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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 9/20/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, Room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 4 088
Miscellaneous

Attachment

PN AAB-[illegible]

Fruit Experiment Station for the Caribbean, Ltd.

Lascelles, St. James
Barbados, West Indies

(809) 432-616[illegible]
TELEX: 2407 "CORAL"

Progress Report

New Techniques for enchanced citrus production in Barbados

and

the Eastern Caribbean

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Project no: 936-5542

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Prepared by

Stan Michelini

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Container experiment

Results were tabulated and analyzed, giving significant differences in plant height and stem caliper for manured treatment. Inoculation tended to produce more growth than treatments without inoculation, the differences being significant in some instances. A full report will be presented in the future.

After measuring the plants, they were pruned to a single stem and grown until a majority of the seedlings were 4 to 6 mm. at grafting height. Seedlings were grafted using Citrus grandis (red pummelo) budwood from the best of the local selections. It is the largest of the edible citrus, possesses a deep red flesh (more intense than Ruby Red grapefruit), and is seedless when grown in solid blocks. All grafted trees were watered, fertilized, and weeded, having been grown in anticipation of planting out.

Island training

Several trips were made to the surrounding islands to liason with the technicians and to help then manage their work and to solve problems. Trips were made to Dominica in January, and March; a trip to St. Lucia was undertaken in March: trips were made to Grenada in December, January and March.

Dominica

On the first trip sites previously sampled, were re-sampled to monitor possible changes in infection data. The technician chosen by the Ministry of Agriculture, Mr. Peter Hill, was assisted with his trap cropping. I inspected his prepared bags, showed him how to inoculate using roots pieces and spores. We inoculated some potted bags and discussed the future plans for the experiments in Dominica. I left Mr. Hill a list of necessities and options. Necessities were the collection of roots, labeling pots with the origin of the root material and / or spores, and timetables for reading root infection and spore collecting. Options included a small seed bed experiment (they sometimes fumigate their seedbeds) and small nursery experiment. The trap crop pots were started but the options were not pursued. The second trip was undertaken from March 5, 1986, by both Dr. Chinnery and myself. We arrived and proceeded to the botanical lab at the gardens. While there we went over the material that Mr. Hill had collected in according to our advance instruction. The wet sieving of immature trap crops

produced spores with sugar gradient technique which were identified as to genus (Glomus), but sufficient spores were not found to attempt species identification. In the afternoon, we took Mr. Hill with us to show him our collection sites, and for him to show us his collection sites. Before leaving Mr. Hill, we discussed the upcoming workshop and his attendance. Later phone calls to Mr. Hill secured the rainfall data we requested to factor that information into our island survey data.

We took samples at all sites visited, which did not (due to time contraints) include all our original sites. Each sample included soil for wet sieving of spores and roots to assay for infection. The root samples were processed by submerging in alcohol and taken with us to the laboratory in Barbados.

Infection data was similar to previous scores although slightly lower than the original samples.

St. Lucia

The one trip was made following our March, Dominica trip. Equipted with sieves and slides, we arrived and proceeded to the laboratory at Union Station. We were joined by Ms. Joan John and Ms. Jania Jean Baptiste, technicians in the Ministry of Agriculture. We wet sieved soil samples they had collected and attempted the sugar gradient technique, but were unable to demonstrate it due to the lack of a working centrifuge, which was obtained subsequent to our departure and is available for future use. There was too much particulate interference to discern more than a few spores. The afternoon was again spent sampling old sites for changes in infection data. We collected root samples for both trap cropping and for assaying root infection. The assays were again made in Barbados, Roots infection was similar to previous analyses in St. Lucia.

We discussed the upcoming workshop with the two technicians and asked that they bring more root samples with them for us to read.

The trap crops were growing in St. Lucia and when we wet sieved to the trap crop medium, we were able to find some Glomus spores. The crops were still young. the sorghum had not yet flowered, and the total spore population was still small.

We left information with them to study, encouraged more trap crops and prepared them for the workshop.

Grenada

The December trip was made together with some more samples for reading. Contact was made with the Ministry to confirm the appointment of Mr. Cox as the technician to be involved with the project. Meeting with Mr. Cox, I discussed the chief goals of the project, I asked Mr. Cox if he could start trap cropping, explained our proceedures and listened to him suggest what he needed to implement to the plan.

On the second trip, I collected more root samples. a portion of each were given to Mr. Cox for him to put in the sterilized media, and the remainder taken to Barbados labs for reading.

The third trip was a repeat of the second, with collections and inoculations of media with root samples. The root data will be reported for two of the samplings as one sample was lost during the transfer from Grenada to Barbados.

Analysis of data obtained showed results similar to original collection.

The trap crops are being grown in the other islands to acquaint the technicians with methods of collecting, propagating, and identification of the VAM found on their island. It is not possible to bring live citrus material into Barbados without difficulty so it was decided to do the cropping in each island. When they have propagated large numbers of spores, we will either send Dr. Chinnery to ID the spores or have the technicians bring them to our September seminar. to be given by Dr. Nemec, Dr. Chinnery and myself.

Trip to Orlando, Florida

In May, I went to an Orlando meeting with Dr. Nemec during which we covered many aspects of the project. We spent most of one day and the morning of another in discussions. The topics included the current status of work throughout the islands, motivational factors to encourage collaborators to produce work with useable results, and our results of previous work.

Specifically we discussed the manuscript I am re-working on the seedbed experiment, including the additions and corrections suggested by reviewers. We discussed the back-

ground papers for the island survey paper, (exchanged copies of articles), statistical analysis of the relevant data, implications of the results, and organization of the paper. We looked over a prepared abstract and Dr. Nemec suggested some changes in wording. We discussed the results of the nursery experiment, and began to look at results for correlations. We designed the plots for the next stage of the project - planting of the nursery trees for long term monitoring of performance.

We discussed the technicians working on the project on the other islands. It was reported that the trap crops should be ready for Dr. Nemec's September visit. We discussed the plans for the September visit, and perused the June workshop schedule. Three days were spent in the library researching related articles.

The workshop

Invitations were mailed well in advance to all potential participants. We received prompt and positive responses from Grenada and St. Lucia, and delayed apology for Mr. Hill not being able to attend from Dominica. We decided to invite two technicians from St. Lucia, because they had been working in the lab together, were both qualified, and seemed to work best as backups for each other.

In Barbados, invitations were sent to all the agricultural organizations and government agricultural officers. Over twenty (20) people attended the opening and subsequent technical programme, including Mr. Cox from Grenada. Ms. John and Ms. Baptiste from St. Lucia, Mr. Thomas and Bisphomb from the Barbados Ministry of Agriculture (BMAFCA) Mr. Barker nursery manager for the BMAFCA nurseries from the BMAFCA, Mr. De Boer from the Barbados Sugar Technology Research Unit.

