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The Introduction of Vesicular-Abruscular Mycorrhizae into Modern Crop Production Practices

J.H. Haas, J.A. Menge, J. Krikun

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The introduction of vesicular-arbuscular mycorrhizae into modern crop
production practices

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THE INTRODUCTION OF VESICULAR-ARBUSCULAR MYCORRHIZAE INTO
MODERN CROP PRODUCTION PRACTICES

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A. ABSTRACT

The project proposed to enhance and evaluate the practical utility of VAM-fungus inoculation. Prevalent Israeli spp. were determined (Glomus macrocarpum, G. mosseae, G. fasciculatum) and single-spore cultures initiated using an improved spore germinating medium. Spore types in avocado orchards in California and Israel were compared and despite the similarities in climate, different spp. were prevalent. The efficacy of several G. macrocarpum isolates, tested at standardized inoculum potential levels, varied greatly; careful inoculum testing for efficacy of isolates suitable to each climate, soil, crop, etc. will be required. Greatly improved inoculum concentrations in soil cultures were obtained under the highest light conditions and in the largest containers tested; several hosts were equally suitable for inoculum production. Field trials in large-size plots in fumigated soils gave good VAM-fungus colonization and plant growth enhancements using mechanical equipment to apply inoculum. Citrus seedlings, cotton, celery, onion and pepper responded well to inoculation when grown in low P soils or in high P-sorbing soils highly fertilized with superphosphate (3 t/ha). Growth and yield benefits in pepper were greater than those obtained by fertigation with 40 ppm P. Field inoculation requires .5-1 m³ soil inoculum/ha; no technical limitations prevent production or application of VAM-fungus inoculum in modern agriculture and growth benefits often can be realized.

B. REPORT

B.1 INTRODUCTION

In our original proposal we identified three major obstacles preventing the use of vesicular-arbuscular mycorrhiza (VAM)-fungus inoculation as a modern crop production practice: "1)reliable, high-quality mycorrhizal inoculum is not yet available in quantities necessary for large-scale field use, 2) efficient and commercially feasible inoculation methods have not been evaluated under field conditions, and 3) the contribution of VAM inoculation to crop growth and yield improvement has not been demonstrated sufficiently in field trials".

To help solve these problems, four research objectives were outlined: "1)to identify locally adapted isolates of VAM-fungi which rapidly colonize roots and stimulate plant growth, 2) to increase the production of high-quality, pathogen-free VAM inoculum, 3) to develop effective, practical field application methods for mycorrhizal inoculum, 4) to determine the nutrition- and yield-improving effects of VAM-fungus inoculations on important crop species in various soils and under modern crop production practices".

During the five years which have elapsed since this proposal was written there has been a large increase in the number of research projects and publications on the subject of VAM. Some of them also have been addressed to the above problems. Here, however, we only review our contributions to solving the problems. [Citations in the text refer to our manuscripts in list and in supplement].

B.2 MATERIALS AND METHODS

The detailed description of materials and methods is presented in the manuscripts attached to this report. We have used commonly accepted techniques in mycorrhiza, plant pathology, horticulture and agronomy research.

B.3 RESULTS

B.3.1 IMPROVING COMMERCIAL MYCORRHIZAL INOCULUM PRODUCTION

B.3.1.1 Collection and isolation of locally adapted strains of VAM-fungi

A considerable number of collections of VAM-fungi had been made in California prior to this project. However, there had been no survey of the avocado crop and since the crop is grown both in California and Israel, the opportunity was taken to begin learning about and collecting the VAM-fungi present in Israel and to investigate the similarities and differences in species present on the crop in both countries.

Soils from orchards in Israel and California, both of mediterranean climate, were sampled and analyzed for the species and quantities of VAM-fungi in them and for their physical and chemical characteristics. In Israel the most common fungi were Sclerocystis sinuosa and Glomus macrocarpum. In California, G. constrictum was present in every orchard examined and G. fasciculatum was next common; Acaulospora spp. and other Glomus spp. also were found, including A. elegans which has never before been reported from California. The differences in VAM fungus species and quantities found on two continents but in areas with similar climates and soil types may be due to host or edaphic factors; different avocado rootstocks are used in the two countries and higher soil fertility levels were present in California soils. The VAM fungus spore population in orchards in both countries averaged 2.8/ml soil. These populations were not correlated with any of the soil physical or chemical characteristics examined nor with avocado cultivar or age. In Israel, no fungi were found in 3 orchards; available P, Ca, Mg and Cu levels were high in these soils and probably reduced the fungus population to a level not detectable in the size samples collected (Haas, Menge, 1984).

