal Colonization: Soil 2

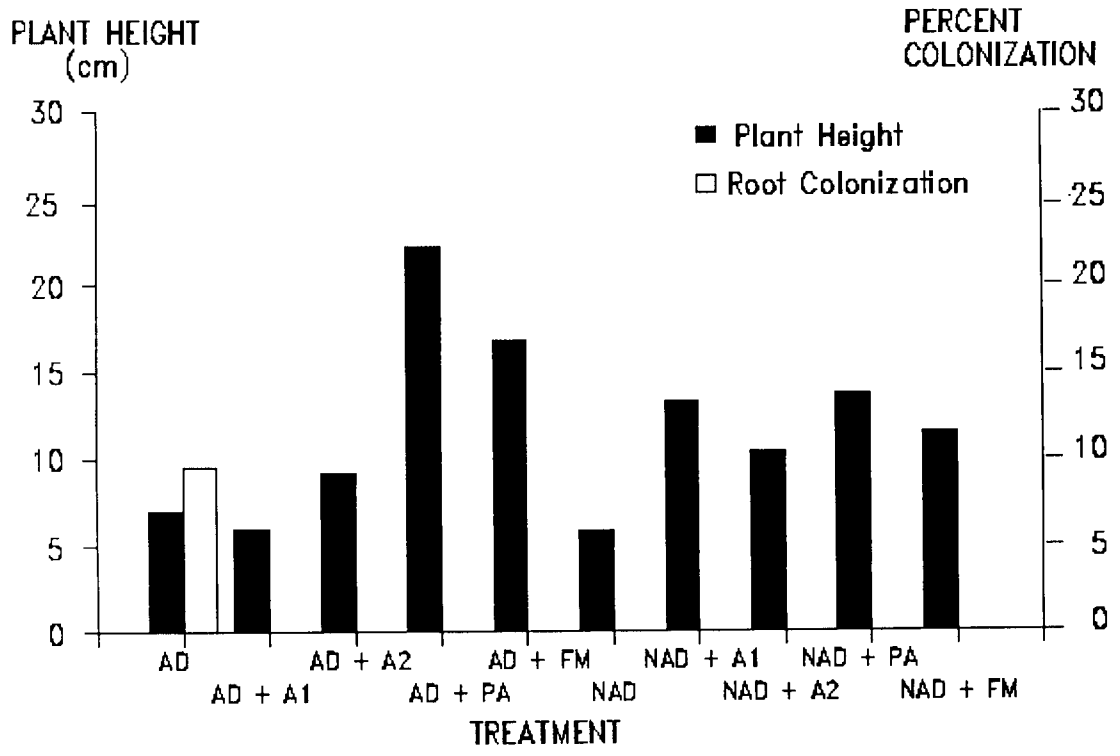

Figure 3.16: Plant Height and VA Mycorrhizal Colonization: Soil 5

especially a combination of N and P may be involved in ARD. This study concurs with their proposal. Nitrogen alone may also be involved in ARD, though P alone is not. P alone may even be deleterious to shoot growth in ARD soils. These findings support the recommendations to growers to sterilize and add 11-55-0 fertilizers to old apple soils. While shoot height is usually measured as an indication of good plant growth, the importance of root growth is often ignored or minimized. In ARD soils N and the combination of N and P do not affect root mass. While P may have no effect on shoot height, it does increase root mass. In this case the plant appears to be maximizing root growth at the expense of shoot growth. This may benefit the young tree in the first year of growth after transplanting and may lead to a healthier tree in subsequent years.

The nutrient status of ARD soils after sterilization is usually ignored. The lowering of pH after autoclaving suggests that organic acids are being released. The increase in P after sterilizing indicates that microbial and organic matter P is being released after treatment. Though these changes may not be of sufficient magnitude to affect apple growth.

Air-drying the soil does not affect pot bioassays for ARD. It is therefore not necessary to recommend to growers that they keep their soil moist prior to doing an ARD test. In the absence of a host plant, drying the soil appears to preserve viable VAM propagules. Drying the soil is not recommended as an alternative to alleviate ARD in the orchard. Pot tests however, show that

drying the soil combined with pasteurizing or adding formalin causes marked increases in shoot height. This treatment could be a viable alternative to growers and should be further tested in field trials.

VA mycorrhizae have been proposed as playing a role in the ARD problem in B.C. Phosphorus and N fertilizers alone do not harm these mutualistic fungi, suggesting that these fertilizers may not be readily available to the plant. However, the combinations of the two does, suggesting that this fertilizer may be immediately available to the plant, thereby eliminating the need for the VAM association.

The relationship between VAM fungus and host shown in this study goes contrary to the usual mutalistic association between the host and fungus. In ARD soils, the plant may be under so much stress that the fungus may be behaving parasitically. The fungus in these soils may be causing a deleterious carbon drain on the plant manifested by the decrease in plant height. The exchangeable P that the plant receives may not be enough to overcome this carbon drain.

This relationship between mycorrhizal fungus and apple host in ARD soils must be further investigated. The elucidation of soil nutrients in ARD soils should be further investigated as well. The levels of soil nutrients before and after sterilization and its effect on both soil micro- and macro- fauna should be further investigated. The ARD causal agent may be detrimentally effected by soil nutrient changes after sterilization.

APPLE GROWTH IN APPLE REPLANT DISEASED SOILS AFTER INOCULATION WITH VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI

INTRODUCTION

Apple replant disease (ARD) can be overcome by fumigating the soil prior to transplanting (Mai and Abawi 1981, Sewell and White 1979, Slykhuis and Li 1985). The symptoms of the disease are ambiguous, the key factor being poor growth, indicative of a root problem. While the disease is biological in nature, no single known pathogen has been implicated in the disease. Apple seedlings will also respond positively to the addition of a nitrogen and phosphorus fertilizer applied after soil fumigation (Slykhuis and Li 1985). This growth response of apple to fumigation and fertilizer treatments, suggests that vesicular-arbuscular mycorrhizal (VAM) fungi may be implicated in ARD.

Fruit trees have high mycorrhizal dependencies. Mycorrhizal avocado trees have been shown to not only grow faster than non mycorrhizal trees but the ability of the mycorrhizal tree to survive transplanting surpasses that of nonmycorrhizal trees (Menge et al. 1978b). Increases in plant growth due to mycorrhizal fungi in both greenhouse and field trials have been demonstrated for citrus as well (Hattingh and Gerdemann 1975).

Apples form mycorrhizae with a wide range of VAM fungal species (Benson and Covey 1976, Hoepfner et al. 1983, Miller et al. 1985b, Plenchette et al. 1982, Reich 1988). Different VAM species induce different growth responses in apples. Generally, total apple growth is enhanced due to the presence of

mycorrhizae. Mosse (1957), and Miller *et al.* (1985b) found that nonmycorrhizal apple plants outgrew or grew as well as mycorrhizal plants initially, but after 8 weeks the mycorrhizal seedlings continued to grow, whereas the nonmycorrhizal seedlings had set terminal buds. Eventually the mycorrhizal plants had greater root, stem and leaf growth compared to the nonmycorrhizal plants.

Inoculation with *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske resulted in better growth of apple seedlings than inoculation with *G. mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe (Benson and Covey 1976). However, apples inoculated with *G. microcarpum* Tulasne and Tulasne showed little increase in plant growth compared to the nonmycorrhizal controls (Covey *et al.* 1981). Miller *et al.* (1985b) and Plenchette *et al.* (1982) found similar trends with the different VAM species used to inoculate apple seedlings.

Plenchette *et al.* (1982) also found that percent mycorrhizal colonization correlated directly to plant height. However, Reich (1988) found that while *G. intraradices* Schenck and Smith resulted in the highest levels of mycorrhizal colonization, apples inoculated with *G. epigaeum* Daniels and Trappe (= *Glomus versiforme* (Karsten) Berch) resulted in the greatest plant growth. It was not the rate of colonization that correlated directly to plant growth, but rather the amounts of root P.

This variation in apple response to VAM inoculation is thought to be a result of the discrepancy between the

extramatrical hyphae found outside the root and the intramatrical hyphae found inside the root. The influence of VAM fungi may not be directly related to the intramatrical hyphae but rather due to the extramatrical hyphal extensions (Miller et al. 1985b).

Another reason for the different responses to VAM fungi is the amount of available P in the soil. The uptake of P is not increased by mycorrhizae in the presence of adequate P. However, apple growth is improved due to mycorrhizae in soils low in P. Hoepfner et al. (1983) have shown that apples grown in fumigated soils required lower P supplements for optimal growth as long as the soil was inoculated with VAM fungi. At higher levels of P, the mycorrhizal colonization was decreased dramatically. They suggest that fumigating soils for apple production should be followed by either P fertilizers or inoculations of VAM fungi.

While apples respond well to VAM fungal inoculations, little work has been done on the growth of inoculated apples in ARD soils. Utkhede (1987) demonstrated that under greenhouse conditions, apples inoculated with Glomus spp. grew significantly better in ARD soils that were not pasteurized as well as in soils that were. Similar results were found in apple seedlings grown only in sterilized ARD soils inoculated with VAM fungi (Hoepfner et al. 1983, Sewell and Roberts 1984,1985). However, in nonsterilized soil, VAM fungi did not enhance apple growth (Sewell and Roberts 1984).

VAM inoculations of old alfalfa soils overcame 'alfalfa sickness' and it is suggested that the indigenous VAM fungi are

not as effective as the inoculated fungi in root infection and stimulation of plant growth (Hwang 1988).

The objectives of this study were to determine if inoculating ARD soils with different VAM fungi would overcome ARD; and to compare the fertilizer 11-55-0, used in standard pot bioassays, with VAM fungi in seedlings response grown in ARD soils.

MATERIAL AND METHODS

THE SOILS

Soil 2 and Soil 5 which were previously identified as having ARD were used in this study. Soil 2 was sterilized and Soil 5 was pasteurized as above (Chapter 3, pages 50 and 52).

THE FUNGI

Both soils were inoculated with three pure VAM fungal species, Glomus clarum Nicholson and Schenck (GC) (1.4 X 10^3 live propagules per ml), Glomus intraradices (GI), (3.3 X 10^2 live propagules per ml) and Glomus versiforme (Karsten) Berch (GV), (1.4 X 10^3 live propagules per ml) which were obtained from Premier Peat, Rivere-du-Loup, Quebec. Soil 5 was also inoculated with a G. monosporum Gerdemann and Trappe (OR), (1.4 X 10^2 live propagules per ml) from the University of B.C. research farm at Oyster River, B.C. Colonized onion root fragments plus inoculated Turface were used as the inoculum source for the 3 species from Quebec, and dried Turface containing live spores were used as the inoculum source for the OR species.

INOCULATING SOILS WITH VAM FUNGI

Sterilized and nonsterilized soil were inoculated with the following rates of VAM fungi: OR, 133 mls/l soil; GI, 57 mls/l soil; both GC and GV, 13 mls/l soil. Ammonium phosphate fertilizer (11-55-0) at 1.5 gm/l (0.165 gm N, 0.36 gm P) soil was given as a fifth treatment. There was also an untreated control (CT). The OR species was used with Soil 5 only. All treatments were replicated 6 times.

SEEDLING GROWTH AND ANALYSIS

One 1 - 2 leaf stage apple seedling was transplanted into each pot and then grown at 24^{o}C on a plant bench. After ten weeks, the height and shoot dry weight were recorded for all seedlings. The roots were fixed in FAA for VAM analysis (see Chapter 2, pp 23). Root dry weights were taken after VAM analysis. All root fragments were placed on filter papers, washed repeatedly to eliminate the lactic acid and then oven-dried at 60^{o}C for 5 days. VAM colonization was determined as previously described (Chapter 2, pp 23).

RESULTS

SHOOT HEIGHT

In Soil 2 shoot height increased significantly due to sterilization (Table 4.1). Similar responses were found in Soil 5 due to pasteurization (Table 4.2).