Mr. Harrington, USAID, Dr. Dales U.W.I. genetic microbiologist John Philips, the originator of the Phillips and Hayman techniques of clearing and staining, several UWI technicians and students, project collaborates, and representatives of the press.

The workshop included presentations of information on mycorrhizae, training in techniques of collection, preparation of root samples, spore collection, spore production, and results of the project work.

Nursery experiment

Statistical analysis of the sixteen (16) different treatments, gave the following results:

6

(1) Fumigated, plus manure gave significantly greater height and caliper, over non-manured.

(2) Both Glomus mosseae and Glomus interadices showed no significant differences when compared with Fumigated, non-inoculated treatments, but did show significant interaction when combined with manure.

(3) Non fumigated plus manure resulted in significantly better growth than non-fumigated without manure, both in height and caliper.

Island survey statistics

A regression analysis was performed on the interaction between infection and environmental factors such as soil nutrients elevation, rainfall, and tree age. Significant Correlations were made with several factors, on each island and with the islands taken as a group. Positive correlations were shown between infection data and tree age, altitude, and manganese. Negative correlations were shown with the rise in pH and soil iron, sulfur, zinc, boron and calcium. A more detailed report will be made in the form of a paper to be orally presented in August.

Next 6 months

We will continue to analise the data collected and to prepare manuscripts containing the information. The trap crops will be mature and the spores should give us species identification on Barbados and the other participating islands.

320 grafted trees will be planted out, twenty (20) from each nursery experiment treatment will be planted in a random block design for long term observation.

A seminar will be held in September, hosted by FRESCA, and UWI, and headed by Dr. Nemec. The same participants will be expected to attend. We will be expecting the island technicians to bring spores from their trap crops and to report on their successes and problems.

It is expected that the programmes on the islands of St. Dominica, Grenada, and St. Lucia will be expanded to include local systematic surveys of local material, propagation of same, and the introduction into the nursery and or science education programmes. Further investigations may tell us why their are dramatic differences in the appearance, health and productivity

of citr the neighboring and apparently similarly evolved soils. ost important differences have been shown to occur between olcanic islands and Barbados, the only coral island in the survey. We hope to pinpoint the cause for the differences.

4.088

Factors affecting mycorrhizal infection of citrus on four islands in the eastern Caribbean

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Received _____; revised _____

Citrus root and soil samples were collected from the Caribbean Islands of Barbados, Dominica, Grenada, and St. Lucia. The roots were examined for the presence of vesicular-arbuscular mycorrhizal (VAM) fungi, and the soil samples were analyzed to determine pH, percentage of organic matter, Ca, Mg, K, N, P, S, B, Cu, Fe, Mn, and Zn. The age of the tree samples, the altitude of each site and its annual rainfall were also recorded. Significant correlations were found between measures of VAM infection and annual rainfall, altitude, soil pH, B, Ca, Cu, Fe, P, and S at the sites on the four islands. Multiple regression equations were determined to predict levels of infection on the basis of site characteristics. Plant health and degree of VAM fungal infection were related, with the healthiest plants being most highly infected.

Keywords: Vesicular-arbuscular mycorrhizae; pH, Calcium, Boron, Rainfall

The mutualistic symbiosis between vesicular-arbuscular mycorrhizal (VAM) fungi and the roots of higher plants has been widely reported (Mosse, 1972; Gianinazzi-Pearson and Diem, 1982; Hayman, 1982; Fitter, 1985). The role of

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VAM fungi in improving plant growth has been attributed to enhanced nutrient uptake and water utilization (Gerdemann, 1968; Safir, et al., 1971b; Timmer and Leyden, 1978; Hardie and Leyton, 1981; Nelsen and Safir, 1982). Occurrence of VAM fungi in soil and their development in roots are often associated with fertility levels in soils (Mosse, 1973; Hayman, 1975; Janos, 1980). High levels of soil phosphorus (Menge, et al., 1978b; Nemec, 1987) and nitrogen (Redhead, 1975; Chambers, et al., 1980) can depress mycorrhizal development, and low soil fertility can increase infection in roots and sporulation in soil (Johnston, 1949; Menge, et al., 1978a; Hayman, 1982). Brazilian soils low in Ca, P, S, N, and Zn were associated with increased VAM fungus infection (Thomazini, 1974). In Australia, Zn deficiency was corrected, Cu and P uptake improved, and the field need for P reduced by an estimated 50% for mycorrhizal as compared to nonmycorrhizal plants (Abbot and Robson, 1982). Improved P uptake and improved growth in low-P soils are frequently cited as major benefits afforded VAM-infected plants (Koucheki and Read, 1976; Janos, 1980; Dodd, et al., 1983), including Citrus (Kleinschmidt and Gerdemann, 1972; Menge, et al., 1978a; Timmer and Leyden, 1978; Nemec and O'Bannon, 1979; Michelini and Nemec, 1987). In addition to directly affecting nutrient availability to the host plant, pH can exert influence on mycorrhizal infection, because some VAM fungus species are pH sensitive (Kruckleman, 1975; Read, et al., 1976); Nemec, 1987. Although lower infection rates have been reported with low pH (Hayman and Tavares, 1985), other workers have found no or variable pH effects (Mosse, 1972; Graw, 1979; Skipper and Smith, 1979; Abbot and Robson, 1982).

VAM fungus-infected plants have improved water transport which provides them with some protection against drought stress (Safir et al., 1971a; Hardie and Leyton, 1981; Levy, et al., 1983) and, under conditions of drought stress, mineral uptake can be maintained (Nelsen and Safir, 1982; Hayman, 1983). Despite these reports of improved drought tolerance, Redhead (1975) found that VAM fungus infection was lower under naturally low rainfall conditions than under conditions of adequate rainfall. Investigations at high rainfall locations showed that mycorrhizal plants outgrew noninfected plants (Janos, 1975; Janos, 1980); however, waterlogged soils are known to depress VAM fungus infection (Redhead, 1975) and can be devoid of these fungi (Khan, 1974).