Spores of Glomus and Sclerocystis species from various soils in the Negev Desert, Israel, including disturbed and undisturbed sites, were collected and described. The species found were G. mosseae (primarily), G. constrictum, G. caledonium, S. sinuosa, S. clavispora, and an undescribed Glomus sp. (Dodd, Krikun, 1984).

Another VAM-fungus collection and survey was conducted in Israel in 1982 to identify species in agricultural soils devoted to herbaceous crops. Soils planted with onion or pepper, two crops for which the requirement for VAM-fungi had been identified, were sampled. Spore populations were usually less than .05 spores/ml soil and too low for reliable identifications to be made with the 200 ml samples used. Infective propagule number, estimated by a most probable number (MPN) technique, was found to be 0-1.7 (median 0.2) propagules/ml soil. To encourage fungus activity and spore production, melon plants were grown for 3-6 months in the sampled soil mixed with sterile sand (1:1). The melon roots were often colonized by VAM fungi and there were 0-13 (0.6) spores/ml of the soil:sand mix, a ten-fold increase over the original quantity found. Generally only one species was found per sample. Glomus macrocarpum was the most common species, followed by G. mosseae and G. fasciculatum. A few spores of other Glomus and Acaulospora spp. occasionally were present. There were no significant differences between the species or quantity of VAM-fungi obtained from either crop or from the various geographic regions of the country. (Haas, Krikun, 1983).

In Israel, spores from the above collections, with special emphasis on the predominant species, were used to initiate soil cultures. Single spore cultures were initiated after germinating the spores on dilute nutrient agar - a technique developed and described by Dodd, Krikun (1984).

B.3.1.2 Evaluation of potential suitability of isolates

Quantification. The bioassay used for determining the inoculum potential of soil cultures of the non-culturable VAM-fungi was studied. The commonly used method consists of seeding a host plant into a soil dilution series and, using the percent of infected plants in each dilution, estimating the non-diluted inoculum level by MPN statistics. Initially, test plants were studied for their ability to become infected by VAM fungi. Five species - wheat, tomato, pepper, onion and melon - were planted in artificially infested soil. Four weeks after seeding, the percent of roots infected with VAM-fungi was determined. Melon had the most infection and was

chosen as the test plant. To determine the optimum duration for the MPN tests, several sources of soil inoculum were diluted with sterile sand in a ten-fold series, seeded with melon, and maintained in a greenhouse chamber at 25° C. Samples of ten plants were taken 3,4,5 and 6 weeks after seeding and the inoculum potential of the soil cultures estimated. The estimated propagule numbers remained fairly constant between 3 and 5 weeks after seeding; this corresponded to the time the second true melon leaf was expanding and had fully expanded. Subsequently, the estimated MPN increased rapidly, indicating that the technique, as expected, underestimates the actual fungus population. We suggested using a plant like melon, which germinates rapidly and becomes infected quickly, and conducting the MPN determination 3-4 weeks after seeding, when inoculum estimations are least affected by the stage of host development. The rapidity with which the tests can be conducted is usually worth the sacrifice in accuracy but for more accurate determinations the tests can be continued for longer time periods. (Haas, Leviou, Krikun, 1983).