The fertilizer treatment resulted in significant increases in shoot height in both sterilized and nonsterilized Soil 2 and in sterilized Soil 5 (Figures 4.1 and 4.2). In Soil 5 pasteurizing and adding fertilizer resulted in an additive

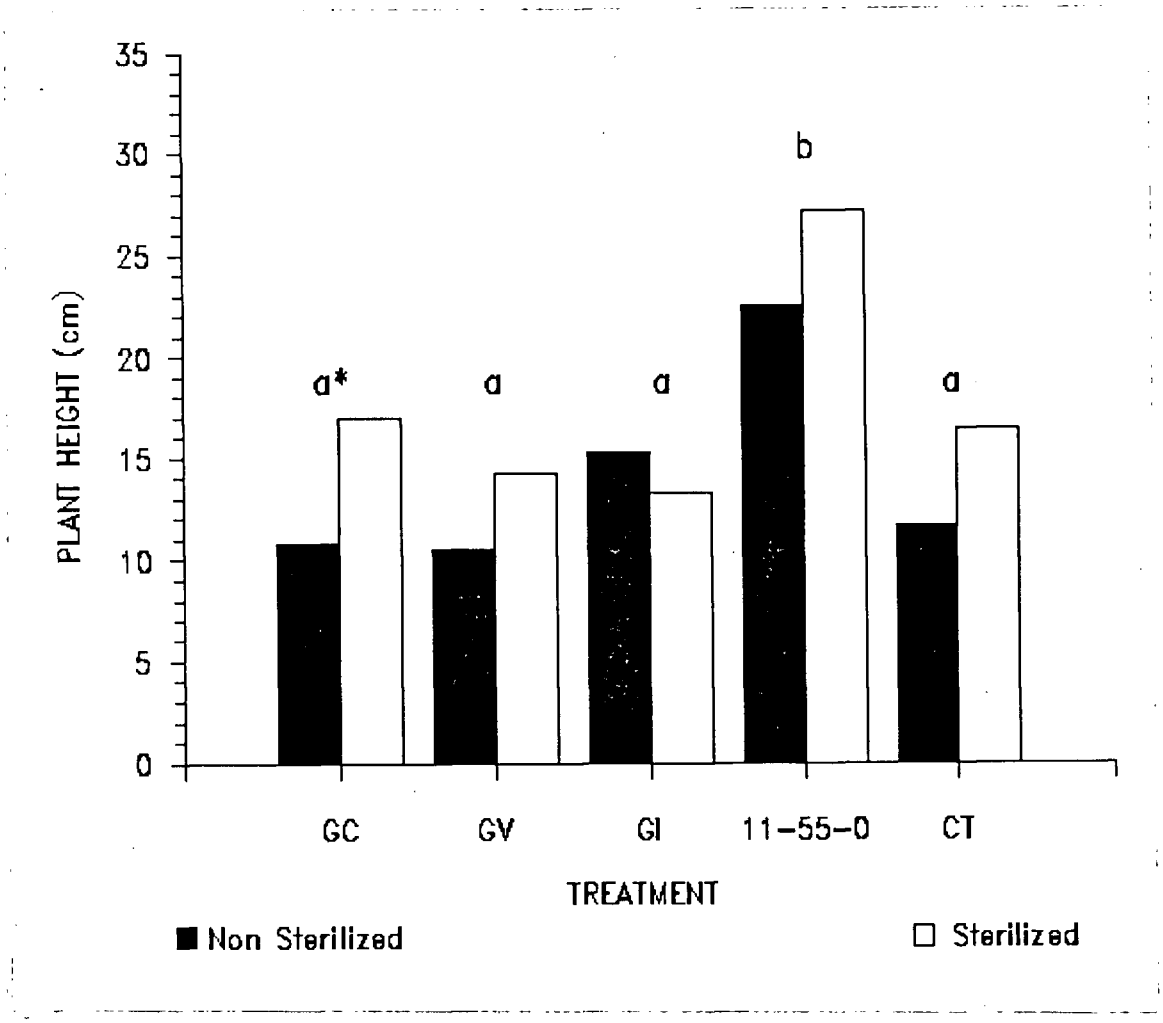

Figure 4.1: Shoot Height at 10 Weeks: Soil 2

* same letter indicates no significant difference for mean of each treatment (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	172.110	172.110	11.784	0.001
Treatment	4	1131.800	282.950	19.373	0.000
Sterility* Treatment	4	123.740	30.935	2.118	0.093
Error	49	715.670	14.605		
Total	58	2114.000			

Table 4.1: Analysis of Variance for Shoot Height: Soil 2

increase in shoot height. No significant differences occurred among any of the other treatments in Soil 2 (Figure 4.1). In Soil 5, any significant increases in shoot height occurred only if the soil was pasteurized. In pasteurized Soil 5, the GV species increased shoot height significantly compared to the GC treatment only (Figure 4.2). The remaining inoculations did not differ significantly from each other.

SHOOT DRY WEIGHT

Patterns of shoot dry weight in both soils followed shoot height closely, increasing significantly when the soil was sterilized or pasteurized (Table 4.3 and 4.4).

Treating Soil 2 with VAM fungi did not result in any changes in shoot dry weight even if the soil was sterilized (Figure 4.3). The fertilizer 11-55-0 however, increased shoot dry weight in both sterile and nonsterile soils.

In Soil 5 there was an increase in shoot dry weight in the GV, GI and 11-55-0 treatments only after pasteurization (Figure 4.4), though this effect was especially striking in the 11-55-0 treatment.

Root Dry Weight

Root dry weight increased significantly due to sterilization in Soil 2 (Table 4.5) and pasteurization in Soil 5 (Table 4.6). However, if Soil 2 was sterilized and given 11-55-0 a reduction in root growth occurrred.

In nonpasteurized Soil 5, none of the treatments affected the root growth (Figure 4.6). However, a significant interaction

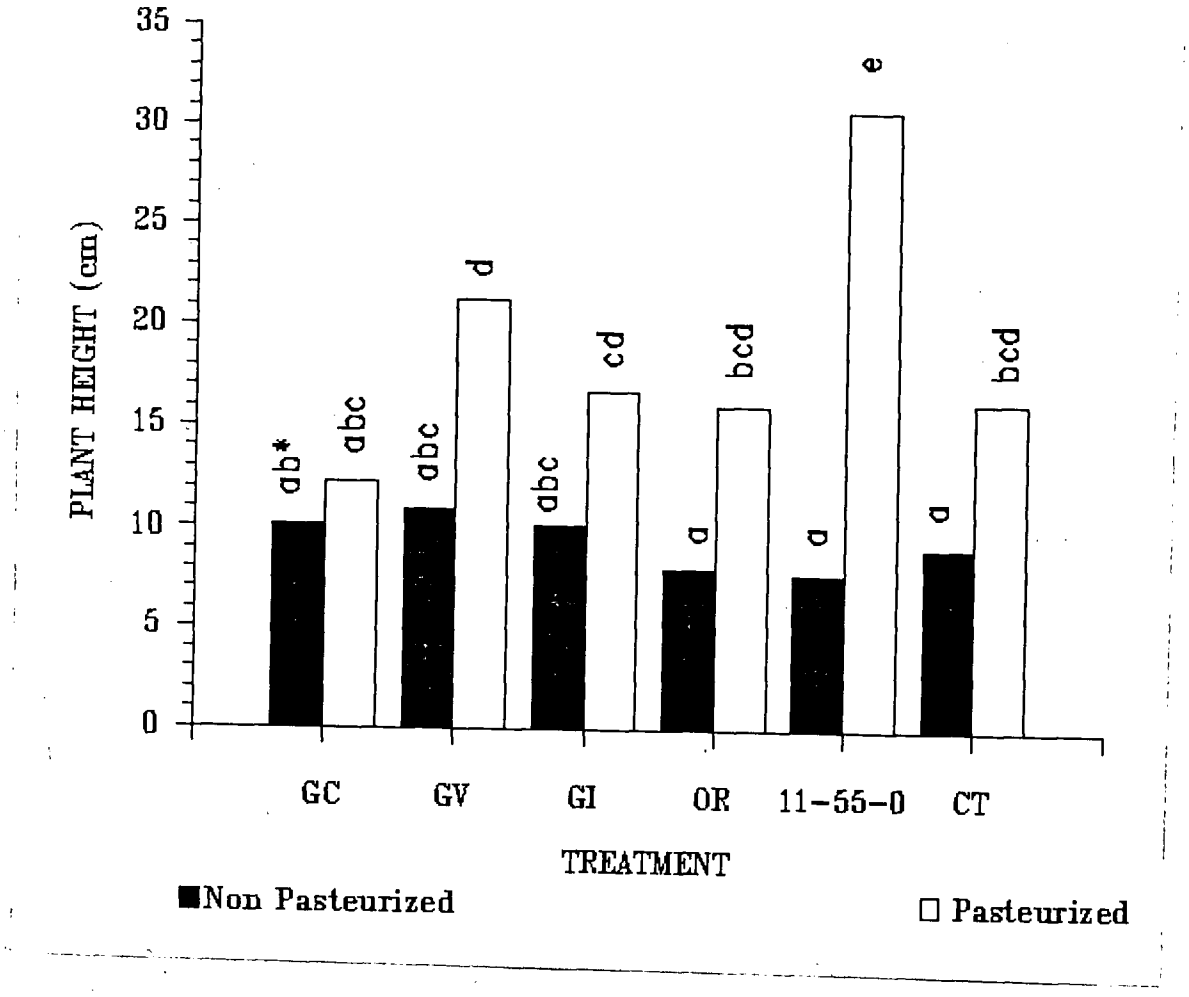

Figure 4.2: Shoot Height at 10 Weeks: Soil 5

* same letter indicates no significant difference ($\alpha = 0.05$ Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	1645.900	1645.900	146.760	0.000
Treatment	5	556.460	111.290	9.924	0.000
Sterility* Treatment	5	770.770	154.150	13.746	0.000
Error	59	661.670	11.215		
Total	70	3629.400			

Table 4.2: Analysis of Variance for Shoot Height: Soil 5

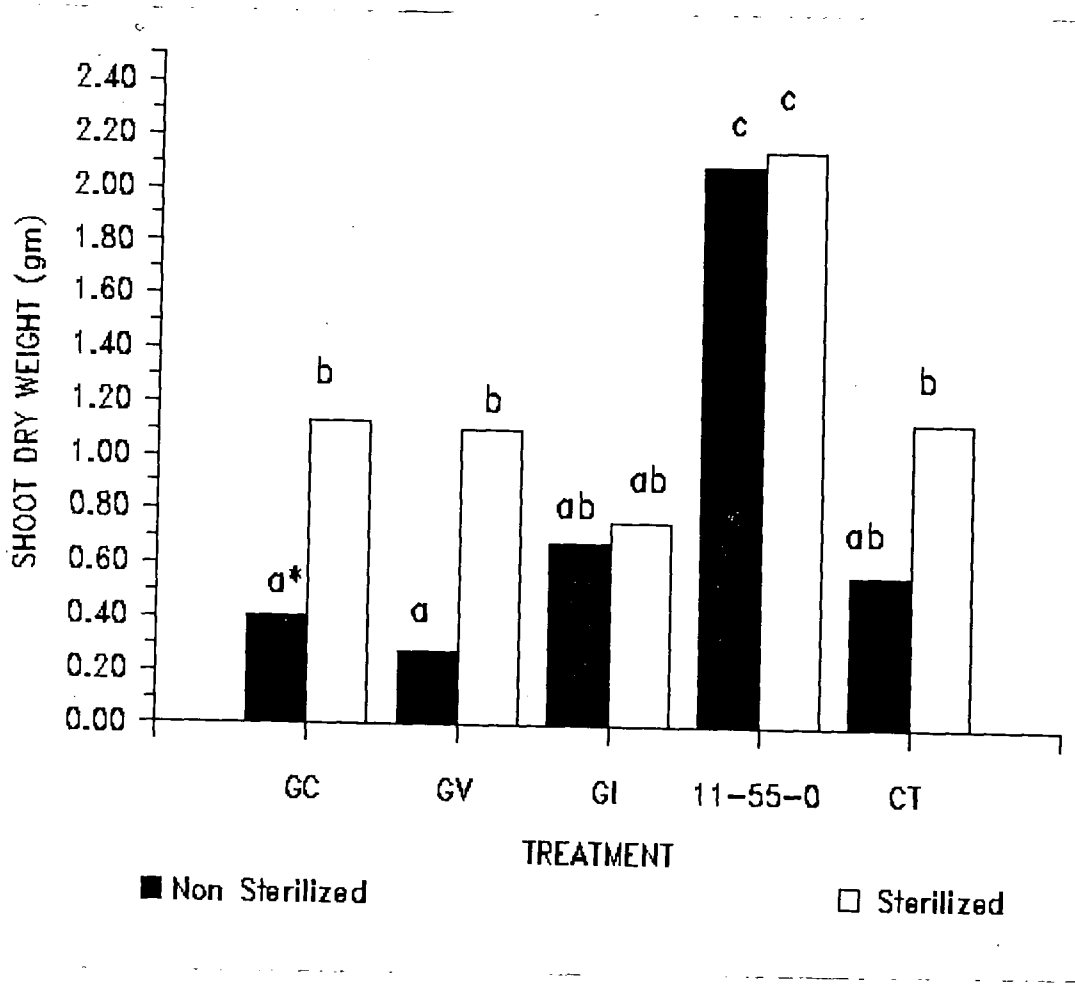

Figure 4.3: Shoot Dry Weight at 10 Weeks: Soil 2

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	3.113	3.113	29.341	0.000
Treatment	4	17.295	4.324	40.756	0.000
Sterility* Treatment	4	1.566	0.392	3.691	0.011
Error	49	5.198	0.106		
Total	58	26.680			