This study was conducted to determine the extent of VAM fungus development in bearing Citrus trees on four islands in the eastern Caribbean where citrus is grown over a wide range of climatic, cultural, and soil conditions. The studies were conducted on three geologically related islands -- Grenada, St. Lucia and Dominica -- which are part of a volcanic arc situated to the north of the Venezuelan tip of South America, and in Barbados, a predominantly coral island lying some 150 km to the east of the chain. Although rainfall is seasonally heavy in the eastern Caribbean, the occasionally extended dry season in combination with constant warmth and average daily wind speeds in excess of 15 km hr^{-1} can lead to extended drought stress in many crops grown on the islands. During their agricultural development, the Caribbean islands became specialized in crop production and this was determined by climatic and edaphic differences intrinsic to the islands. Thus, Barbados inhabitants grew sugarcane; Grenada, cacao and spices; St. Lucia, bananas and mangoes; and Dominica, citrus. On all of the islands, citrus is grown in small plantings ranging from a few trees at each site to 10 acres (4 ha). Citrus growth, yield, and overall appearance are better in

Dominica than on the other islands, most noticeably when compared with Barbados.

Infection parameters were correlated with site characteristics, including pH, rainfall, elevation, and soil nutrient status, to determine which were most limiting to infection and those which expressed optimum development of infection in roots.

Methods

Plantings were sampled on each island to encompass elevation and rainfall extremes and a variety of soil types. In each planting, one to three trees were sampled for healthy fibrous roots and soil in the root zone to a depth of 15 cm.

In May 1984, soil and roots from 21 trees were obtained in 13 Barbados plantings ranging in elevation from sea level to 305 m and rainfall from 800 to 2000 mm per year, and 17 trees were sampled from nine plantings in St. Lucia with elevations from 30 to 520 m and rainfall from 1280 to 2400 mm per year. The following December, seven trees from seven plantings in Grenada at elevations from 30 to 305 m and annual rainfall from 2300 to 2700 mm, and 16 trees from eight Dominica plantings ranging from 30 to 700 m in elevation and with an annual rainfall of 1970 to 5120 mm were sampled. Irrigation was available in six of the 13 Barbados sites, but was not present in the plantings on the three volcanic islands.

Elevations above sea level, accurate to ± 15 m, were obtained from contour maps of each island. Rainfall data from 1984 records were taken from the weather stations closest to each of the collection sites. At the time of sampling, tree age was estimated and recorded. Information on rootstock type was obtained by examining root sprouts and trunk appearance or from Ministry of Agriculture personnel and nurserymen. Interviews with these personnel

confirmed that Seville sour orange (Citrus aurantium L.) was the principal rootstock used in these island plantings.

Roots from each tree were separated from the soil, washed in water, fixed in 35% formalin, acetic acid, 95% ethanol (FAA), and sent to the U.S. Horticultural Research Laboratory in Orlando, Florida, for analysis of infection. Roots were cut into 1-cm-long pieces and cleared and stained according to the Phillips and Hayman (1970) method. One hundred root pieces per tree were examined for vesicle number, relative hyphal development, and scored for percentage of infection. Vesicles and hyphae were rated on a scale of 0 to 3, where 0 = no vesicles and absence of hyphae, 1 = 1 to 50 vesicles and light hyphal development, 2 = 51 to 100 vesicles and moderate hyphal development, and 3 = over 100 vesicles and extensive hyphal development.

Soil samples were bagged and sent to Agro Services International, Inc., Orange City, Florida, for an S-1 analysis of 11 mineral elements, pH, and organic matter. A 0.25 M $NaHCO_3$, 0.01 M EDTA and 0.01 M NH_4F extract was analyzed for P, K, Cu, Fe, Mn and Zn; N, Ca, and Mg were determined on a 1M - KCl extract, and S and B were analyzed using a $CaH_4(PO_4)_2$ extract.

Soil samples collected at the sites in each island were wet-sieved to obtain VAM fungal spores for determining identity of genera and, possibly, species present around roots.

The data for the islands were compared for VAM status, edaphic parameters, altitude and annual rainfall of the sample sites by analysis of variance and any significant differences elucidated by Tukey's H.S.D. (Zar, 1984). Simple regressions of the three measures of fungal infection and the islands' soil nutrients, pH, altitude, rainfall, and tree age were computed. All islands were considered singly and as a group for these analyses. Additionally, stepwise multiple regression analyses using all nutritional and

environmental data from the combined islands were performed to determine the equations which best predicted the effect of soil and environmental conditions on VAM infection.

Results

The roots of all the Citrus trees sampled were infected by VAM fungi. The infection data (Table 1) show differences between Barbados and the volcanic islands. The mean hyphal rating for Barbados was significantly lower than those for Dominica ($P < 0.001$), Grenada ($P < 0.001$), and St. Lucia ($P < 0.05$). Among the volcanic islands, St. Lucia's mean hyphal rating was significantly lower than Dominica's ($P < 0.001$) and Grenada's ($P < 0.05$). The roots of trees sampled in Barbados had a significantly lower percentage of infection than those from Dominica ($P < 0.001$), Grenada ($P < 0.01$), and St. Lucia ($P < 0.01$). There were no significant differences in mean vesicle ratings between the islands. No structures of the fungus present in the roots were distinctive enough to make species identifications of the VAM fungi, i.e. no chlamydospores were present. Almost all the spores found in the sample sites were identified as Glomus, but some Sclerocystis sporocarps and a few Gigaspora azygospores were present in samples from the other islands. Due to very low spore populations and their high senescent condition, identification to species was not feasible.

The environmental and soil characteristics of the sites sampled are summarized in Table 2. There were very few significant environmental or soil differences found among the three volcanic islands. Mean annual rainfall at the Dominican sites was greater than for those in Grenada ($P < 0.01$) or St. Lucia ($P < 0.001$), and soil Mg levels at the sites in St. Lucia were higher than those in Dominica ($P < 0.05$). Most of the significant differences between islands involved Barbados. This island had a significantly higher soil pH

than Dominica ($P < 0.001$), Grenada ($P < 0.05$), and St. Lucia ($P < 0.001$), which is related to the higher levels of soil Ca found in Barbados compared with each of the other islands ($P < 0.001$). Soil S levels were significantly higher in Barbados than Dominica ($P < 0.05$), and the mean level of soil B was higher in Barbados than in Dominica ($P < 0.001$), Grenada ($P < 0.01$) or St. Lucia ($P < 0.01$). Barbados also had lower levels of soil Fe than those recorded for Dominica ($P < 0.05$), Grenada ($P < 0.05$) and St. Lucia ($P < 0.001$). Mean annual rainfall at the Citrus sites was lower in Barbados than in any of the other islands ($P < 0.001$).