Evaluation of inoculum efficacy. Strains of G. macrocarpum grown in soil cultures from single spores were tested for efficacy in promoting growth of bell pepper seedlings. These comparisons were made with known numbers of propagules/ml of soil inoculum as determined by MPN. Cultures propagated under similar growth conditions had from .02-25 propagules/ml soil inoculum as measured by the MPN technique. Seven soil cultures with similar inoculum potential levels (median seven propagules/ml) were used undiluted, and at 10^{-1} and at 10^{-2} dilutions. Also, cultures with originally dissimilar propagule levels (.02-15/ml) were diluted to uniform inoculum potential levels and tested for efficacy. Significant variation in the efficacy of isolates within the species was found. The superior isolates could be recognized 4 wk after seeding on the basis of plant height but the final rating was done with plant dry weights after 7 wk growth. The ranking of the isolates differed depending upon the inoculum potential at which they were tested; this will complicate the selection of superior isolates for commercial use because they will

have to be tested at the inoculum potentials at which they will be used in the field. (Haas, Krikun, 1984).

The efficacy of VAM-fungi indigenous to soils of the semi arid northern Negev of Israel was examined in greenhouse tests. The data indicated the presence of a highly efficient VAM population in the calcareous loess soil common to the region. The indigenous fungi greatly enhanced phosphorus (P) uptake of pepper plants in three out of four soils tested at four P levels, in comparison with non-VAM plants. In the loess soil, additions of superphosphate equivalent to 3 t/ha could not alleviate the absence of the VAM association. Increasing internal P leaf levels were correlated closely with a stimulation of flower bud production in pepper. Subsequent planting of onion in the same soils with no further P addition revealed the total dependency of this species on the VAM symbiosis. Mycorrhizal infection reduced by half the R/S ratios of onion plants, mainly by the enhancement of shoot growth. These findings were studied and discussed with regard to the evolution of the mycorrhizal association and its effect on plant morphology and physiological processes. (Dodd, Krikun, Haas, 1983; Krikun, Haas, Bar-Yosef, 1983).

B.3.1.3 Development of methods for producing large quantities of high quality inoculum

There can be large variation in inoculum quantities developing in soil cultures. Beginning with single spores, isolates of G. macrocarpum were grown in soil culture for about 8 months using alternating cropping with melon and sudan grass. Soil cultures contained from .02-25 (median 7) propagules/ml soil at the end of the growth period (Haas, Krikun, 1984).

Techniques for maximizing inoculum production were studied in California. Mycorrhizal inoculum of G. fasciculatum, as measured by root infection and sporulation on sudan grass, increased with increasing light intensity. Compared to natural glasshouse daylight, spore numbers of G. fasciculatum also increased dramatically when mycorrhizal sudan grass was exposed to extended photoperiods with mercury vapour or metal halide lamps of high intensity. Results from the light

experiments indicated that the level of sugars, but not amino acids, in root exudates from 2-month-old sudan grass plants was correlated with the spore production of G. fasciculatum. It appears that growing sudan grass at greater light intensities and under extended photoperiods will increase the quantity and quality of commercial G. fasciculatum inoculum." (Ferguson, Menge, 1982a).

Mycorrhizal plants grown in larger pots had greater dry weight, spore production/g soil and spore production/plant than plants grown in smaller pots. Roots of sudan grass were infected more rapidly and subsequent production of spores was greater when soil inoculum (spores, mycorrhizal roots, and soil) was used rather than surface disinfested spores, even when inoculum spore concentrations were equivalent. (Ferguson, Menge, 1982b).

Spore production was high in all the crop plants tested as potential host plants for G. deserticola soil inoculum production. Peanuts, asparagus, pepper, barley, tomato, alfalfa, and sudan grass produced from 30 to 48 spores/g soil after 4 mo. Thus it may be possible to use any of these plants for production of inoculum. When sudan grass inoculum was compared with Troyer citrange inoculum the former was more effective in stimulating growth and infection of Troyer citrange host plants. (Ferguson, dissert. I).

B.3.2 COMMERCIAL APPLICATION OF VAM-FUNGUS INOCULUM

B.3.2.1 Inoculum potential requirements

Spore inoculum. When Troyer citrange seedlings were inoculated with G. deserticola spore inoculum at seven different inoculum densities, high spore concentrations (1.2-2 spores/g soil) were required to significantly increase plant dry weight. This corresponded to root infection of 40-58%. When Brazilian sour orange seedlings were inoculated with G. deserticola, G. fasciculatum and G. constrictum, the greatest root infection and growth response was observed at approximately 1 spore/g soil. The growth response by G. constrictum was relatively constant for spore densities of .2-2 spores/g soil. The low inoculum density of G. constrictum required to colonize roots and stimulate host growth recommends it as

the more efficient, more economical organism for commercial application. However, G. constrictum does not sporulate prolifically, nor does it stimulate large growth responses in host plants compared to G. fasciculatum. (Ferguson, dissert. I).