Table 4.3: Analysis of Variance for Shoot Dry Weight: Soil 2

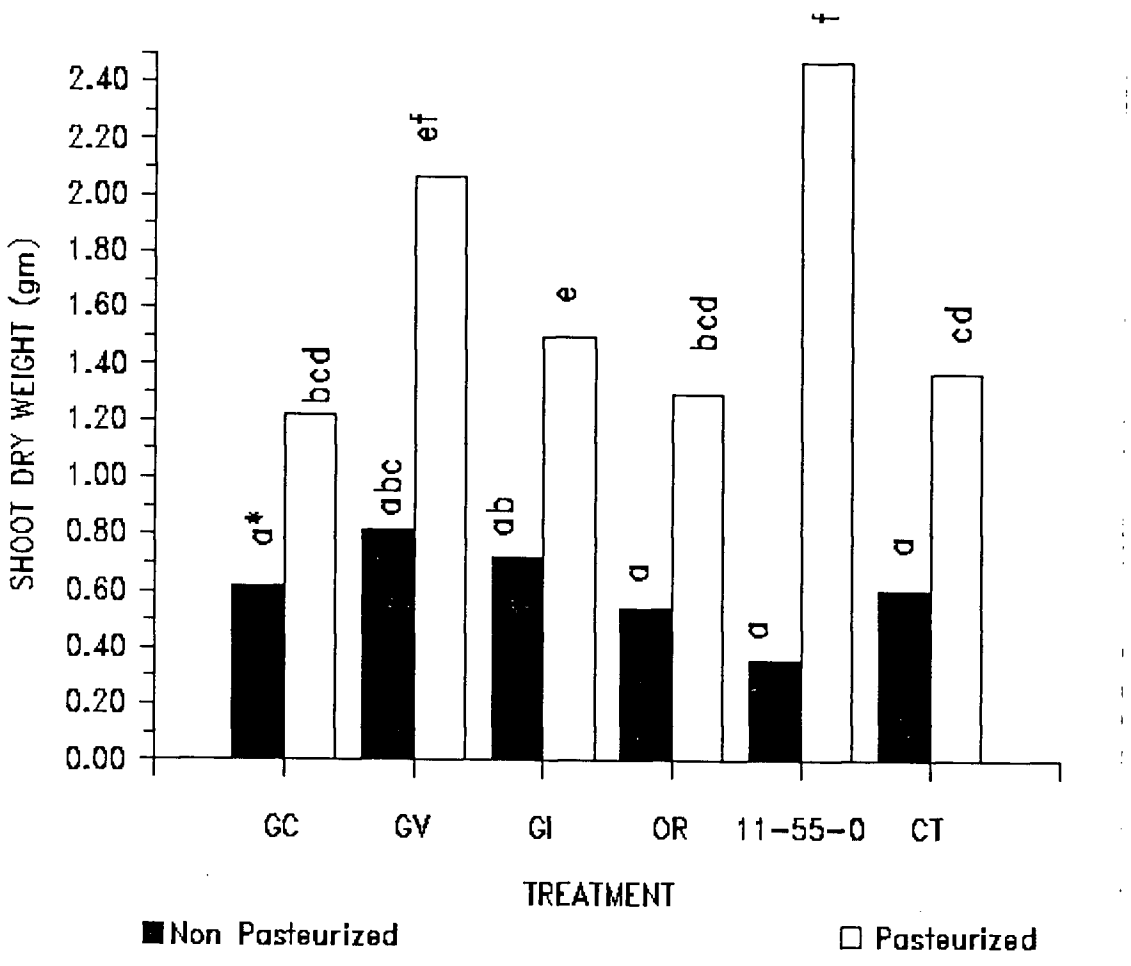

Figure 4.4: Shoot Dry Weight at 10 Weeks: Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	19.467	19.466	219.140	0.000
Treatment	5	3.453	0.691	7.775	0.000
Sterility* Treatment	5	4.815	0.963	10.840	0.000
Error	59	5.241	0.089		
Total	70	32.963			

Table 4.4: Analysis of Variance for Shoot Dry Weight: Soil 5

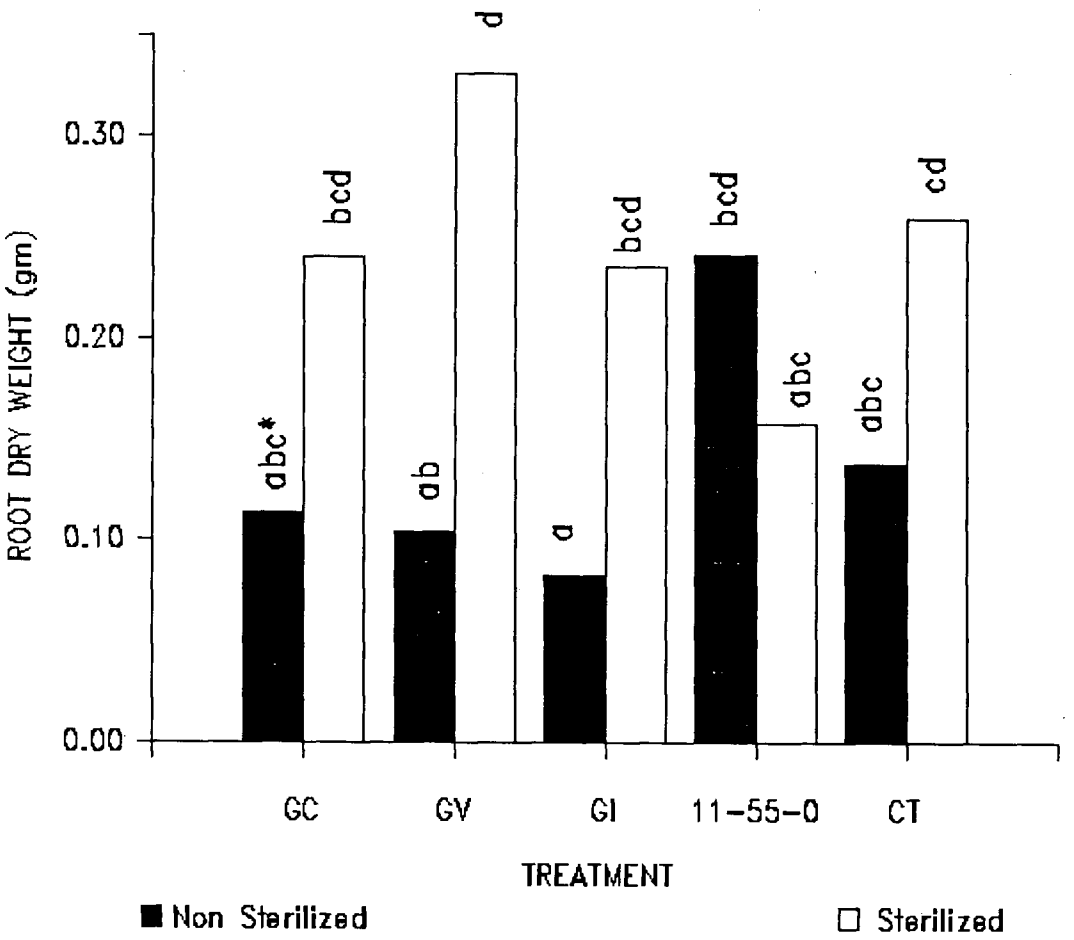

Figure 4.5: Root Dry Weight at 10 Weeks: Soil 2

* same letter indicates no significant difference ($\alpha = 0.05$ Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	0.187	0.187	30.355	0.000
Treatment	4	0.027	0.007	1.109	0.363
Sterility* Treatment	4	0.151	0.038	6.139	0.000
Error	49	0.302	0.006		
Total	58	0.666			

Table 4.5: Analysis of Variance for Root Dry Weight: Soil 2

between pasteurizing this soil and adding the fungi GV and GI occurred. Root dry weight increased significantly in these treatments.

VAM Fungi

Sterilizing Soil 2 did not affect the mycorrhizal colonization of roots (Table 4.7), but it did in Soil 5 (Table 4.8). There were significant interactions between sterilization and treatment for both soils.

In Soil 2 the GV, in sterilized soils and the GI, in both sterilized and nonsterilized soils increased root colonization significantly compared to the 11-55-0 treatment and the sterilized control (Figure 4.7). No difference in mycorrhizal colonization ocurred in this soil among the various fungi. The VAM colonization was not affected in sterilized soils if they were re-inoculated. Sterilizing this soil or adding 11-55-0 significantly decreased VAM fungi.

Soil 5 had a low indigenous population of VAM fungi. In this soil the GC increased mycorrhizal colonization even in the non pasteurized soils (Figure 4.8). There were significant increases in VAM colonization among the other treatments only if the soil was pasteurized. Though the various VAM fungi did not differ significantly from each other. The GI was the best colonizer in the pasteurized soil only. There were significant increases in VAM colonization with the OR and GI after pasteurization.

VAM FUNGI AND PLANT GROWTH

In both ARD soils used in this study, VAM fungi did not

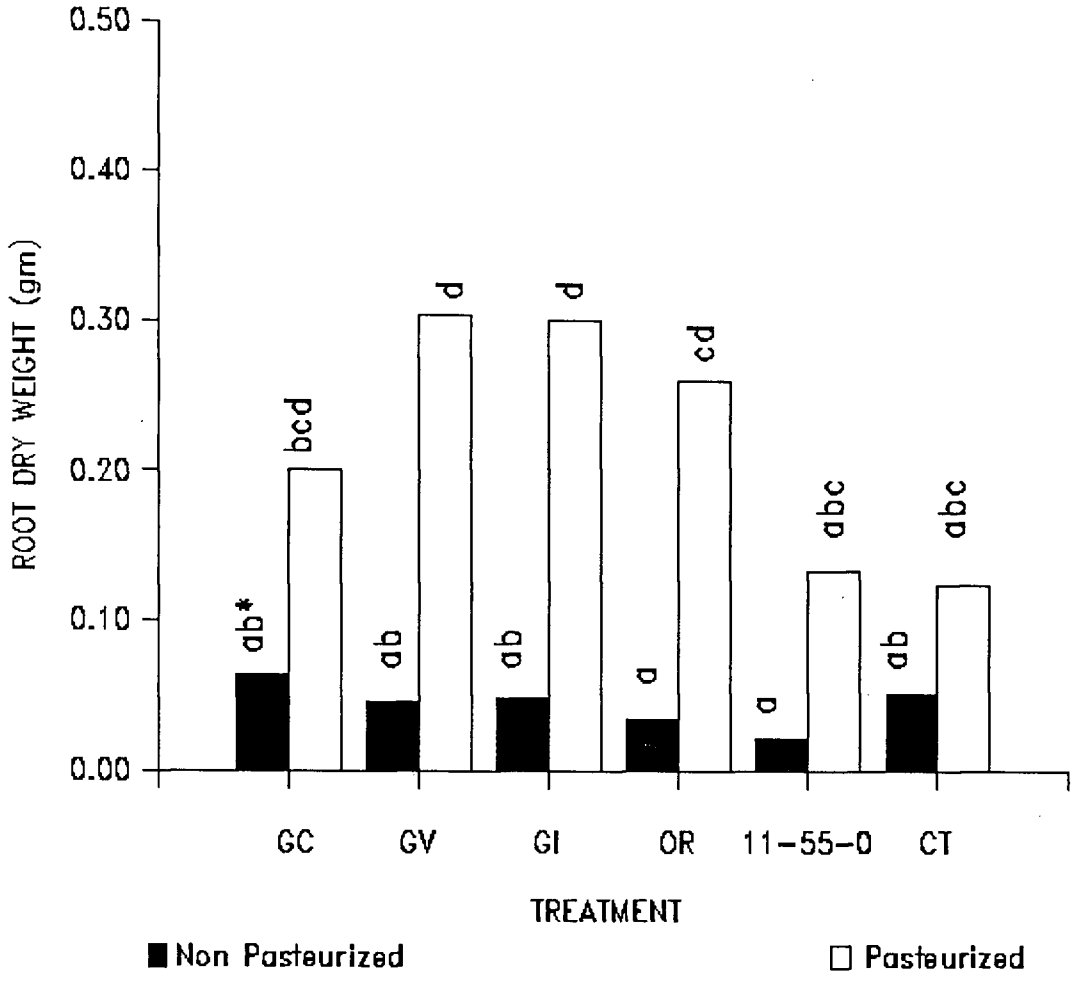

Figure 4.6: Root Dry Weight at 10 Weeks: Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	0.530	0.530	92.001	0.000
Treatment	5	0.112	0.022	3.892	0.004
Sterility* Treatment	5	0.089	0.018	3.106	0.015
Error	59	0.340	0.006		
Total	70	1.079			