The significant relationships between infection data and the 16 soil and environmental factors from the sites on the four islands are presented in Table 3. Considering the combined data from the islands, significant positive correlations were evident with respect to vesicle ratings and increases in altitude, rainfall and Cu, while an inverse correlation was found with increased soil P. Hyphal ratings were positively correlated with increases in rainfall and altitude, and inversely correlated with increases in soil Ca, S, B, and pH levels. Percentage of infection data exhibited the same correlations found by hyphal ratings and a positive correlation with soil Fe. Precipitation showed stronger relationships with regard to percentage of infection ($P = 0.0004$) and hyphal ratings ($P = 0.0001$) than vesicle scores ($P = 0.0413$). Conversely, altitude had its greatest effect on vesicle scores ($P = 0.0019$) as compared with percentage of infection ($P = 0.0229$) and hyphae ($P = 0.0306$). There were no significant correlations between the three measures of infection and tree age, soil organic matter, magnesium, potassium, nitrogen, manganese or zinc.

Significant relationships between infection data and environmental and edaphic factors varied between islands and involved Mg, Zn, Cu, Fe, and altitude (Table 4). The best equations from stepwise multiple regression analysis are given in Table 5.

Discussion

The significant differences found between Barbados and the other islands in soil Ca and pH result from the soils in Barbados being mainly coral derived, whereas those of Dominica, Grenada, and St. Lucia are of volcanic origin. These soil differences, accompanied by significantly lower rainfall, may have contributed to the overall lower percentage of infection and presence of hyphae in the Barbados root samples. Elmes and Mosse (1984) found reduced VAM fungus infection in Zea mays, grown by a nutrient film technique, when the Ca level exceeded 150 mg L^{-1}. They concluded that this was due to changes in concentration of P in their solution, but this does not exclude the possibility of a direct Ca effect on VAM infection.

Precipitation had a marked effect on the presence of fungal structures in the roots, and was one of only two factors found to affect all three infection measurements (Table 3). Without adequate water, the soil in Barbados shrinks in volume from 10 to 15% (J.C. Hudson, personal communication) creating physical stress and root damage. Therefore, it is possible that the 5-month dry season which preceded sampling in Barbados affected the root condition and may have had a deleterious effect on infection and establishment of VAM fungi in the roots. In Kenya, Newman, et al. (1986) found a reduction of percentage and intensity of infection in the roots of three species of savanna grass during the dry season, which they attributed to the extended drought. A similar reduction in both percentage and intensity of VAM fungal infection was found in the roots of sugarcane immediately following the rainy season in

Barbados (Chinnery, et al., 1987). In Italian sand dunes, Giovannetti (1985) found reduced VAM fungus infection of Helichrysum stoechas (L.) Moench, Ammophila arenaria (L.) Link and Eryngium maritimum L. during the summer drought period. Newman, et al. (1986) also suggested that the increased VAM infection levels found at the start of the wet season resulted from a flush of nutrient release from accumulated leaf litter following moisture-induced decomposition (Swift, et al., 1981; Newman, et al., 1986).

The significant correlations between increasing rainfall and increased values of the three measures of VAM infection indicate the importance of adequate moisture in root growth and VAM infection. While VAM fungal presence in the roots was related to high rainfall, roots in some poorly drained soils in Grenada and St. Lucia were decomposed. Conversely, the internally well-drained Dominican soils yielded healthy, non-necrotic roots even from areas receiving an annual rainfall in excess of 5000 mm.

Throughout the data, vesicle occurrence was less variable than hyphal density. The similarity in vesicle ratings between islands may reflect the occurrence of different VAM species, e. g. Gigaspora spp. do not produce vesicles or differences in fungal growth stages (Bonfante-Fasolo, 1984). However, high rainfall is associated with increased elevation, with the combination resulting in higher hyphal and vesicle ratings. The greater association of vesicles with altitude indicates a possible temperature interaction with vesicle formation.

While soil pH has been shown to affect VAM species composition, the pH range found in soils in Barbados is well within levels tolerated by VAM fungi (Graw, 1979; Skipper and Smith, 1979; Hayman and Tavares, 1985; Nemec, 1987). Boron is reportedly toxic to Citrus at levels as low as 0.5 ppm in the soil solution (Kelley and Brown, 1928 cited by Chapman, 1968; Gupta, et al., 1985).

In the soils sampled from several of the Barbados plantings, B exceeded this level. While absorption of B is reduced by high soil Ca, B is known to accumulate under low rainfall conditions. If rhizosphere B levels were toxic to roots in these plantings, it is possible that the VAM fungi are affected via reduced plant growth.

The increase in VAM vesicle numbers with increasing soil Cu reflects the insensitivity of VAM to Cu levels. The decrease in vesicle number with increasing P agrees with many previous investigations (Gianinazzi-Pearson and Diem, 1982; Hayman, 1983; Nemec, 1987). The lower Fe concentrations in Barbados are pH related, since at high pH insoluble Fe compounds are formed. The higher S values found in Barbados soils as compared with those levels present in the volcanic soils probably reflect greater fertilizer use, rather than intrinsically higher soil S levels.

The use of single-factor linear regressions can often lead to misleading conclusions in this type of study, because significant correlation between a VAM infection measure and one of the site parameters could be a result of two or more other factors influencing the relationship. The literature documents many complex interactions between mineral elements in plant nutrition, e. g. Taper and Leach (1957) demonstrated a Ca/Fe/Mn interaction and Chinnery and Harding 1980 a Fe/Mn/P interaction, both of which are also affected by pH.

Menge, et al. (1982) used multiple regression and a derivative, multifactor reciprocal response equations, to predict dependency of Troyer citrange (Poncirus trifoliata (L.) Raf. X Citrus sinensis (L.) Osbeck) on the VAM fungus, Glomus deserticola Trappe, Bloes and Menge (ex. G. fasciculatus (Thaxter) Gerd. and Trappe), in California citrus soils and nursery mixes. Their best equations involved extractable soil P (bicarbonate), Zn, Mn, Cu, and pH.

The multiple regression equation (Table 5) predicting percentage of VAM infection for the eastern Caribbean islands exhibits an inverse relationship with soil Ca, which was significantly higher in Barbados where infection was significantly lower, and soil P as expected from the literature (e.g. Gerdemann, 1968; Hayman, 1975; Menge, et al. 1978b; Hardie and Leyton, 1981; Fitter, 1985). The single factor regressions (Table 3) found percentage of infection to be significantly related to seven of the 16 site characteristics, including soil Ca but excluding soil P (P = 0.0806). A similar multiple regression equation was found for hyphal ratings across the island, but this also includes direct dependence on rainfall, which was the most significant independent variable in the simple regressions. The equation predicting vesicle ratings is very different from those predicting the other two infection measures, because it is related to the lack of inter-island differences in vesicle ratings. In this case, soil P was significant by itself, but is not included in the multiple regression equation, whereas pH is included despite not being significant in the single factor analysis (P = 0.4954). However, pH was significant by itself for both percentage of infection and hyphal ratings, yet not included in their multiple regressions.