Soil inoculum. Soil inoculum promoted greater sudan grass root colonization than spore inoculum. Soil inoculum with as few as .1 spores/g soil initiated VAM whereas with spore inoculum root infection was not observed until after 2 mo and then only where at least .2 spores/g soil were used. Three and 4 mo after inoculation, statistically greater root infection again was found at lower inoculum density levels in plants inoculated with soil inoculum than in plants inoculated with spore inoculum. In another experiment, the final quantity of VAM in plants inoculated with sudan grass soil and spore inoculum was similar but the dry weight of seedlings treated with soil inoculum was greater; this also suggests greater initial infection and growth stimulation from soil inoculum. (Ferguson, dissert. I).

The effect of different quantities of soil inoculum of G. deserticola was tested with citrus seedlings by pre-plant incorporation in the seed bed. Root infection, 10 mo after inoculation, was similar in all treatments regardless of inoculum density level. The greatest growth response occurred at the highest inoculum potential (432 g soil inoculum = 43250 spores/m^2). All lower inoculum density treatments (27 to 216 g soil inoculum) resulted in growth responses but they did not differ from one another (Ferguson, dissert. IV).

Soil inoculum of G. macrocarpum was tested with pepper during a 7 wk growth period. There was a significant positive correlation between initial number of propagules/ml soil and all plant height and weight parameters measured. Growth of mycorrhizal plants was significantly greater than non-mycorrhizal plants when more than .5 propagules/ml were present. With more than 5/ml, 70 percent of the ten isolates were effective. A level of about one propagule/ml was suggested for field infestation of pepper seed beds; greater benefits can be expected from higher levels up to at least 10-15 propagules/ml. (Haas, Krikun, 1984).

Other forms of inoculum. Field and greenhouse inoculations with lyophilized sudan grass roots containing G. deserticola initiated VAM on citrus and stimulated

growth responses. However, the infection and responses were statistically poorer than spore or soil inoculum. (Ferguson, dissert. IV).

Inoculum viability. Infection in plants whose seeds were sown between 1 and 10 wk after soil infestation was consistently high (>78%). Citrus seed germination required 6-8 wk and therefore the inoculum remained viable for 16-18 weeks after application (Ferguson, dissert., IV).

B.3.2.2 Use of VAM-fungus inoculation in field-seeded crops

Citrus nursery. All inoculation methods, except for that involving lyophilized inoculum, resulted in a statistically similar host plant growth response that was from 521-636% greater than that of uninoculated plants. Seedlings of all three citrus types that had been inoculated using a seed planting machine had significantly greater infection and growth response than uninoculated seedlings. Root infection levels were greater in plants inoculated with the post-plant (seedling) banding, broadcast (300 and 900 g), and soil drill and spore drill methods than in plants inoculated with the pre-plant banding, seed inoculation, or lyophilized roots treatment. Infection in all treatments was greater than 55% except for seedlings inoculated with lyophilized roots, where 37% infection was observed (Ferguson, dissert. IV).

Direct seeded pepper. In methyl bromide (MB)-fumigated and non-fumigated (NF) field plots in high P-sorbing soil, soil inoculum (G. macrocarpum) and pepper seeds were placed in furrows opened by a mechanical seeder. After germination, fertigation was initiated via drip irrigation lines with 1, 15 or 40 ppm phosphoric acid or with sulphuric acid (pH 3.8). Two mo after seeding plant top weight was measured after severing 5 plants/plot at the soil line. Simultaneously root systems from these plants were removed from the 0-40 cm soil depth and used to measure root colonization by VAM fungi. Non-VAM plants receiving 1 ppm P in the fertigation system were stunted severely as were those fertigated with acidified nutrients. Although the acidification was scheduled to reduce the pH at the emitters to 3.8,

measurements showed that the pH was 4.5 and above during the first 6 weeks and only thereafter 3.8.