Table 4.6: Analysis of Variance for Root Dry Weight: Soil 5

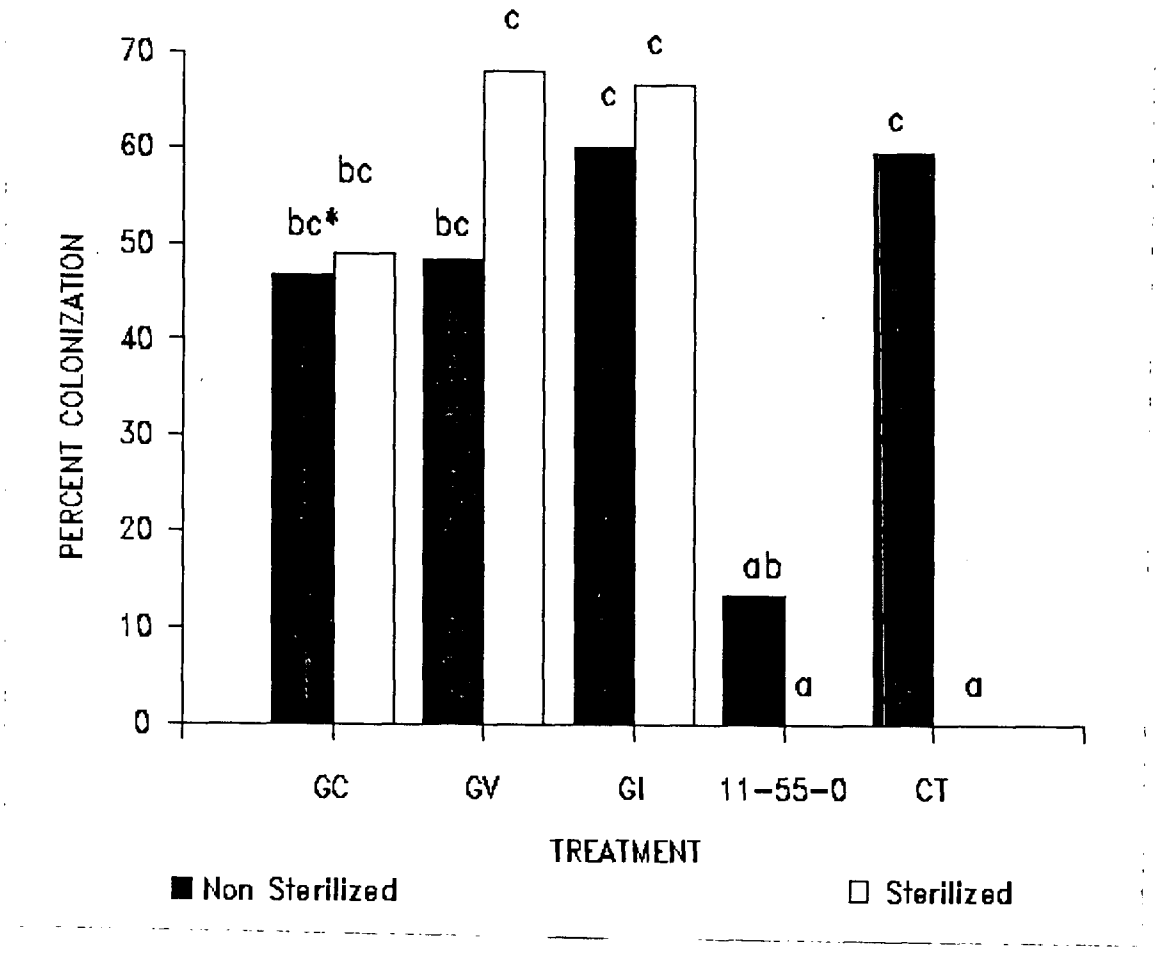

Figure 4.7: Percent Mycorrhizal Colonization: Soil 2

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	1121.200	1121.200	3.089	0.085
Treatment	4	24354.000	6088.500	16.775	0.000
Sterility* Treatment	4	11261.000	2815.200	7.756	0.000
Error	49	17785.000	362.960		
Total	58	54239.000			

Table 4.7: Analysis of Variance for Mycorrhizae: Soil 2

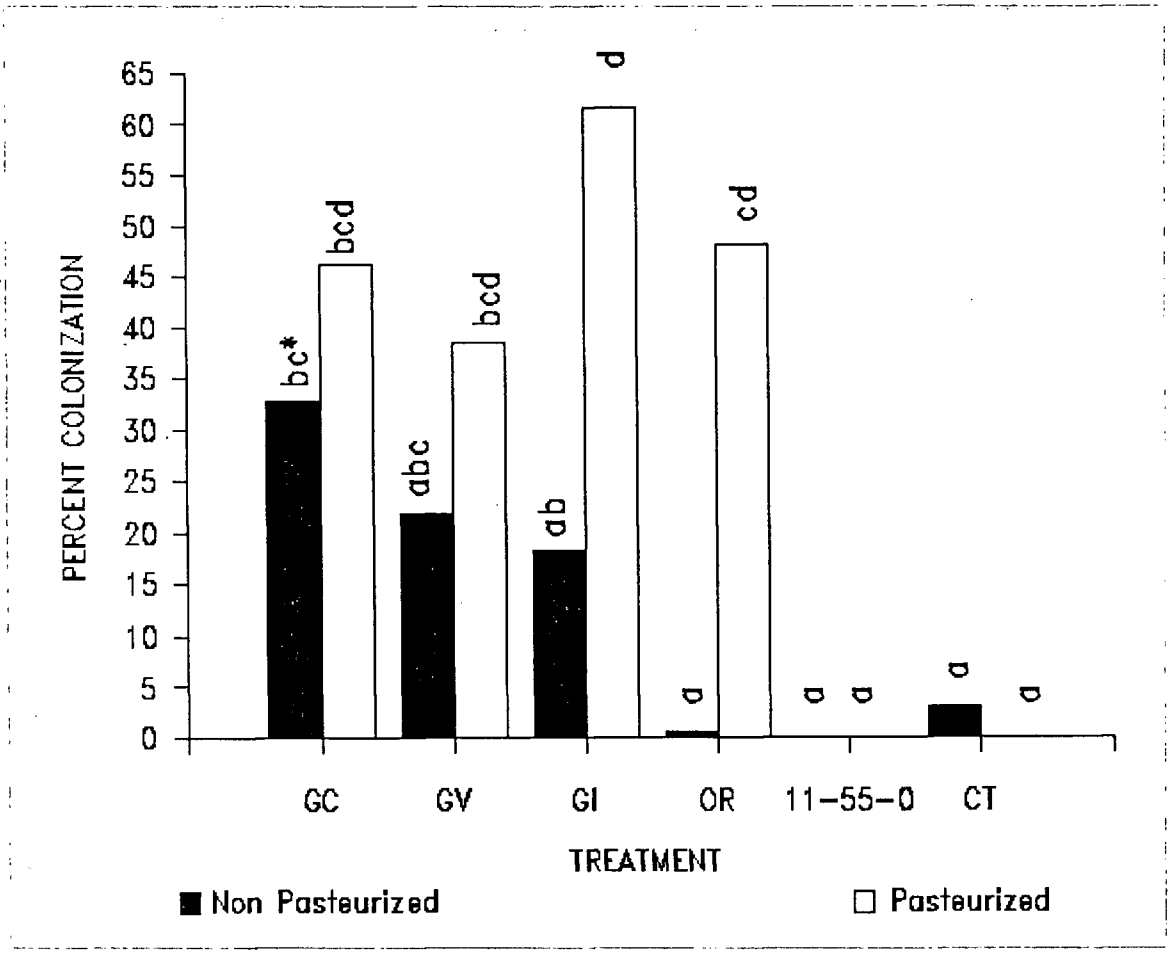

Figure 4.8: Percent Colonization: Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Source	DF	Sum of squares	Mean square	F-ratio	Probability
Sterility	1	6590.600	6590.600	31.584	0.000
Treatment	5	19293.000	3858.600	18.491	0.000
Sterility* Treatment	5	6690.400	1338.100	6.412	0.000
Error	59	12312.000	208.670		
Total	70	45255.000			

Table 4.8: Analysis of Variance for Mycorrhizae: Soil 5

was not sterilized or pasteurized concurring with Sewell and Roberts (1984,1985) (Figure 4.9 and 4.10).

While there was no linear correlation between mycorrhizal colonization and shoot or root growth, it appears as if those plants with the lowest VAM colonization show the greatest shoot growth in nonsterilized ARD soil (Figure 4.9). It may be that the VAM fungi are behaving as pathogens in nonsterilized ARD soils.

In ARD pot bioassays, soils treated with the fertilizer 11-55-0 gave the greatest increase in shoot growth compared to any VAM inoculations (Figures 4.9 and 4.10). This fertilizer however, caused drastic reductions in the VAM colonization in sterilized and non sterilized soils.

While the various fungi did not induce as great a response in shoot growth as the fertilizer treatment, they induced the greatest root growth (Figures 4.11 and 4.12). There is an opposite response of seedlings to fertilizer except in the nonsterile treatment of Soil 2.

DISCUSSION

The growth responses that occurred in inoculated mycorrhizal apple plants were unexpected. In both ARD soils used in this study, VAM fungi did not induce the expected plant growth increases in pot bioassays if the soil was not sterilized or pasteurized concurring with Sewell and Roberts (1984,1985) (Figure 4.9 and 4.10). This goes contrary to Utkhede's (1987) study. Utkhede (1987) used a mixture of Glomus spp. at 1:1 (vol/vol) inoculum:soil. It may be that in ARD soils VAM