The differences between the results of the simple and multiple regression analyses are a consequence of interactions between the factors measured. For hyphal ratings and percentage of infection, increases in both soil pH and Ca are seen to reduce levels of infection, yet only Ca is found in their multiple regressions. This is a result of soil Ca levels being the main cause of the observed differences in soil pH. Likewise, rainfall and altitude are both significantly correlated with vesicle and hyphal ratings, but only one of the pair occurs in each of the multiple regression equations, a result of altitude and rainfall being highly correlated on the islands. In each of these cases, multiple regression enables the most important of the pair to be detected.

This study has confirmed the findings in the literature that VAM infection is influenced by a range of environmental conditions. On the four islands, percentage of infection was shown to increase with increased altitude and rainfall and to decrease with increases in pH and soil Ca. Soil Ca and P levels alone predict 46.3% of the variation in percentage of infection.

Acknowledgments

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Table 1 Means (and standard errors) for three measures of vesicular-arbuscular mycorrhizal infection in *Citrus* roots collected from four islands in the eastern Caribbean, and for all samples combined.[1]

VAM fungus parameters rated[2]	Eastern Caribbean Islands				
	Barbados	Dominica	Grenada	St. Lucia	Combined
Vesicle Rating	0.22 a (0.04)	0.24 a (0.14)	0.49 a (0.13)	0.41 a (0.07)	0.32 (0.04)
Hyphal Rating	0.48 a (0.08)	2.02 b (0.16)	1.64 b (0.32)	0.94 c (0.13)	1.02 (0.10)
Percentage Infection	37.3 a (4.6)	93.25 b (6.2)	73.6 b (12.8)	67.8 b (5.9)	60.3 (4.2)
No. of trees sampled	21	16	7	17	61

[1]For each parameter, means in the same row followed by the same letter are not significantly different ($P > 0.05$) by Tukey's multiple range test.

[2]Vesicles and hyphae rated on a scale of 0-3, where 0 = no vesicles and hyphae, 1 = 1-50 vesicles and light hyphal development, 2 = 51-100 vesicles and moderate hyphal development, 3 = >100 vesicles and extensive hyphal development. One hundred 1-cm-long root pieces were rated for each tree.

Table 2. Environmental and soil characteristics of *Citrus* sites on four eastern Caribbean Islands.[1]

Environmental and soil parameters	Eastern Caribbean Islands				
	Barbados	Dominca	Grenada	St. Lucia	Combined
Rainfall (mm 1984)	1387 (67)	3436 b (414)	2439 c (67)	2178 c (113)	2089 (122)
Altitude (m)	133 a (20)	237 a,b (91)	224 a,b (38)	276 b (42)	207 (22)
pH	7.1 a (0.1)	5.2 b (0.3)	6.0 b (0.4)	5.6 b (0.2)	6.2 (0.2)
Organic matter (%)	2.6 a (0.3)	2.4 a (0.5)	1.7 a (0.2)	2.4 a (0.3)	2.4 (0.2)
Calcium (meq $100ml^{-1}$)	29.7 a (2.2)	5.3 b (1.0)	11.0 b (2.4)	12.0 b (1.4)	17.9 (1.7)
Magnesium (meq $100ml^{-1}$)	4.7 a,b (0.5)	1.7 a (0.3)	3.7 a,b (0.6)	6.4 b (1.4)	4.7 (0.5)
Potassium (meq $100ml_{-1}$)	0.98 (0.17)	0.64 a (0.17)	0.48 a (0.09)	1.17 a (0.22)	0.92 (0.10)
Nitrogen ($\mu g\ ml^{-1}$)	5.4 a (0.4)	9.1 a (2.0)	6.1 a (0.3)	19.1 a (10.5)	10.45 (3.4)
Phosphorus ($\mu g\ ml^{-1}$)	48.8 a (12.9)	38.9 a (13.7)	8.0 a (3.1)	43.9 a (13.9)	40.3 (7.2)
Sulphur ($\mu g\ ml^{-1}$)	83.0 a (17.8)	15.1 b (1.9)	14.0 a,b (3.8)	51.8 a,b (15.1)	53.6 (9.3)

Table 2. Continued.

Boron	1.42 a	0.37 b	0.45 b	0.66 b	0.89
($\mu g\ ml^{-1}$)	(0.18)	(0.11)	(0.15)	(0.07)	(0.10)
Copper	8.72 a	17.83 a	7.56 a	8.09 a	9.74
($\mu g\ ml^{-1}$)	(2.19)	(7.69)	(2.11)	(1.09)	(1.53)
Iron	20.38 a	96.38 b	88.14 b	108.82 b	69.17
($\mu g\ ml^{-1}$)	(9.92)	(19.08)	(23.41)	(16.27)	(9.37)
Manganese	13.62 a	7.20 a	17.17 a	32.21 a	17.76
($\mu g\ ml^{-1}$)	(6.98)	(2.58)	(2.05)	(7.49)	(3.90)
Zinc	6.20 a	8.11 a	19.39 a	14.07 a	10.75
($\mu g\ ml^{-1}$)	(1.18)	(2.88)	(5.24)	(5.05)	(1.94)

[1]Values presented are means and (standard errors).

[2]Barbados and St. Lucia plantings sampled May 1984 and Dominica and Grenada sampled December 1984.

[3]For each parameter, means in the same row followed by the same letter are not significantly different ($P > 0.05$) by Tukey's multiple range test.

Table 3 Results of simple linear regressions relating three measures of vesicular-arbuscular mycorrhizal infection in *Citrus* roots from four islands in the eastern Caribbean and 16 site characteristics.[1]

Environmental and soil parameters	a	b	R^2	P
		Mean vesicle rating		
Annual rainfall	0.12915	0.00009	0.07731	0.0413
Altitude	0.16271	0.00076	0.17879	0.0019
Soil phosphorus	0.39218	-0.00178	0.10169	0.0188
Soil copper	0.24537	0.00770	0.08627	0.0308
		Mean hyphal rating		
Annual rainfall	-0.12211	0.00054	0.40135	0.0001
Altitude	0.72897	0.00138	0.08645	0.0306
Soil pH	2.93332	-0.31061	0.20624	0.0009
Soil calcium	1.66319	-0.03629	0.34688	0.0004
Soil sulphur	1.19126	-0.00328	0.08458	0.0325
Soil boron	1.41939	-0.45452	0.18056	0.0018

Table 3 Continued.