The pepper plants were heaviest where they received 40 ppm P from one dripper line/row and of these the VAM-fungus inoculated plants were the largest. The plants irrigated with 40 ppm P but with one irrigation line/2 rows received an equal quantity of water (and therefore P) per plant but grew similarly to those irrigated with 15 ppm P. The greater distance between drippers combined with the high P-sorption capacity of the soil, effectively reduced the 40 ppm P available to the plant to a level similar to 15 ppm P. This quantity was sufficient to avoid stunting only if VAM were present.

As expected, the root systems of plants in NF plots and in fumigated but infested soil were mycorrhizal. However plants in MB-treated soil were also mycorrhizal although at a much reduced level. Increasing levels of P in the fertigation system reduced the general level of fungus colonization, especially in the MB-treated non-infested plots.

Fifteen wk after seeding there were no differences in root colonization between plants as a result of the various P treatments. The plants in artificially infested soil were more mycorrhizal than those in naturally infested NF soil; MB plants were still less mycorrhizal than the others.

Commercial fruit yield, as measured over the entire harvest season, were highest where MB plots were infested with VAM. Maximum yields were obtained where the infested plots had been fertilized with 15 ppm P, 40 ppm P (one drip line/2 rows), or acidified nutrients during the first 2 months of crop growth. These were not the plots which had the maximum biological yield at 2 months; there was a poor correlation between early growth and final yield ($r=.7$, n.s.).

The pepper plant-collapse disease, which necessitates fumigation of the crop, did not occur during the harvest season. Therefore the yields in the NF plots were higher than normal and provided an unplanned comparison of natural VAM-fungus inoculum with artificial inoculum. With all the parameters measured, there was an advantage with artificial infestation.

In addition to induction of greater total yield, the infestation with VAM stimulated the production of larger fruit; 50% of all the harvested fruit were of export quality (extra large and large sizes) where soil had been infested. (Haas, Krikun, Bar-Yosef, 1984).

Cotton, wheat and tomato. Two sites in California were selected to conduct a large-scale trial of VAM inoculation on MB-fumigated soil. Inoculum, a mixture of G. fasciculatum, G. constrictum, and Gigaspora margarita, was applied before planting using a tractor-mounted fertilizer banding machine. Direct seeded cotton was successfully inoculated with VAM-fungi and yields increased as much as 35%. However, infection development was slow and probably prevented even larger growth and yield stimulation. Tomato seedlings, transplanted into the soil, were not infected despite being placed directly upon VAM inoculum with very high infection potential. Wheat was infected but because of poor conditions for wheat growth, adequate conclusions could not be drawn about the response of the crop to VAM in fumigated soil. (Menge, 1983).

Melon, onion, pepper and celery. Field experiments in Israel were conducted to compare crop growth of mycorrhizal and non-VAM crops. Fumigated plots were used as the control treatment for comparison with VAM plants in a highly infective loess soil with additions of four levels of superphosphate (0-3 t/ha). Onion, pepper and celery were highly responsive to VAM and the non-VAM plants of these species grew poorly even with the heighest level of P-fertilization. (Krikun, Haas, 1985).

Inoculation of non-sterile soils. Wheat, pepper and mung bean were planted into 10 California citrus orchard soils with and without added VAM-fungus inoculum. After 4 mo of growth, artificially inoculated plants in 5 of the 30 soil/plant combinations were significantly heavier than those in naturally infested soil. Over the entire test, inoculation increased the size of plants by 27%. Two soils containing low P and low indigenous VAM-fungus populations, benefitted consistantly and dramatically but other soils with high populations of mycorrhizal fungi and high P concentrations also benefitted. VAM infection at the termination of the experiment

was not well correlated with growth response; it appears that rapid early infection is far more important than the amount of infection at harvest. (Menge, 1983).

B.3.2.3 Use of VAM-fungus inoculation in transplanted crops

Citrus nursery. All inoculation methods, except for the treatment involving the use of lyophilized roots, resulted in a growth response that was from 283-366% greater than that of uninoculated seedlings. Dry weight values in this transplant experiment were lower than dry weight values in the direct seeded experiment primarily because of frost damage. Root infection levels greater than 57% were observed in all treatments, except for the seed inoculation and lyophilized roots treatment, where 49 and 37% infection, respectively, were observed (Ferguson, dissert. IV).