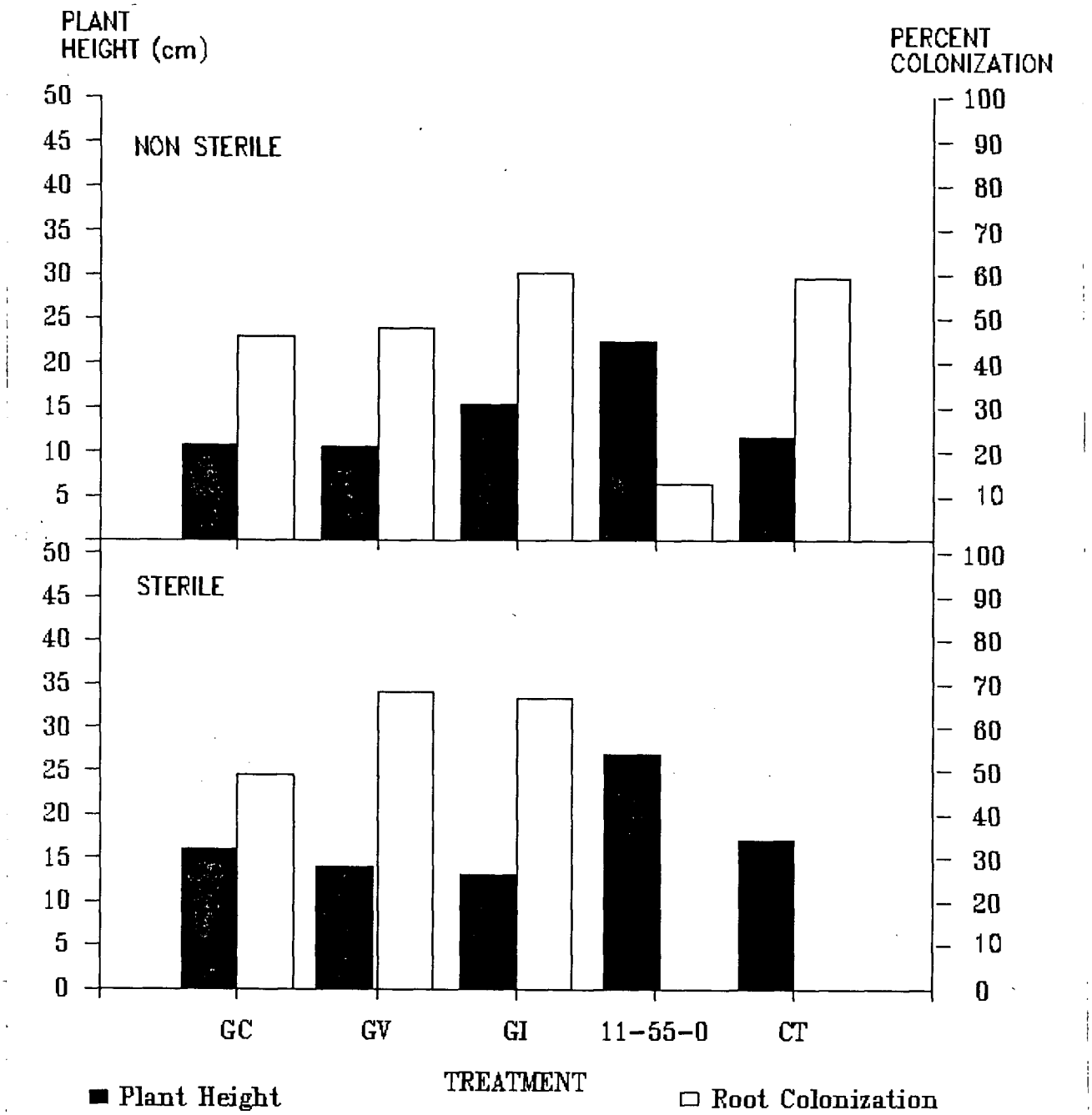

Figure 4.9: Mycorrhizal Colonization and Plant Height: Soil 2

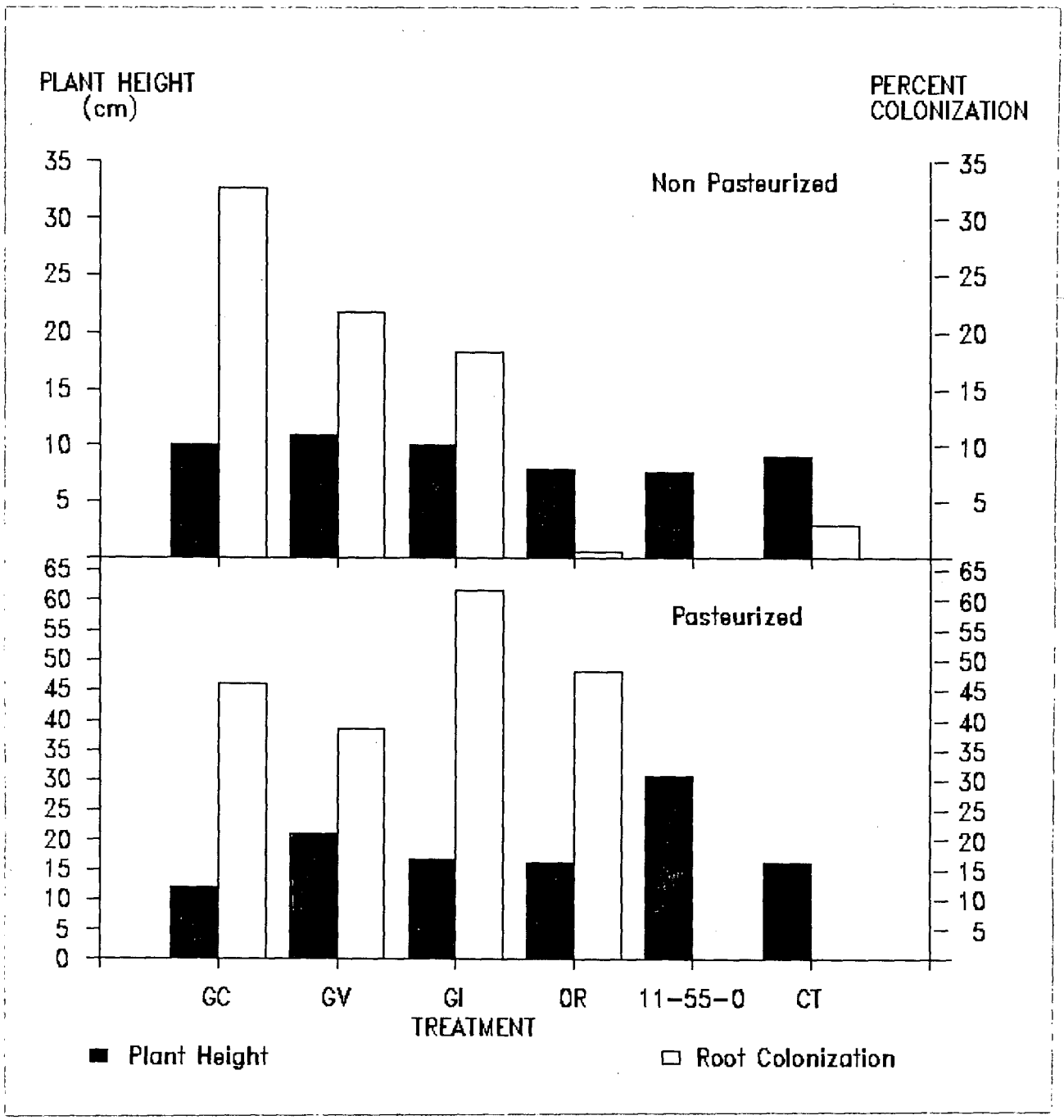

Figure 4.10: Mycorrhizal Colonization and Plant Height: Soil 5

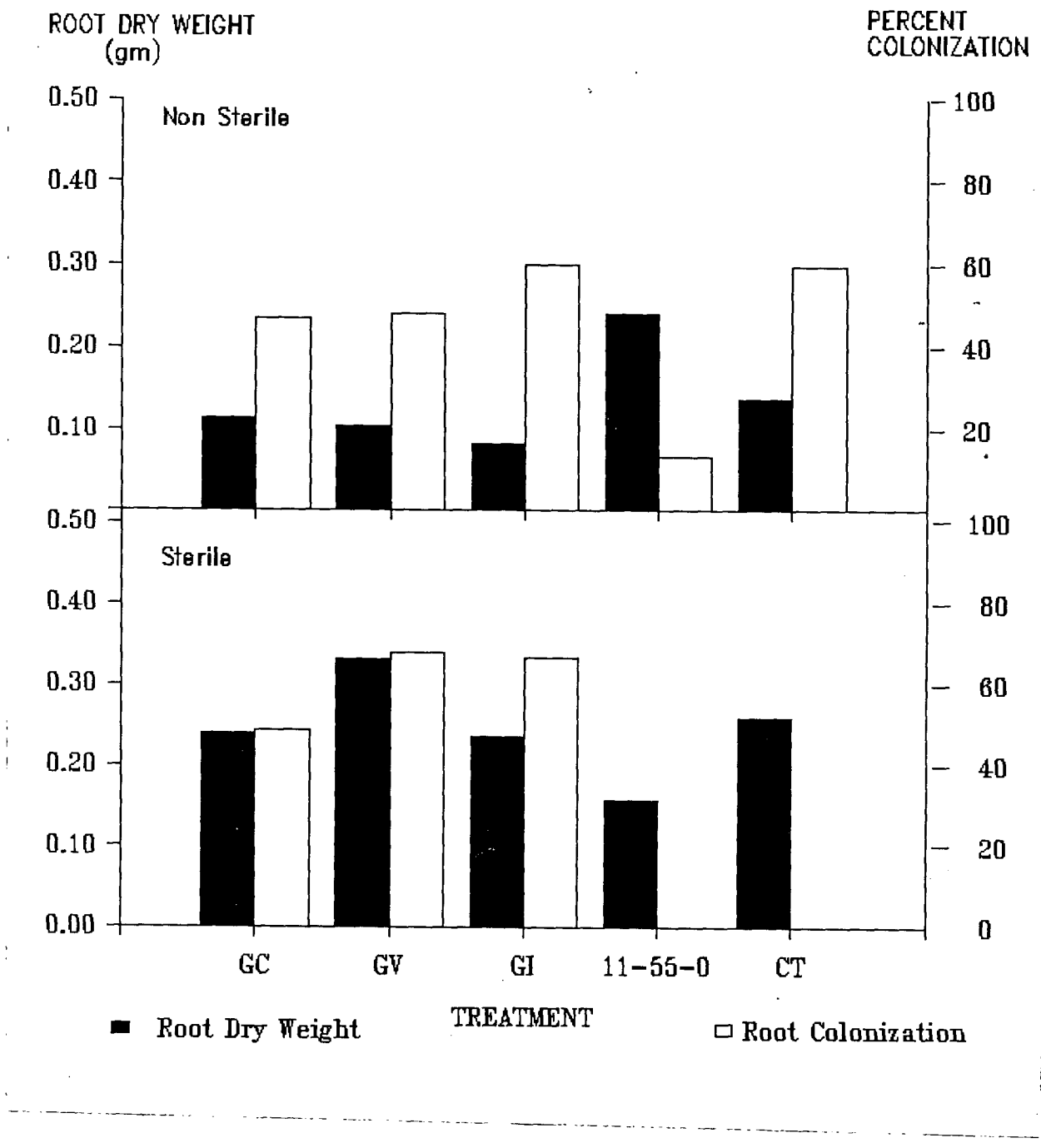

Figure 4.11: Mycorrhizal Colonization and Root Dry Weight: Soil 2

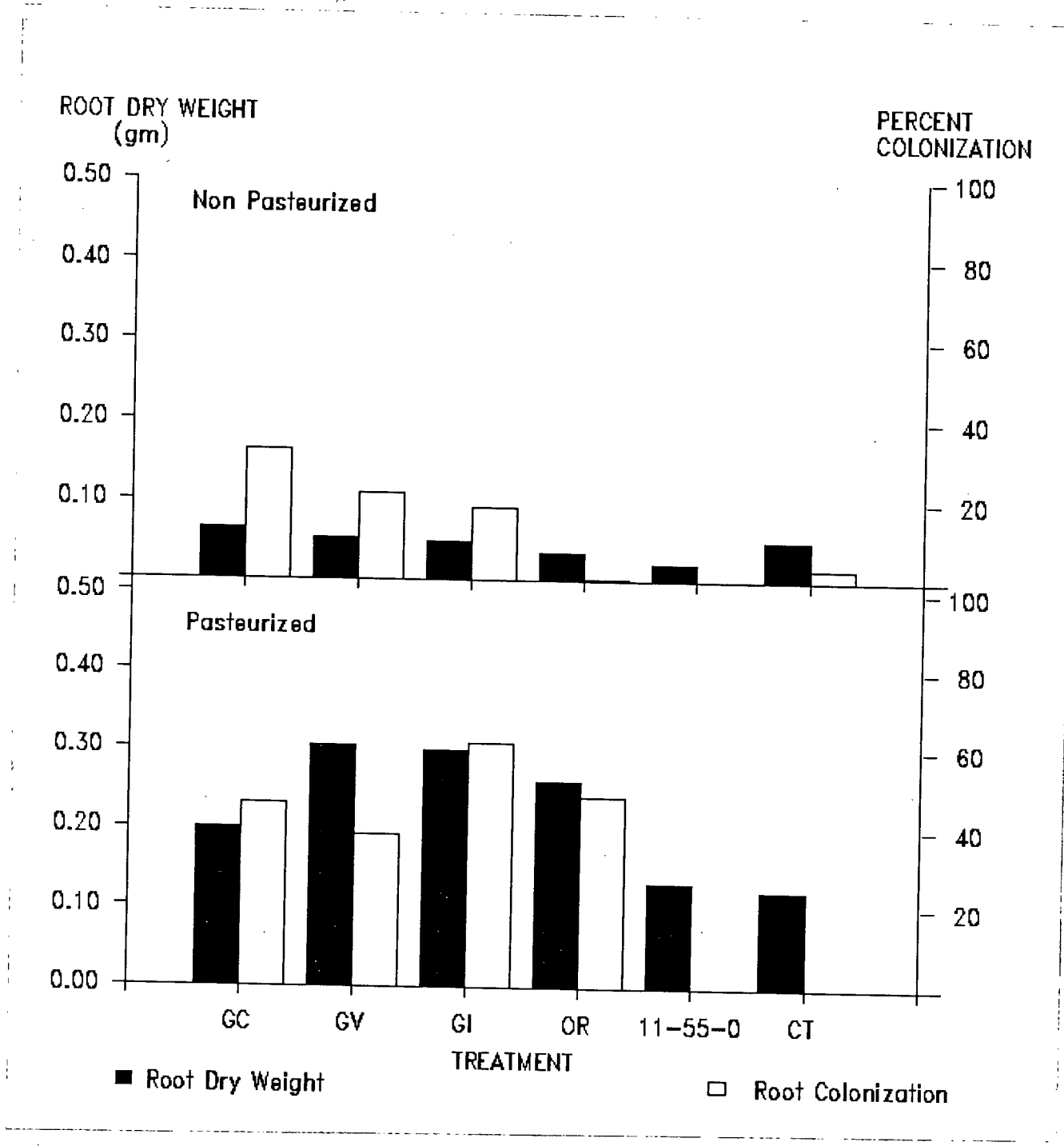

Figure 4.12: Mycorrhizal Colonization and Root Dry Weight: Soil 5

propogules must surpass a threshold level in order to induce plant growth increases. In this study, as in Sewell (1985), pure cultures of VAM fungi were used, eliminating any other microbe effects. It may be that in Utkhede's (1987) study the inoculum may have had other plant growth promoting micro-organisms.