		Infection (%)		
Annual rainfall	17.9635	0.02027	0.34139	0.0004
Altitude	48.1634	0.05877	0.09557	0.0229
Soil pH	141.6710	-13.17465	0.22760	0.0005
Soil calcium	87.6133	-1.52880	0.37745	0.0002
Soil sulphur	67.4537	-0.13320	0.08532	0.0318
Soil boron	77.1086	-18.88539	0.19123	0.0014
Soil iron	49.0044	0.16237	0.13091	0.0077

[1]Only those cases where the dependent and independent variables are significantly correlated are presented.

Table 4 Significant regressions between three measures of vesicular-arbuscular mycorrhizal infection in *Citrus* roots and site characteristics on individual islands in the eastern Caribbean.

Island	Fungus parameter vs. site characteristic	Regression coefficient and probability	
		b	P
Barbados	Mean vesicle rating vs. soil magnesium	-0.03926	0.0458
	Percentage of infection vs. soil magnesium	-4.28586	0.0400
Dominica	Mean vesicle rating vs. soil copper	0.01694	0.0018
	Mean vesicle rating vs. soil zinc	0.04014	0.0138
	Mean hyphal rating vs. soil copper	0.01536	0.0355
Grenada	Mean hyphal rating vs. soil zinc	-0.04921	0.0300
St. Lucia	Mean vesicle rating vs. altitude	0.00079	0.0412
	Mean vesicle rating vs. soil iron	-0.00211	0.0315

Table 5 The best multiple regression lines for predicting vesicle and hyphal ratings and percentage of mycorrhizal infection across the sites sampled in the four islands.

VAM fungus parameters	Regression equation	R^2	F	d.f.	P
Vesicle ratings	Y = -0.0059 - 0.0153 Age + 0.0008 altitude + 0.0349 pH + 0.0098 Cu	0.307	5.309	4,48	0.0015
Hyphal ratings	Y = 1.0085 + 0.0003 rainfall - 0.0233 Ca - 0.0036 P	0.490	15.662	3,49	0.0001
Infection (%)	Y = 95.7150 - 1.5916 Ca - 0.0512 P	0.463	21.589	2,50	0.0001

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October 8, 1985

23 OCT 1985

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PROGRESS REPORT FOR CITRUS MYCORRHIZAE RESEARCH
(PROJECT NO. 936-5542)
APRIL THROUGH SEPTEMBER

1) Container Experiment

Flats of Florida certified sour orange and Citrus macrophylla (Cm) seedlings were sampled and results tabulated for hyphae and vesicle rating. The inoculated flats were highly infected (see Fig. 1). The uninoculated flats picked up some mycorrhizal contamination, and were treated with soil drenches of Benlate to clear up the infection. Samples taken during the transplanting showed that Benlate reduced hyphae and vesicles.

Old bagasse (the residue from sugar cane stems after processing) had been collected in May, 1984. It was mixed by "Bobcat" with mined sand of local origin and placed into one large pile. The 60% bagasse/40% sand mixture was analyzed for nutrients and found to be adequate in all 11 tested, except nitrogen (Fig. 2).

Two subpiles, each containing about 1/4 of the total media were separated; one for fumigation, the other left non-fumigated. The remainder was mixed in the ratio of 4 buckets mixed medium to one bucket of old chicken house scrapings (manure + bagasse), and separated into fumigated and non-fumigated piles. This mix gives proportions of 50% bagasse, 30% sand and 20% manure.

The Bobcat then cleared an adjoining site for the installation of the container experiment.

The media piles were hand leveled to a depth not exceeding 70 cm and watered. The manure mixture was observed to be hot and began composting. Left to settle for 2 weeks, some of the heat had dissipated. Plastic was laid over the 1 of each of the 2 "with manure" and "without manure" piles, secured in trenches to prevent gas escape, and methyl bromide introduced at the rate of 1.5 pounds per cubic yard (540 g./cubic meter). Two weeks later the plastic was removed.

The cleared site for the container experiment was further levelled by hand and plastic stretched over the area. New 4 inch concrete blocks were laid in straight rows with walkways between them. New plastic containers were transported to the site and rinsed with a chlorox solution.

Containers were filled with the appropriate media, and seedling transplants introduced so that all treatments had a similar mix of sizes. One treatment was potted at a time, with the cart used to transport containers rinsed with chlorox solution between each treatment.

Each treatment consisted of between 40 and 60 sour orange plants, equally divided into three replicates, and between 21 and 40 Cm seedlings, also equally divided. Losses of the Cm seed were due to the long delays between shipping and receiving the seed from plant quarantine, as well as post-germination mortality. However, sufficient numbers survived to give experimental validity.

All plants were watered in, with stem caliper and plant height recorded. There were only single stemmed seedlings.

The irrigation system was installed, with a fertilizer injector used to fertigate through individually supplied pressure compensating emitters rated at 4 l./hr. $\pm$ 10%. Plants were irrigated as necessary, and fertilized with KNO_3 at the rate of 3 grams/pot every 2 weeks.

Weeding was performed as necessary without using herbicides. Most weeds were coming from the perimeter, as even the non-fumigated pots had little weed growth.

Stem caliper and plant height were measured again in mid-August. Growth rates for the different treatments have been recorded, but not analyzed. At this stage, using mean growth figures, it is evident that the largest growth differences will be between the plus manure and without manure treatments.

The growth experiment will be terminated in October, with final growth measurements and mycorrhizal infection rates determined and recorded. Subsequently, trees will be grafted and set out in orchards for long-term observations on differences between mycorrhizal and non-mycorrhizal plants.

2) <u>St. Lucia Training</u>

During the last week of April, a training trip was undertaken by investigator Michelini. Contacts previously established during the soil surveys of St. Lucia and Dominica were asked to arrange for the preparation of sterilized media and to make available soil and root samples from locations around the island. The media and samples were brought to the Ministry of Agriculture Field Stations.

In St. Lucia, everything was prepared as requested. A group of 8, including the propagation unit manager, technicians, and students were present. A 45 minute presentation to introduce the concepts and experimental results of mycorrhizae work was delivered. Explantations were given regarding the importance of mycorrhizae, their distribution, and methods of determination. Emphasis was placed on practical applications and methods of insuring survival of native mycorrhizae populations.

The lab work began with the sampling of container grown citrus (using native, unsterilized soil as a major component of the medium). Soil and roots were added to 250 ml. beakers, stirred, and the liquid decanted into a series of sieves. The three sieves (#60, top; #270, middle; and #325, bottom) serve to exclude large particles and trap spores and other similar sized particles.

The spore washings were discarded, and the spore trappings examined under a compound microscope, which was used due to the unavailability of a dissecting microscope. Due to the limited field and poor lighting, we were not able to find any spores. Rather, we looked at prepared slides from Barbados, which showed stained hypha infestation and very high vesicle counts.

As an alternative to spores being introduced into autoclaved medium, we took the citrus roots from the containers, washed them, and cut them into short lengths. Roots were buried in the media and corn seed planted.