Pepper seedlings for transplanting. Seeds were sown in nutrient-poor sand or nutrient-rich peat/vermiculite amended or not amended with G. macrocarpum. The VAM seedlings were irrigated with three levels of nutrient solution, and transplanted into four levels of P-amended soil, each of which was irrigated with two levels of nutrient solution minus P. Mycorrhizal seedlings in sand were responsive to increasing nutrient levels; in nutrient-rich peat the seedlings did not respond to additional fertilization. The greatest seedling development accompanied by good fungus colonization was in nutrient-poor medium irrigated with the highest nutrient solution tested (18 mM N, 1.2 mM P, and 7 mM K). Non-VAM plants almost ceased growing between the 4th and 5th weeks, whereas VAM plants increased in weight by 41-188%. After transplanting, sand grown seedlings benefited from VAM when 300 mg P/kg or more was added to the soil but peat-grown plants did not. Fruit development was delayed in all non-VAM plants compared with VAM ones. (Haas, Bar-Tal, Bar-Yosef, Krikun, 1984).

B.3.2.4 Interaction of VAM-fungi and other beneficial fungi.

Mycorrhizal and disease-control fungi were investigated to determine the feasibility of simultaneous application of these beneficial fungi in commercial applications. Glomus macrocarpum and G. fasciculatum were the VAM-fungi and Trichoderma harzianum and Talaromyces flavus the antagonists. The effect of each fungus on the other was

tested using different inoculum levels of each and by infesting soil with them simultaneously or at various intervals. Mycorrhizal development in melon, pepper and cotton was not affected by the presence of either of the antagonistic fungi at any of the inoculum potential levels tested. External hyphae of the VAM-fungi were not affected either. The presence of VAM-fungi did not affect the populations of *T. harzianum* found in sand in which mycorrhizal plants had been growing for 3-4 wk but there was a reduction in the antagonist populations in a peat:vermiculite mixture. *Talaromyces flavus*, which was tested only in sand, also had fewer propagules when in the presence of a mycorrhizal plants compared to non-mycorrhizal ones. (Appleboim, thesis).

B.4 DISCUSSION

In 1980, when our proposal was submitted, a substantial body of knowledge existed proving that VAM improves plant nutrition and growth. The beneficial effects had primarily been noted in greenhouse trials and in soils low in P where the VAM effectively increased the root surface active in P absorption. We have identified another situation where VAM are important - soils with high P-sorption characteristics. These soils are common in semi-arid climates where high quantities of active calcium and magnesium often are present in the soil. Even superphosphate applications of 3 t/ha or drip irrigation with 15 ppm P as phosphoric acid could not replace the lack of VAM following fumigation of these P-sorbing soils.

This project was addressed to the introduction of VAM inoculation in modern crop production practices; two extreme situations can be recognized. One, intensively grown crops where fumigation is practiced or artificial growth-media are employed and the natural VAM inoculum is reduced or non-existent, and two, extensive cropping where the natural inoculum can be supplemented with more effective inoculum. Most effort was devoted to the inoculation of intensively grown crops; the granted funds were insufficient to support the work proposed on nonsterile soils and the need for this work remains.

Field tests on a relatively large scale of c. 50 m²/plot were established with artificial inoculation of pepper in Israel and citrus, cotton, wheat and tomato in

California. With pepper comparisons with non-fumigated soil and fumigated, non-infested soil were made with fertigation levels above and below the normally recommended levels. Prior to our work with VAM and pepper, the pepper stunting which followed MB fumigation had been attributed to bromide toxicity. When we showed that either higher P fertigation or VAM inoculation could eliminate the stunting, growers were able to fertigate with 40 ppm P using available technology. Although the benefits of VAM inoculation over-and-above those obtained with P fertigation still have to be put into practice, the technology for doing so largely has been developed. This was shown in the work on citrus nursery stock inoculation using mechanical dry-fertilizer application equipment to deliver VAM-fungus inoculum. Citrus were known to be mycorrhiza dependent and the addition of inoculum to fumigated soil, as expected, often resulted in growth benefits. When the fertilizer placing equipment was used with high quality inoculum, VAM-fungi colonized 55% of the roots and the inoculated plants were 500-600% larger than noninoculated plants.