Mosse (1957) indicated that mycorrhizal apples are slow growers that will after 8 weeks outgrow nonmycorrhizal plants. This study was terminated after 10 weeks and this may have been too soon to see the effects of the VAM fungi on shoot growth in ARD soils. It appears that inoculated apples will first build a solid root mass which may eventually lead to healthier shoot growth.

ARD soils usually have indigenous mycorrhizal fungi and these fungi are not effective in stimulating plant growth. The ARD factor, which appears to be inhibiting the effectiveness of these fungi, appears to be inhibiting the inoculated fungi in nonsterilized ARD soils as well.

There are predatory microfauna in soils that limit the growth of VAM fungi. The collembola Folsomia candida feeds on the external hyphae of VAM fungi, thereby reducing the effectiveness of the fungus (Warnock et al. 1982). The feeding activity of predatory microfauna may explain why VAM inoculations did not induce plant growth increases in nonsterilized ARD soils, but did in sterilized ARD soils. It may also explain why plants with high levels of VAM colonization do not induce high growth increases. It is possible that the intramatrical hyphae in plants grown in

ARD soils do not correlate with extramatrical hyphae which may be reduced by predators. In the quest to understand ARD, soil microfauna are ignored. It is possible, that the microfauna, in acting as predators of VAM fungi, reduce the extramatrical hyphae and the effectiveness of the VAM fungus. The interactions of the microfauna in ARD soils should be studied further in the ARD complex.

Baylis (1967) documented that in cases of adequate soil P VAM inoculations will cause a depression in plant growth. Both soils used in this study had high soil P. It may be that the lack of growth response of inoculated seedlings to VAM could have been a result of the high levels of soil P.

Sewell (1984), proposes that Pythium spp., as the causal agent of ARD, exert their effect by destroying the root cortical cells and any subsequent root growth is severly restricted. Through the destruction of these cells, the VAM associations are destroyed. Results from this study indicate that this is not the case. VAM colonization is high in plants affected by ARD. In fact it appears that the higher the VAM colonization the lower the apple growth in nontreated ARD soils. If a facultative pathogen such as Pythium or Fusarium is introduced with a VAM fungus, the fungus may aid in or cause growth depressions, rather than induce growth increases (Hall 1981). It is possible that a facultative pathogen is present in these soils, affecting the plant growth in such a manner that the VAM fungi are unable to overcome any pathogenic effects. If VAM colonization goes beyond a certain

threshold, then they may also behave as pathogens rather than symbionts.

VAM fungi do not benefit their hosts in ARD soils and in fact may behave as pathogens. The larger the VAM colonization the more nutrient exchange sites, and the more C is taken from the host. If no or minimal amounts of P are being exchanged then the fungus may be causing a deleterious C drain on the plant.

VAM fungi may effectively compete for photosynthates with their hosts (Buwalda et al. 1982). Colonization of roots by VAM fungi leads to the decrease in carbohydrates within the shoots while not affecting carbohydrates in the roots. This may explain the results found in this study. It is possible that apple seedlings grown in ARD soils are under sufficient stress that the VAM fungus effectively competes for carbohydrates and this leads to depressed growth especially in shoots.

The fertilizer 11-55-0, at the rate applied, gave the greatest increase in plant height. This fertilizer appears to be available for plant use immediately or very soon after application. From its effect on the VAM fungi, it appears that it moves rapidly through the roots. Menge et al. (1978a) suggests that it is the concentration of P within the root and not soil P that is the limiting factor in mycorrhizal colonization. This could explain why the VAM fungi are eliminated or drastically reduced by this fertilizer.

The fertilizer 11-55-0 gives a flush of shoot growth at the expense of root growth in plants grown in ARD soils. This

response of the seedlings to 11-55-0 in ARD soils should be treated with caution. In orchards that are being planted to dwarf trees, well developed root systems are imperative to the survival of the tree. If 11-55-0 causes a reduction in root growth, these trees may not do well in orchards in the long term. Reduced roots are susceptible to toppling by wind and to invasion of pathogens. While the short term response of shoot growth to 11-55-0 is striking, long term effects of 11-55-0 on different clonal stocks must be examined.

In summary, the factors responsible for ARD may be inhibiting the performance of the VAM fungi in nontreated ARD soils. The extramatrical hyphae were not examined in this study, but they may be negatively affected in these soils. Microfaunal predators may be feeding on the extramatical hyphae, thereby reducing the effectiveness of the VAM fungus. Future work is necessary to determine the correlation between intra- and extramatrical hyphae in apples grown in ARD soils.

The VAM fungi may be behaving as pathogens by increasing the effects of indigenous or introduced facultative pathogens. Or they may be effectively competing for carbohydrates with the host, causing a deleterious C drain, giving little P in return for the C received.

The VAM fungi are however, inducing root growth, which in the long term establishes healthier plants better able to survive transplanting and the invasion of root pathogens.

While VAM fungi do not overcome ARD in soils used in this

study further long term field studies must be performed with these fungi and other ARD soils. An optimal level of 11-55-0 fertilizer should be established in which plant growth increases occur, but at which no adverse effects on the VAM fungi occur.

CONCLUSIONS

The procedures leading to the production of apples are numerous and complicated. In the nursery, certain minimum standards must be maintained to ensure healthy rootstock reaches the orchard. Heavy applications of fertilizers, pesticides and irrigation are part of current nursery practices, and lead to the production of healthy rootstock. These practices however, are not conducive to the survival and proliferation of VAM fungi. In the stoolbed nursery used in this study all beds are fumigated prior to establishment. The VAM fungi are severely limited in this nursery as a result of this practice. The soil in the budded nursery used in this study is never fumigated so the VAM fungi in this nursery are not adversely effected.

Within 6 months of fumigation, VAM population should begin to reestablish and proliferate. In the stoolbed nursery, this does not appear to be happening. Reestablishment of the fungi appears to be erratic with minimal spread through the nursery. It is hypothesized that cultural practices, such as pesticide, fertilizer and irrigation applications throughout the growing season, may be responsible for this pattern.

The difference in mycorrhizal colonization among the different rootstocks found in stoolbed nurseries would reflect this erratic pattern of VAM proliferation. This difference in VAM colonization among the different rootstocks is due primarily to the physical location of each rootstock block in relation to a viable source of inoculum rather than a genotypic receptiveness

of host to fungus. However, root morphology and spread plays an important role in the meeting of fungus and host. Those rootstocks that have the ability to spread throughout the soil area are more likely to encounter a live VAM propagule increasing their probability of forming mycorrhizae.

Malling 2 has this capability, and this is most likely the reason for its consistently higher VAM colonization compared to other rootstocks. Through hormone production, VAM fungi may be indirectly influencing the root morphology of each clonal rootstock. Those rootstocks that form high VAM colonizations may have different levels of growth hormones that change their root morphology. A self-serving relationship might exist in which the fungus colonizes the host, changes the root hormone levels, influencing the ability of the clone to spread, and thereby increasing the probability of an encounter between root and fungus.

The difference in VAM colonization amongst the different clones after cold storage again is not a genotypic factor, but rather a reflection of management practices. Harvesting of rootstocks in stoolbeds is not random, but occurs by rootstock. Each rootstock is therefore processed and stored as a block. Those rootstocks, that are stored under inappropriate conditions may have their VAM associations adversely affected. Malling 4 was stored under dry conditions, and this may have caused the decrease in VAM colonization. Malling Merton 111 was placed in cold storage first, in the part of the chamber that receives the

least amount of oxygen. The length of time in cold storage, plus a decrease in the oxygen levels could account for the decrease in VAM colonization in this rootstock.

Establishing productive orchards in a minimal time frame is an intensive procedure. There are a number of soil-based problems that cause failure of newly transplanted apple orchards. These problems can be separated by causal agent. Those abiotic agents, such as soil chemical, nutrient and physical imbalances are important though not of direct concern in the ARD concept.

The ARD concept is constantly changing and is difficult to elucidate. Clearly, affected transplants are never killed, though their roots are poorly branched and their shoot growth is severely inhibited. These symptoms are easily overcome by soil fumigation and in some cases by the application of soil P as 11-55-0 even though available soil P is high. The biotic agents causing these symptoms may be separated on the basis of diagnosis; those pathogens that are easily identified and those organisms that are not. If transplants are not growing well due to the presence of a specific organism, this is not ARD.

Unfortunately there is a tendency by many to simplify a complex phenomena. It is not acceptable to use the term ARD as an umbrella term for any transplanting disorder that is overcome by soil fumigation.

Each soil identified as having ARD is unique with its own chemical, physical and biological characteristics. The word disease does not give a good indication of the complexity of the

problem and should be replaced with disorder. It may be time to start appending the term ARD with its causal agent. Thus an apple replant problem caused by nematodes should be termed as 'apple replant disorder caused by nematodes'. If the causal agent can not be identified then the terminology should simply be 'apple replant disorder caused by unknown factors (ARD-UF)'.

All 5 soils used in this study were identified as having ARD caused by unknown factors. All 5 soils had extremely high amounts of phosphorus yet test seedlings grown in 4 of the 5 soils showed increases in shoot growth after the addition of 11-55-0 and 34-0-0 fertilizers. While giving an increase in shoot height, the 11-55-0 fertilizer reduced root growth and VAM colonization in test seedlings at the rate used. The 34-0-0 fertilizer, while not giving as great a shoot response compared to the 11-55-0, does not appear to be so severe on VAM associations. While not giving a strong shoot growth in nonsterilized soils, the fertilizer 0-45-0 results in increased root growth and does not adversely affect VAM colonization. VAM fungi appear to mimic the fertilizer 0-45-0 in ARD soils used in this study.

VAM fungi will not overcome ARD. In nonsterilized soil, there is little shoot growth in response to inoculated VAM fungi. However, root growth is increased by these fungi. It is apparent that inoculated VAM fungi will not overcome ARD in nonsterilized soils, and that they do not give as great a shoot growth response as the 11-55-0 fertilizer in sterilized soils. However, it is proposed that with the strong root based plants induced by the

VAM fungi, eventually these plants will show strong shoot growth as well.

In nonsterilized ARD soils, VAM fungi are not inducing the expected growth of the host. It is hypothosized that VAM fungi in non-sterilized ARD soils are acting as pathogens rather than symbionts. The benefits of having this association is not mutual. The fungus is deriving its carbon source from its host, but the P gain by the host may have minimal affects on apple growth. The fungus is able to spread throughout the root, but this is not an indication of spread throughout the soil.

It is further proposed that VAM predators in ARD soils are indirectly involved in ARD. The VAM predators, may be significantly reducing the extramatrical hyphae thereby reducing the route for soil P movement into the host. They are eliminated upon sterilization, and the fungus is then able to perform as expected and mycorrhizal plants will show increased plant growth.

The role of soil P in these soils must be elucidated. All 5 soils used in this study had adequate to high amounts of soil P, yet seedlings responded positively to applications of N and P fertilizers. The fertilizer 11-55-0 was applied at a rate of 3,000 kg/ha. This enormous amount of fertilizer, while giving a growth response in pot tests, surely does not reflect the required amounts of 11-55-0 in the field.

It is also apparent that methods used to determine the amounts of available P in the soil are not reflecting the P requirements of the apple plant. It is therefore proposed that

either the standards used to determine P requirements by apple seedlings be re-evaluated and changed accordingly, or that soil P should not be used as a criterion to measure P requirements of young apple seedlings. It may be more appropriate to measure root P in test seedlings to determine the levels of P required and available to the plant. Measuring root P would also elucidate the role of VAM fungi in plants grown in these soils.