During the next visit to St. Lucia, the "trap cropped" mycorrhizal root pieces should have infected the corn roots and sporulated. Should a good population of spores be found, identification of genus and species should be possible. Dr. Chinnery, the technician recently trained in the U.S. laboratories of Dr. Nemec and Dominican Training Dr. Norm Schenk, will be present to make identifications.

Following the session in St. Lucia, Michelini travelled to Dominica where he met with Tony Sorhaindo of the Ministry of Agriculture. Mr. Sorhaindo had prepared the media as requested, and had decided to utilize the I.I.C.A. lab and their technician. The technician had experience sampling for nematodes, and was familiar with sieving procedures.

The root and soil samples were gathered from the field station. The samples were sieved as previously stated. Using a Bausch and Lomb dissecting microscope, we were able to pick out about 10 spores per sieving; this is considered a large number of spores for a field sample. Spores from at least 2 different genera were observed. Spores were washed into the containers and corn seed planted over them. A follow-up visit is scheduled for November, during which time initial identification and further trap cropping will take place.

From the large spore population, it appears that in addition to extremely high infection rates found in Dominica (93%), the VAM fungi also sporulate abundantly.

3) Barbados

Throughout this reporting period, the technician in Barbados was given samples of citrus roots and asked to locate soils and wet sieve them to locate spores. The seedbed and flat samples, as well as local citrus soil samples yielded few spores. Those that were found were stored for possible identification.

A tour was arranged to locate soil samples, not from citrus, but from plants likely to have high spore populations. Seaside grasses were sampled from 13 locations in 7 parishes. Hypha development and vesicle counts were much higher than found in any citrus root samples. From each sample, one sub-sample was cleared and stained and the other sub-sample planted in sterile media in the FRESCA greenhouse. Sorghum seed was planted over the roots. During the next reporting period, identification of the spores will be made.

4) Seedbed Experiment

Seedbed growth measurements taken in February led us to conclude that beneficial growth responses in the fumigated beds were due to inoculation with the various mycorrhizae treatments. After the collection of growth data, which showed the differences to be levelling off, and the infection data, which showed that the controls were infected by February, it was decided to stop measuring growth response but to continue to monitor mycorrhizal infection. Clearing and staining root samples indicated that the infection increased during the reporting period, with percent infection of roots, hyphae development, and vesicle formation increasing steadily with time.

The citrus trees were removed and the area planted with "Florigraze" perennial peanut. It is anticipated that heavy mycorrhizal infection will promote better Rhizobium performance, as has been stated in the literature. The perennial peanut will be planted as a ground cover in orchard situations. It has been found to fix an extra 300 pounds N per acre per year in Florida, and should prove useful in N fertilization in orchards.

5) Presentation of Results

In September, the results of the seedbed experiment were presented at the joint meeting of the Caribbean Food Crops Society and the American Society for Horticultural Science, Tropical Region, held in Trinidad and Tobago. The presentation generated much discussion. Dr. Balderston, a South American researcher, noted that after applying nematicides, bananas were showing signs of P deficiency, and he hypothesized that the nematicide killed the mycorrhizae, resulting in P deficiency in their low P soils.

Dr. Carr, an official from the host country's commercial agriculture company, Caroni, requested more information, reprints, and advice regarding Caroni's new direction away from sugar and into citrus plantings. In their nursery, their pathologist was suggesting that the soil should be fumigated, but Caroni will now proceed with more caution. They may not fumigate at all, or they may add inoculum to fumigated soil.

Dr. Carr also brought up a question regarding the use of nematicides on sugar cane, noting that some treated plots had not done as well as control plots. Noting that sugar cane is highly mycorrhizal, and that furidan is toxic to VAM, it was suggested that there may be a link between the treatment and negative growth response.

The final manuscript of the paper presented, entitled "Effects of VAM Fungal Inoculation on Sour Orange Seedlings in the Tropics" will be forwarded to USAID after going through the USDA's peer review mechanism.

6) Dr. Nemec's Visit

In July, Dr. Nemec arrived in Barbados to check on the progress of the experiments and to liaison with Dr. Chinnery and Mr. Michelini with regard to future project plans. After viewing the seedbed experiment, for which the

data had been gathered but the seedlings left in the ground, he looked at the container experiment in progress. It was apparent at that time that the manured treatments were greener than the non-manured treatments.

Dr. Nemec, Dr. Chinnery, and Mr. Michelini met at Dr. Chinnery's lab to discuss techniques for the isolation of VAM fungal spores. Noting that few of the samples were containing spores, we decided to concentrate on sampling grasses rather than citrus. Preliminary grass samples taken during the visit confirmed the presence of large spore populations from selected locations. Lab Isolation and Introduction techniques were reviewed with Dr. Chinnery and his technician.

Arrangements were made for Dr. Chinnery's upcoming U.S. training. Discussions were held regarding the USDA procedures for publishing papers. It was agreed that an oral presentation in Trinidad would not prejudice the acceptance of the paper for publication at a later date.

7. The Next Six Months

The container experiment will be terminated and relevant data collected and analyzed. Trees will then be grafted and planted in orchards for long- and short-term observations. Short-term observations will include transplant response, survival, time of fruiting, disease resistance, and drought tolerance. Long-term observations will include survival, response to diseases, overall yield, and growth.

The other islands will be visited, trap crops examined, more trap crops initiated, and updated reports on our progress will be presented. Should new genera or species be encountered, we will of course report the findings.

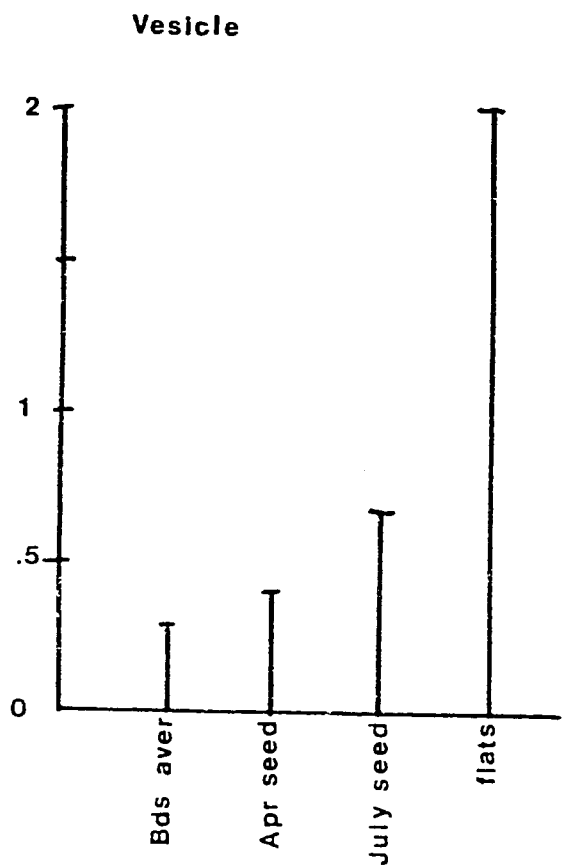
The results of the container experiment will be quantified and written in a short paper, if the results are significant. We will be looking at the health of the roots, as well as growth and infection indices.