Intensively grown annual crops often are seeded in nurseries and transplanted to the fields and greenhouses. The transplanting process provides an elegant and very inexpensive means of transferring mycorrhizal inoculum to the field because almost no extra labor input is involved. High quality seedlings are produced in high P-fertilized media because young plants require at least 40 ppm available-P, but phosphates are known to reduce VAM-fungus colonization of hosts. Our experiments with pepper supported the negative effect of P on VAM but showed that excellent seedling production and good VAM-fungus colonization could be achieved. Instead of adding P to the growth media before seeding, seedlings were produced in P-deficient media but fertigated with solutions containing 40 ppm P. Other work on transplanted crops showed that disease control fungi (Trichoderma and Talaromyces) could be incorporated into the growing media along with VAM-inoculum and neither fungus would antagonize the other. Therefore the concept of VAM seedling production for modern agriculture is ready for large-scale field trials. We expect that crop growth benefits will be realized similar to those attained in our experiments.

The quest for high quality inoculum is a never-ending one. We have considerably increased the inoculum potential of soil cultures by growing them under high light and long photoperiod conditions, and by growing the host plants in large containers rather than small pots. Our experience has shown that there are better and poorer hosts for inoculum production but that a variety of plants are satisfactory and the commercial producer can select one of them as the host which best suits his purposes.

One of the problems of inoculum production is the maintenance of pathogen-free cultures. Our improvement of the technique for germinating surface sterilized spores and our use of them to initiate cultures indicates that pathogen free production is feasible if care is taken to avoid contaminating the culture during the several months required to propagate the spore-derived cultures.

The variation in efficacy which was present in single spore cultures of Glomus macrocarpum presents evidence of a newly recognized difficulty in inoculum production. Selecting cultures for commercial use depending upon soil type and nutrient status, crop species, agronomic practices, climate, etc. already can be complicated. High isolate variability will require thorough testing of isolates before use; however, we have developed the techniques for conducting those tests and showed that they can be performed rapidly.

Our experiences in greenhouse and field inoculations with soil cultures allows us to approximate the amount of inoculum required for field application. The actual quantities/ha will vary with planting density as well as inoculum potential of the soil cultures, but the amounts will not vary greatly from our approximations. With pepper and cotton, 50 ml (c. 45 g) and 114 g of soil inoculum, respectively, per running meter of row, and with citrus, 27 g inoculum/m² of seed bed gave significant growth benefits in MB-fumigated soils. With commercial planting densities, about 1 m³ inoculum/ha would be required for row crops and 2 m³/ha for seed beds. These are maximum quantities because our application techniques were inefficient in terms of inoculum use; most of the inoculum was placed between seeds and relatively little under the seeds themselves. A more accurate approximation of the quantities

required may be made from our use of soil inoculum to infect seedling-growing trays to produce pepper for transplanting. Four ml/seedling was used; for a crop planted with 100000 seeds/ha 400 l inoculum/ha would be used. Therefore, some 500-1000 l inoculum/ha is a reasonable estimate of inoculum requirement although greater amounts of inoculum may increase growth responses as some of our experiments have shown.

We believe that the introduction of VAM-fungus inoculation into modern production practices has been proven possible and beneficial. Its eventual use will depend upon cost/benefit relations. In response to those who believe the cost will be too high we suggest examining the initial reaction to the technology of drip irrigation. Today extensive crops like cotton are being irrigated with drippers.

B.5 PUBLICATIONS AND MANUSCRIPTS CITED

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C. EVALUATION OF ACHIEVEMENT

All of the objectives of the original proposal were achieved and almost all of the planned experimentation was conducted although some of the work was performed differently than envisaged. The budget restrictions imposed by the actual grant awarded reduced the scope of the work. In Israel, only one field trial could be conducted with high-quality inoculum. On the other hand, the Israeli work benefitted greatly from the unplanned cooperation of B. Bar-Yosef, Plant Nutrition and Soil Scientist, in designing and conducting experiments on P-nutrition effects.