In summary, nursery management practices may be detrimental to VAM associations formed by rootstocks. The difference in clonal VAM associations is not a genotypic receptiveness to VAM fungi but rather due to environmental factors.

There are many factors responsible for failure in establishing new apple orchards. Fumigation and additions of N and P fertilizers will result in increases in shoot growth in ARD soils. Root growth and VAM associations may be adversely affected by these fertilizers. VAM fungi will not overcome ARD-UF in affected soils, though shoot growth is increased if the soil is fumigated first. These growth responses are not as great as that induced by 11-55-0 fertilizer. In the short term, the inverse response occurs in root growth.

As it is incorrect to diagnose a single factor as being responsible for the ARD complex, so is it incorrect to search for single cures for this complex phenomena.

The ARD complex becomes more complicated as evidence presented in this study indicates that VAM fungi can behave as pathogens in these soils rather than as symbionts. Further work

is needed to elucidate the role of these fungi in these soils.

REFERENCES

Baylis, G.T.S. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66: 231-243.

Benson, N.R. and R.P. Covey Jr. 1976. Response of apple seedlings to zinc fertilization and mycorrhizal inoculation. HortSci. 11: 252-253.

Benson, N.R., R.P. Covey Jr., and W. Haglund. 1978. The apple replant problem in Washington State. J. Amer. Soc. Hort Sci. 103: 156-158.

Buszard, D.J. and P. Jensen. 1986. A note on the incidence of apple replant disease in Quebec orchards. Phytoprotection. 67: 133-136.

Buwalda, J.G. and K.M. Goh. 1982. Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soi Biol. Biochem. 14: 103-105.

Cataska, V., V. Vancura, Z. Prikryll and G. Hudska. 1988. Artificial induction of apple replant problem by Penicillium claviforme inoculation. Plant and Soil. 107: 127-136.

Cataska, V., V. Vancura, G. Hudska and Z. Prikryl. 1982. Rhizosphere micro-organisms in relation to the apple replant problem. Plant and Soil 69: 187-197.

Cooper, K. M. 1983. Mycorrhizal fungi can improve growth of horticultural crops. Orchardist. N.Z. 56: 410-413.

Coker, E.G. 1958. Root studies: XII. Root systems of apple on Malling rootstocks on five soil series. J. Hort Sci. 33: 71-79.

Covey, R.P., B.L. Koch and W.A. Haglund. 1984. Control of apple replant disease with formaldehyde in Washington. Plant Disease 68: 981-983.

Covey, R.P., B.C. Koch and H.J. Larsen. 1981. Influence of vesicular-arbuscular mycorrhizae on the growth of apple and corn in low phosphorous soils. Phytopath. 71: 712-715.

Deal, D.R., W.F. Mai and C.W. Boothroyd. 1971. A survey of biotic relationships in grape replant situations. Phytopath. 62: 503-507.

Eno, C. F. and H. Popenoe. 1963. The effect of gamma radiation on the availability of nitrogen and phosphorus in soil. Soil

Sci. Soc. Amer. Proc. 28: 299-301.

Gennari, M., M. Negre and R. Ambrosoli. 1987. Effects of ethylene oxide on soil microbial content and some soil chemical characteristics. Plant and Soil. 102: 197-200.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Granger, R..L., C. Plenchette and J.A. Fortin. 1983. Effect of a vesicular-arbuscular (VA) mycorrhizal fungus (Glomus epigaeum) on the growth and leaf mineral content of two apple clones propagated in vitro. Can. J. Plant Sci. 63: 551-555.

Grochowska, M., G.J. Buta, G.L. Steffens and M. Faust. 1984. Endogenous auxin and gibberellin levels in low and high vigour apple seedlings. In: Acta Hort. 146: International Workshop on Controlling Vigor in Fruit Trees. Ed: Faust, M. Int. Soc. Hort. Sci.Wageningen, Netherlands. pp 125-134.

Hall, I.R. and A. Kelson.1981. An improved technque for the production of endomycorrhizal infested soil pellets. N.Z. J. Agric. 24:221-222.

Hartman, H.T. and D.E. Kester. 1975. Plant Propagation: Principles and Practices. Prentice-Hall Inc., Englewood Cliffs, New Jersey. pp.465.

Hattingh, M.J., and J.W. Gerdemann. 1975. Inoculation of brazilian sour orange seed with an endomycorrhizal fungus. Phytopath. 65: 1013-1016.

Havis, I., Morris H.F., R. Manning and T.E. Denman. 1958. Responses of replanted peach trees to soil treatments in field tests in Texas. Proc. Amer. Soc. Hort. Sci. 71: 67-76.

Hoepfner, E.F., G.L. Koch and R.P. Covey. 1983. Enhancement of growth and phosphorus concentration in apple seedlings by vesicular-arbuscular mycorrhizae. J. Amer. Soc. Hort. Sci. 108: 207-209.

Hoestra, H. 1968. Replant diseases of apple in the Netherlands. Meded. Landbouw. Wagen., Nederland. Veenman and Zonen Wageningen.

Hoestra, H. and Oostenbrink. 1962. Nematodes in relation to plant growth. IV. Pratylenchus penetrans (Cobb) on orchard trees. Neth. J. Agric. Sci. 10: 286-296.

Hwang, S. F. 1988. Effects of vesicular arbuscular mycorrhizae

and metalaxyl on growth of alfalfa seedlings in soils from fields with "Alfalfa Sickness" in Alberta. Plant Dis. 72: 448-452.

Jaffe, B.A., G.S. Abawi and W.F. Mai. 1982. Role of soil microflora and Pratylenchus penetrans in an apple replant disease. Phytopath. 72: 247-251.

Kelly, C.C. and R.H. Sprilsbury. 1949. Soil Survey of the Okanagan and Similkameen Valleys: British Columbia. B.C. Dept. Agric. Rep. 3. Victoria, B.C.

Kleinschmidt, G.D. and J.W. Gerdemann. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. Phytopath. 62: 1447-1453.

Kormanik, P.P. and A.C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In. Methods and Principles of Mycorrhizal Research. Ed. N.C. Schenck. APS. St. Paul, Minnesota. pp 37-46.

La Rue, J.H., W.D. McClellan and W.L. Peacock. 1975. Mycorrhizal fungi and peach nursery nutrition. Cal. Agric. 29: 6 - 7.

Lavkulich, L.M. 1978. Phosphorus soluble in Sodium Carbonate. In Methods Manual: Pedology Laboratory. Dept Soil Science. Univ. B.C. Vancouver, B.C.

Liegel L.H. 1983. Effect of dry-heat sterilization on chemical properties of Puerto Rican soils. Commun. Soil Sci. Plant Anal. 14: 277-286.

Luttermerding, H.A. 1981. Soils of the Langley Vancouver Map Area: Report 15. vol (6) B.C. Min. Environ., Victoria, B.C.

Luttermerding, H. A. 1980. Soils of the Langley-Vancouver Map Area. bull 18.vol (2) B.C. Min. Environ. Victoria, B.C.

Mack, A.R. 1963. Biological activity and mineralization of nitrogen in tree soils as induced by freezing and drying. Can. J. Soil Sci. 43: 316-324.

Mai, W.F. and G.S. Abawi. 1981. Controlling replant diseases of pome and stone fruits in northeastern United States by preplant fumigation. Plant Dis. 65: 859-863.

Menge, J.A. 1982. Effect of soil fumigants and fungicides on vesicular-arbuscular fungi. Phytopath. 72: 1125-1132.

Menge, J.A., D. Stierle, D.J. Bagyaraj, E.L.V. Johnson and R.T. Leonard. 1978a. Phosphorus concentration in plants responsible for inhibition of mycorrhizal infection. New.

Phytol. 80: 575-578.

Menge, J.A., R.M. Davis, E.L.V. Johnson and G.A. Zentmyer. 1978b. Mycorrhizal fungi increase growth and reduce transplant injury in avocado. Cal. Agric. 32: 6-7.

Miller, D.D., P.A. Domoto and C. Walker. 1985a. Mycorrhizal fungi at eighteen apple rootstock plantings in the United States. New Phytol. 100: 379-391.

Miller, D.D., P.A. Domoto and C. Walker. 1985b. Colonization and efficacy of different endomycorrhizal fungi with apple seedlings at two phosphorus levels. New. Phytol. 100: 393-402.

Mosse, B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature 179: 922-924.

Mountain, W.B. and H.R. Boyce. 1958. The peach replant problem in Ontario. VI. The relationship of Pratylenchus penetrans to the growth of young peach trees. Can. J. Bot. 36: 135-151.

Nemec, S. 1987. Effect of storage temperature and moisture on Glomus species and their subsequent effect on citrus rootstock seedling growth and mycorrhiza development. Trans. Brit. Mycol. Soc. 89: 205-212.

Pepin, H.S., W.F. Sewell, and J.F. Wilson. 1975. Soil populations of Thielaviopsis basicola associated with cherry rootstock in relation to effects of the pathogen on their growth. Ann. Appl. Biol. 79: 171-176.

Plenchette, C., V. Furlan and J.A. Fortin. 1982. Effects of different endomycorrhizal fungi on five host plants grown on calcined montmorillonite. J. Amer. Soc. Hort. Sci. 107: 535-538.

Reich, L. 1988. Rates of infection and effects of 5 vesicular-arbuscular mycorrhizal fungi on apples. Can. J. Plant Sci. 68: 233-239.

Reid, C.P.P. and G.D. Bowen. 1979. Effects of soil moisture on V/A mycorrhiza formation and root development in Medicago. In: The Soil-Root Interface. Ed. Harley, J.L. and R.S. Russell. Academic Press, London. pp 211-220.

Robertson, W.J., C.D. Boyle and H.L. Brown. 1988. Endomycorrhizal status of certified strawberry nursery stock. J. Amer. Soc. Hort. Sci. 113: 525-529.

Rom, R.C. and S.A. Brown. 1973. Burr-knot characteristics of six clonal rootstocks. Fruit Var. J. 27: 84-86.

Rom, R.C. and S.A. Brown. 1979. Factors affecting burrknot formation on clonal Malus rootstock. HortSci. 14: 231-232.

Rom, R.C. and G.R. Motichek. 1987. Cultivar effect on adventitious root development of clonal apple rootstocks. HortSci. 22: 57-58.

Ross, R.G., K.B. McRae, and R.J. Newberry. 1984. Growth of apple trees affected by replant disease after lifting, site fumigation, and replanting. Can. J. Plant Path. 6: 257-258.

Ryan, C.L.J. 1975. Specific replant diseases in Hawkes Bay. Part II. Pot tests used for detecting replant disease. Its identification and distribution. Orchardist N.Z. 48: 165-168.

Salonius, P.O, J.B. Robinson, and F.E. Chase. 1967. A comparison of autoclaved and gamma-irradiated soils as media for microbial colonization experiments. Plant and Soil. 2: 239-249.

Savory, B.M. 1967. Specific replant diseases of apple and cherry. Rep. E. Malling Res. Stn. for 1966. pp. 205-208.

Sewell, G.W.F. 1981. Effects of Pythium species on the growth of apple and their possible causal role in apple replant disease. Ann. Appl. Biol. 97: 31-42.

Sewell, G.W.F. 1984. Replant disease: Nature, etiology and importance. Brit. Crop Protect. Conf.: Pests and Dis. 3: 1175-1182

Sewell, G.W.F. and A.L. Roberts. 1984. Effects of phosphorus and VA mycorrhiza on apple growth. Rep. East Mall. Res. Sta. 1983 pp 101-102.

Sewell, G.W.F. and A.L. Roberts. 1985. Small responses to soil sterilisation in apple seedling bio-assays of orchard soils. Rep. East Mall. Res. Sta. 1985 pp. 113-115.

Sewell, G.W.F. and G.C. White. 1979. The effects of formalin and other soil treatments on the replant disease of apple. J. Hort. Sci. 54: 333-335.

Sewell, G.W.F. and G.C. White. 1981. Effects of formalin and other soil treatments on the poor growth or replant disease of apple. Rep. E. Mall. Res. Stn. for 1980. pp 169-170.

Skipper, H.D. and D.T. Westermann. 1973. Comparative effects of propylene oxide, sodium azide and autoclaving on selected soil properties. Soil Biol. Biochem. 5: 409-414.

Slykhuis, J.T. and T.S.C. Li. 1985. Responses of apple seedlings to biocides and phosphate fertilizers in orchard soils in British Columbia. Can. J. Plant Path. 7: 294-301.