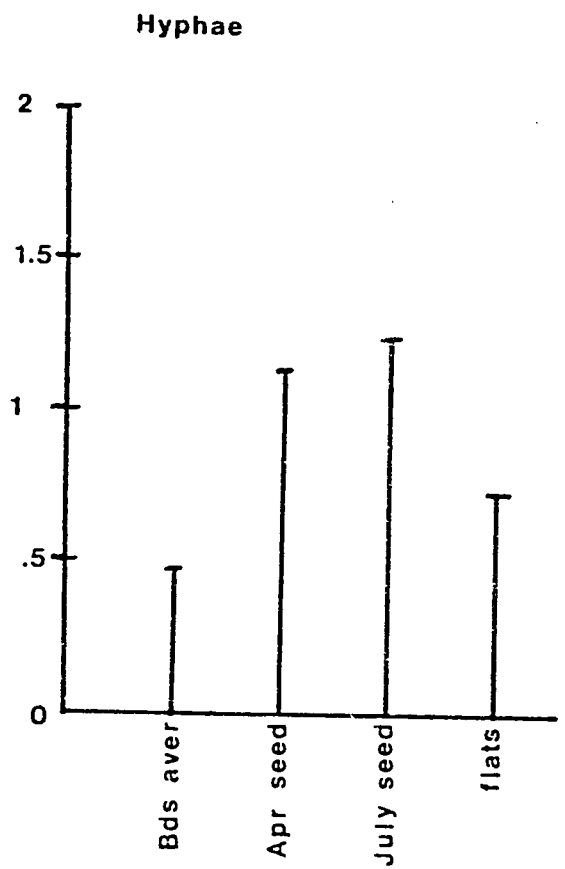
A short session will be held for Barbados' Ministry of Agriculture personnel, outlining VAM benefits and highlighting the results obtained with VAM.

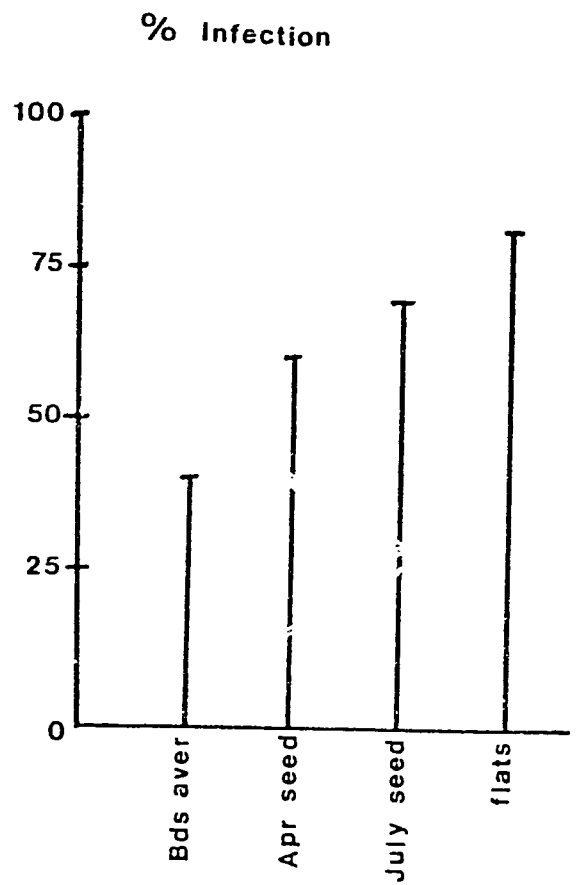
Preparations will be made for a presentation of our mycorrhizae survey results. We have been notified of the acceptance of the title by the American Society for Horticultural Science for its August 1986 meeting at U.C. Davis. Abstracts are due by February.

A follow-up visit with Dr. Nemec will be arranged to discuss the results of our work. This will be along the same pattern of Dr. Nemec's July visit; looking at the experiments, going over data, and expanding on techniques for future laboratory work. It will be particularly useful to discuss the subtle points of both experimental technique and field observations, as well as the specific direction of the research.

0723b







Comparison of Mycorrhizae (Barbados)

Figure 1

AGRO SERVICES INTERNATIONAL, INC. 215 E. Michigan Ave. P.O. Box 667 Orange City, FL

USDA Horticulture Research Lab
2120 Camden Road
Orlando, FL 32803

Stan Michelini

Sample 26

Crop to Fert. _____ Field & Sample No. S-B

Yield Goal _____ Farm Location Sand - Bogasse mix

Last Crop _____ Barbados

Approx. Yield _____ Date Sample Rec'd. _____

Lime Applied _____ Date Returned 6/15/84

Soil Condition: Act. C.E.C. 11.7 meq/100 ml; Base Satn. 100 %, Acid. Satn 0 %; pH 7.1; O.M. _____ %; Sol. Salts 225 ppm

ELEMENTS	SOIL ANALYSIS Lab No. B6 59-d	meq/100 ml	ug/ml	INTERPRETATION GUIDE (Below / Critical Level / Optimum / Above)	FERTILIZER SUGGESTIONS MIX	Lbs./acre kg/ha
Act. Acidity	A.A.	0				
Calcium	Ca	9.0			Calcium	
Magnesium	Mg	1.73			Magnesium	
Potassium	K	.92			Potash (K_2O)	
Sodium	Na					
Ca/Mg Ratio	Ca/Mg	5.2			Dolomitic Lime	
Mg/K Ratio	Mg/K	1.9			Calcitic Lime	
Nitrogen	N		3		Nitrogen	
Phosphorus	P		206		Phosphate (P_2O_5)	
Sulphur	S		192		Sulphur	
Boron	B		.60		Boron	
Copper	Cu		1.8		Copper	
Iron	Fe		135		Iron	
Manganese	Mn		51.0		Manganese	
Zinc	Zn		6.2		Zinc	
Other						

Texture:

Comments:

This report is accepted by the client under the condition that Agro Services International Inc. is responsible only for the accuracy of the analysis of the sample as received, such liability limited to the cost of the analysis. No other warranties expressed or implied, are given.