The first objective achieved was the identification, in Israel, of isolates of VAM-fungi which rapidly colonize roots and stimulate plant growth. Also, the value of screening for effectiveness in growth stimulation was evaluated and a mixture of superior isolates was used successfully in a field trial on pepper. In California this work had been done prior to the BARD grant although the search for better isolates is a continuing one and several isolates were evaluated for efficacy in situ and in greenhouse trials with soil cultures.

Objective two, improving methods of producing quantities of high quality, pathogen-free inoculum, was achieved by showing that soil inoculum is the superior form, that numerous hosts can be used for inoculum production, that increasing light quantities increases spore production in soil cultures, that single spore cultures can be produced in a relatively brief period and therefore there is a great feasibility of maintaining clean cultures, and that efficacy variation between isolates makes it imperative to carefully test cultures for quality to ensure maintenance of high quality.

The third objective, developing effective, practical field application methods for inoculation, was successfully achieved for direct seeded row crops and for transplants produced in seedling trays. Ordinary fertilizer applicators for deposition of bands of fertilizers in or adjacent to the seed row were used to infest soil; plants grown in the soil were infected. For trays of seedlings, inoculum was mixed with sand or peat:vermiculite mixtures and colonized seedlings

were obtained. Inoculum viability in infested soil was shown to be retained over many weeks; therefore no major obstacles remain in using field application methods.

Field testing of the nutrition- and yield-improving effects of inoculation was our final objective. It was achieved by comparing melon, onion, pepper and celery growth and nutrition in fumigated and nonfumigated loess soil and showing the essentiality of VAM in these soils when growing the latter three crops; also, by comparing cotton at 2 sites and citrus at one in California, and pepper in Israel. The California experiments were in P-deficient soils and yield improvements were achieved. In Israel, the tests of VAM-fungus inoculation was combined with low, medium and high P-fertigation treatments and improvements in yield were obtained over those ascribable to P-nutrition alone.

D. DESCRIPTION AND RESULTS OF COOPERATION

Personal meetings between the cooperators took place before the project was funded and led to the preparation of the project and the initiation of the joint survey of avocado-growing soils. Partially BARD-funded meetings included separate 1-2 week visits by each Israeli cooperator in the USA and by Menge in Israel. Although repeated exchanges of information were made by mail during the course of the project the personal contacts resulted in a) significantly greater proficiency of the Israel group in working with VAM-fungi [their experience was extremely limited before the work began], b) the development of the improved techniques for infesting soil and achieving growth benefits, and c) the greater awareness by the cooperators of the large quantity of arable lands which are high P-sorbing and the large number of crops which are likely to benefit from VAM-fungus inoculation.

E. BENEFIT TO AGRICULTURE

A 'spin-off' from our work has given immediate benefits to agriculture. The solution to pepper stunting following methyl bromide (MB) fumigation followed our proof that P nutrition and not bromide toxicity was the cause of the problem. Although the available quantities of VAM-fungus inoculum are not sufficient for treating the pepper crop and gaining the benefits of both P fertigation and VAM inoculation, the immediate agricultural problem has been solved and good pepper crops can be grown. It should be noted that the awareness of the effect of fumigation on VAM-fungi and P nutrition has reduced complaints about the toxic effect of MB treatment on several crops. We believe that the time should and will come that many crops grown on fumigated soil will routinely be inoculated with VAM-fungi. Our work has shown that the technique is feasible and easily integrated into modern crop production practices.

The results on inoculum production techniques have been used to improve the quality of the commercial VAM-fungus inoculum available from at least two firms in the U.S.A.

Longer-term benefits to agriculture will result from the increased awareness of the importance of temporary P-deficiencies in crop growth. The recommended quantities of available P in soil for optimum crop growth have increased substantially over the past few years, especially for intensively grown crops. The commercial use of VAM-fungus inoculation can halt the continuing increase in recommended soil P levels by allowing the mycorrhizal plant to make more efficient use of the available P already present in the soil.

F. PUBLICATIONS AND MANUSCRIPTS - SUPPLEMENT