Traquair, J. A. and S.M Berch. 1988. Colonization of peach rootstock by indigenous vesicular-arbuscular mycorrhizal (VAM) fungi. Can. J. Plant Sci. 68: 893-898.

Trudgill, D.L. 1984. A pot investigation into the nature of raspberry (Rubus idaens L.) replanting disorders in Scotland. Crop Res. (Hort. Res.) 24: 105-118.

Upstone, M.E. 1974. The testing of orchard soils for specific apple replant disease. 1965-1974. ADAS. Sci. Ser. Ann. Rep: pp 181-185.

Utkhede, R. 1987. New lines of research on apple replant disease. In Successful Replanting. Ed. McPhee, B. and T. Swales. Okan. Simil. Coop. Grow. Assoc. pp 32-34.

Warnock, A.J., Fitter, A.H. and M.B. Usher. 1982. The influence of a springtail Folsomia candida (Insect, Collembola) on the mycorrhizal association of leek Allium porrum and the vesicular-arbuscular mycorrhizal endophyte Glomus fasciculatus. New. Phytol. 90:285-292.

Warcup, J.H. 1957. Chemical and biological aspects of soil sterilization. Soils and Fertilizers. 20: 1-5.

Williams-Linera, G., and J.J. Ewel. 1984. Effect of autoclave sterilization of tropical andept on seed germination and seedling growth. Plant and Soil. 82: 263-268.

Zar, J.H. 1984. Biostatistical analysis. Prentice Hall. Englewood Cliffs. N.J. pp. 239-240.

APPENDIX

Soil pH for Old Rows and Alleyways Soils 1 - 4

1	2	3	Mean
7.40	7.34	7.39	7.37
7.13	7.09	7.10	7.11
7.41	7.20	7.44	7.38
7.50	7.41	7.43	7.43
7.43	7.47	7.47	7.45
7.06	7.11	7.08	7.09
7.23	7.27	7.26	7.25
7.32	7.33	7.28	7.31
7.36	7.28	7.24	7.29
6.83	6.89	6.90	6.86
7.42	7.43	7.43	7.43
7.30	7.36	7.35	7.34
	MEAN		7.27

Table A.1a: Soil pH for Old Rows: Soil 1

1	2	3	Mean
7.41	7.51	7.48	7.47
7.49	7.51	7.48	7.49
7.47	7.43	7.54	7.49
7.27	7.20	7.28	7.25
6.62	6.68		6.64
7.40	7.28	7.35	7.34
7.70	7.09	7.19	7.33
7.21	7.16	7.19	7.19
7.30	7.19	7.28	7.26
7.05	7.00	7.06	7.04
7.10	7.11	7.10	7.10
7.39	7.30	7.35	7.34
6.60	6.49	6.52	6.56
7.20	7.17	7.13	7.20
	MEAN		7.19

Table A.1b: Soil pH for Old Alleyways: Soil 1

1	2	3	Mean
7.32	7.42	7.33	7.36
7.50	7.39	7.30	7.40
6.99	6.54	7.32	6.94
7.60	7.59	7.59	7.60
7.00	7.23	7.27	7.16
7.39	7.48	7.34	7.40
7.25	7.33	7.32	7.30
7.44	7.40	7.42	7.42
7.00	7.02	6.99	7.02
7.40	7.41	7.45	7.42
7.07	7.11	7.12	7.10
7.34	7.28	7.17	7.26
7.18	7.14	7.14	7.15
7.28	7.29	7.35	7.30
7.61	7.50	7.54	7.55
	MEAN		7.29

Table A.2a: Soil pH for Old Rows: Soil 2

1	2	3	Mean
7.57	7.51	7.49	7.52
7.32	7.30	7.33	7.32
7.54	7.55	7.53	7.54
7.31	7.33	7.34	7.34
7.58	7.63	7.60	7.60
7.45	7.49	7.54	7.49
7.29	7.23	7.39	7.30
7.19	7.08	6.98	7.08
7.53	7.50	7.49	7.51
7.59	7.54	7.55	7.55
7.74	7.74	7.81	7.76
7.44	7.49	7.48	7.47
7.45	7.48	7.40	7.44
	MEAN		7.46

Table A.2b: Soil pH for Old Alleyways: Soil 2

1	2	3	Mean
5.90	5.81	6.06	5.92
6.86	7.26	6.92	7.01
5.19	5.67	5.88	5.58
6.95	7.04	6.99	6.99
6.46	6.19	6.31	6.32
5.73	5.63	5.64	5.66
6.11	6.14	6.13	6.12
5.63	5.64	5.68	5.65
6.41	6.30	6.31	6.34
6.45	6.25	6.25	6.31
5.99	5.61	5.78	5.79
7.27	7.08	7.12	7.15
7.65	7.54	7.64	7.61
	MEAN		6.34

Table A.3a: Soil pH for Old Rows: Soil 3

1	2	3	Mean
6.15	6.20	6.18	6.17
6.55	6.77	6.63	6.65
6.91	6.99	6.98	6.96
7.30	7.41	7.50	7.40
5.78	5.55	5.64	5.65
6.78	7.06	7.34	7.06
5.54	5.42	5.47	5.47
6.05	5.94	6.01	6.00
5.84	5.78	5.80	5.80
5.76	5.76	5.83	5.78
7.21	7.10	7.09	7.13
7.00	6.80	6.83	6.87
5.94	5.78	5.77	5.83
	MEAN		6.37

Table A.3b: Soil pH for Old Alleyways: Soil 3

1	2	3	Mean
7.71	7.71	7.69	7.70
7.10	7.14	7.13	7.12
7.45	7.37	7.44	7.42
7.65	7.36	7.36	7.45
7.71	7.56	7.64	7.63
7.65	7.72	7.66	7.67
6.96	6.94	6.96	6.95
7.53	7.39	7.39	7.43
7.54	7.51	7.56	7.53
7.06	7.02	7.04	7.04
7.55	7.52	7.51	7.52
6.83	6.79	6.71	6.77
7.39	7.62	7.31	7.44
7.78	7.77	7.74	7.76
	MEAN		7.39

Table A.4a: Soil pH for Old Rows: Soil 4

1	2	3	Mean
6.95	6.97	7.03	6.98
7.61	7.57	7.60	7.59
7.29	7.21	7.26	7.25
7.48	7.38	7.35	7.40
7.15	7.05	7.04	7.08
7.43	7.39	7.36	7.39
7.52	7.49	7.56	7.52
7.21	7.15	7.13	7.16
6.96	6.89	6.81	6.88
7.35	7.33	7.14	7.27
7.14	7.13	7.13	7.13
7.40	7.23	7.26	7.29
7.05	7.97	7.01	7.04
7.04	6.98	7.02	7.01
	MEAN		7.21

Table A.4b: Soil pH for Old Alleyways: Soil 4

Shoot Height For Soils 1 - 4 with Various Fertilizer and Sterilization Treatments

	Non Sterile	Sterile
Control	8.25 a*	14.13 a,b
11-55-0	17.00 b,c	21.88 b,c
34-0-0	14.50 a,b,c	23.67 c
0-45-0	8.33 a	17.50 b,c

Table A.5: Mean Shoot Height (cm): Soil 1

	Non Sterile	Sterile
Control	8.53 a,b	13.54 a,b,c
11-55-0	15.83 b,c	19.67 c
34-0-0	14.00 a,b,c	18.08 c
0-45-0	7.08 a	14.45 a,b,c

Table A.6: Mean Shoot Height (cm): Soil 2

	Non Sterile	Sterile
Control	20.28 a,b*	21.00 a,b
11-55-0	32.83 b	30.08 b
34-0-0	29.58 b	31.83 b
0-45-0	12.75 a	25.75 b

Table A.7: Mean Shoot Height (cm): Soil 3

	Non Sterile	Sterile
Control	8.53 a*	13.54 a
11-55-0	15.83 a	19.67 a
34-0-0	14.00 a	18.08 a
0-45-0	7.08 a	14.45 a

Table A.8: Mean Shoot Height (cm): Soil 4

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Root Dry Weight for Soil 1 and 2 with

Various Fertilizer and Sterilization Treatments

	Non Sterile	Sterile
Control	0.160 a*	0.230 a
11-55-0	0.200 a	0.268 a
34-0-0	0.190 a	0.217 a
0-45-0	0.413 a	0.326 a

Table A.9: Mean Root Dry Weight (gm): Soil 1

	Non Sterile	Sterile
Control	0.218 a*	0.356 a
11-55-0	0.315 a	0.223 a
34-0-0	0.227 a	0.367 a
0-45-0	0.413 a	0.400 a

Table A.10: Mean Root Dry Weight(gm): Soil 2

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Mycorrhizal Colonization in Soil 1 and 2

After Various Fertilizer and Sterilization Treatments

	Non Sterile	Sterile
Control	42.60 b*	0.00 a
11-55-0	5.66 a	0.00 a
34-0-0	32.25 b	0.00 a
0-45-0	33.78 b	0.00 a

Table A.11: Percent Mycorrhizal Colonization: Soil 1

	Non Sterile	Sterile
Control	52.53 c*	0.00 a
11-55-0	0.55 a	0.00 a
34-0-0	33.17 b	0.00 a
0-45-0	37.95 b	0.00 a

Table A.12: Percent Mycorrhizal Colonization: Soil 2

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Shoot Height After Soil Sterilization and Adding VAM Fungi and Fertilizer

	Non Sterile	Sterile
GC	10.8 a*	17.0 a,b
GV	10.5 a	14.2 a
GI	15.3 a,b	13.3 a
11-55-0	22.5 b,c	27.1 c
CT	11.6 a	16.4 a,b

Table A.13: Mean Shoot Height (cm): Soil 2

	Non Pasteurized	Pasteurized
GC	10.1 a,b	12.2 a,b,c
GV	10.9 a,b,c	21.3 d
GI	10.1 a,b,c	16.8 c,d
OR	8.0 a	16.1 b,c,d
11-55-0	7.7 a	30.8 e
CT	9.1 a	16.2 b,c,d

Table A.14: Mean Shoot Height (cm): Soil 5

* same letter indicates no significant difference ($\alpha = 0.05$ Tukey's (HSD) test)

Shoot Dry Weight After Soil Sterilization and Adding VAM Fungi and Fertilizer

	Non Sterile	Sterile
GC	0.403 a*	1.137 b
GV	0.275 a	1.106 b
GI	0.688 a,b	0.763 a,b
11-55-0	2.098 c	2.153 c
CT	0.574 a,b	1.140 b

Table A.15: Mean Shoot Dry Weight (gm): Soil 2

	Non Pasteurized	Pasteurized
GC	0.619 a*	1.220 b,c,d
GV	0.817 a,b,c	2.068 e,f
GI	0.718 a,b	1.500 d,e
OR	0.542 a	1.295 b,c,d
11-55-0	0.355 a	2.467 f
CT	0.603 a	1.366 c,d

Table A.16: Mean Shoot Dry Weight (gm): Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Root Dry Weight After Soil Sterilization and Adding VAM Fungi and Fertilizer

	Non Sterile	Sterile
GC	0.113 a,b,c*	0.240 b,c,d
GV	0.104 a,b	0.332 d
GI	0.082 a	0.235 b,c,d
11-55-0	0.241 b,c,d	0.157 a,b,c
CT	0.137 a,b,c	0.259 c,d

Table A.17: Mean Root Weight (gm): Soil 2

	Non Pasteurized	Pasteurized
GC	0.064 a,b*	0.200 b,c,d
GV	0.053 a,b	0.303 d
GI	0.048 a,b	0.299 d
OR	0.035 a	0.260 c,d
11-55-0	0.022 a	0.132 a,b,c
CT	0.051 a,b	0.123 a,b,c

Table A.18: Mean Root Dry Weight (gm): Soil 5

* same letter indicates no significant difference (α = 0.05 Tukey's (HSD) test)

Mycorrhizal Colonization After Soil Sterilization and Adding VAM Fungi and Fertilizer

	Non Sterile	Sterile
GC	46.70 b,c*	48.93 b,c
GV	48.20 b,c	68.03 c
GI	60.07 c	66.68 c
11-55-0	13.38 a,b	0.00 a
CT	59.35 c	0.00 a

Table A.19: Percent Mycorrhizal Colonization: Soil 2

	Non Pasteurized	Pasteurized
GC	32.68 b,c	46.10 b,c,d
GV	21.80 a,b,c	38.47 b,c,d
GI	18.32 a,b	61.52 d
OR	0.55 a	48.13 c,d
11-55-0	0.00 a	0.00 a
CT	3.00a	0.00 a

Table A.20: Percent Mycorrhizal Colonization: Soil 5