The Relationship Between Cultural Practices and Arbuscular Mycorrhizal (AM) Activity in Orchards Under Different Management Systems

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Summary

It has been demonstrated that soil disturbance, excessive fertilization and the use of some agrochemicals affect the survival and function of arbuscular mycorrhizal (AM) fungi. To clarify the effect of different cultural practices on mycorrhizal activity in orchards, spore population dynamics, soil and plant mineral content and mycorrhizal infection were surveyed monthly from May to October, 2000 in kiwifruit, Japanese chestnut, Iyo tangor, satsuma mandarin, Japanese persimmon, apple, peach, Japanese plum and grape plantings, subjected to different management regimes. In intensively managed orchards, levels of major elements in the soil were generally high, owing to periodic fertilization; consequently, the content of these elements in the leaves was within the recommended range for normal growth and function. High levels of AM infection were maintained throughout the season in the apple orchard that received no fertilizer or fungicide. The infection levels on roots of kiwifruit vines decreased in July and August after three fungicide sprays in June; it also decreased in Iyo tangor in July following four fungicide applications in May and June. These orchards had no under-story cover plants. However, the infection level of peach trees under sod culture remained relatively high even after five fungicide sprays from May through August. Thus, population density of AM spores in the soils seems to be low in the clean cultivated orchards, subjected to frequent fungicide sprays, whereas it was high under sod culture. We conclude that excessive fertilization, frequent fungicide sprays and clean culture have an adverse effect on AM spore populations and tree infection in orchards.

Key Words: cultural practices, mycorrhiza, plant nutrition, soil fertility, under-story plants.

Introduction

Arbuscular mycorrhizal (AM) fungi are soil borne microorganisms that form symbiotic relationships with a majority of cultivated plants (Brundrett, 1991). The plant-fungal association is characterized by a bi-directional flow of inorganic minerals from the fungus to the host plant, and photosynthates from the plant to the fungal partner (Sylvia et al., 1998). The fungus plays an important role in the acquisition of nutrients from the soil (Read et al., 1992) and is particularly useful in low input, sustainable agriculture.

Cultural practices, particularly the use of fertilizers, can adversely affect AM fungal activity. The greatest impact of mycorrhizal activity on plant growth occurs when P and other immobile mineral elements are in limited supply. Hence, Rajapakse et al. (1989) found a

negative correlation between soil P and levels of root infection by AM fungi with increasing P levels.

Tillage, likewise, has a negative effect on AM activity. O'Halloran et al. (1986) reported that corn, grown in non-tilled soil, had higher shoot P concentrations as compared with plants in conventional production. McGonigle et al. (1990) suggest that tilling the soil disrupts the extra-radical mycelium that is responsible for connecting to a newly developing root system. Therefore, a possible consequence of land preparation prior to sowing would be a lag phase in the establishment of the symbiotic relationship.

Crop rotation and fallowing also affect the diversity and function of mycorrhizal fungi. Mycorrhizal populations have been shown to react favorably to conversions between low input and conventional tillage systems (Kurle and Pfleger, 1994). Weed proliferation that is common in low-input tillage helps to restore fungal populations to original levels following conventional tillage. Periods of long, clean fallowing, often maintained in low rainfall areas to conserve both soil moisture and nutrients, lead to a gradual decline in mycorrhizal inoculum. Since the 1940's, it has been

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observed that crops, planted immediately after long fallows, grow poorly (Leslie and Whitehouse, 1965). In spite of tests that indicate the presence of more than adequate soil P, cereal crops develop classic P deficiency symptoms, such as stunted growth, dark green leaves with terminal die-back, delayed maturity, and poor grain development. Similar observations have been made for crops grown on flooded soils, suggesting that some of the mechanisms responsible for the problem may be the same (Wetterauer and Killorn, 1996). The cause of the syndrome has not been fully explained. However, the significant reduction in mycorrhizal inoculum observed after extended fallow (Thompson, 1987) or flooding (Ellis, 1998), and the positive response by these soils to inoculation explains the cause of the syndrome as being partly due to the gradual elimination of indigenous AM fungi.

The interaction between agrochemicals and AM fungi has been studied with mixed results. Some fungicides promote mycorrhizal activity (Sugavanam et al., 1994; Rutto et al., 1999), whereas most have an inhibitory effect (Udaiyan et al., 1995; Schreiner and Bethlenfalvay, 1997); others have no effect (Kucey and Bonetti, 1988).

The objective of this survey was to study the interactions between different orchard management practices, tree species, soil fertility, and the population dynamics and function of indigenous AM fungi.

Materials and Methods

Site characterization

The survey was conducted on trees growing in the Ehime University Farm, about 20 m above sea level in southern Japan, where the climate is characterized by hot, humid summers and cold, dry winters. The orchard soil is a sandy loam, classified as a eutric fluvisol (FAO/UNESCO) with a pH (soil:water = 1:5) of 5.7, a mean bulk density of a 1.08 g cm^{-3} and horizon A thickness of $0.2 (\pm 0.05) \text{ m}$. Rainfall and temperature data for the duration of the survey are presented in Fig. 1.

Orchard management

Different orchards were subject to different management practices at the time of the survey. In weed management, varying levels of clean culture were maintained in all orchards except in the peach orchard where the areas around the trees were kept clean and in the apple orchard where mowing was the only mode of weed control. Two herbicides, one a mixture of diquat (7%) and paraquat (5%) and the other a mixture of terbacil (40%) and dichlorophenyldimethyl urea (DCMU) (39%) were sprayed in all plots except the apple, peach and chestnut orchards during the survey. The fungicides, used for the control of fungal diseases, included Kocide (*C. iyo*, *C. unshiu*, *D. kaki*, *V. labrusca*), Manzab (*C. iyo*, *C. unshiu*), Zineb (*A.*

chinensis, *C. iyo*, *C. unshiu*, *D. kaki*, *P. persica*, *P. salicina*, *V. labrusca*) Pencozeb (*C. iyo*, *C. unshiu*, *P. persica*, *P. salicina*), Benlate (*A. chinensis*, *C. iyo*, *C. unshiu*), Rovral (*A. chinensis*, *D. kaki*, *P. persica*, *V. labrusca*), and Thiophanate methyl (*A. chinensis*, *D. kaki*, *P. persica*, *P. salicina*, *V. labrusca*). The miticides and insecticides were Kelthane (*C. iyo*, *C. unshiu*), Sanmite (*C. iyo*, *C. unshiu*), Phenothiocarb (*A. chinensis*, *C. iyo*, *C. unshiu*), Elsan (*P. persica*, *P. salicina*), Cypermethrin (*A. chinensis*, *D. kaki*, *P. persica*, *P. salicina*, *V. labrusca*), Acephate (*C. iyo*, *C. unshiu*), machine oil (*C. iyo*, *C. unshiu*), Acetamiprid (*C. iyo*, *C. unshiu*), Supracide (*A. chinensis*, *C. iyo*, *C. unshiu*, *D. kaki*, *P. persica*, *P. salicina*, *V. labrusca*), Carbaryl (*P. persica*, *P. salicina*) and Biphenthrin (*D. kaki*). The compound fertilizer NPK (8-6-6) was applied twice in the Iyo tangor and satsuma mandarin orchards at 1.0 kg and 0.7 kg per tree, respectively. Similarly, NPK (15-15-15) was applied once in the peach orchard at the rate of 0.9 kg per tree.

Soil and leaf sample collection

Soil, root and leaf samples were collected monthly from five plants of kiwifruit (*Actinidia chinensis* Planch.), Japanese chestnut (*Castanea crenata* Sieb. et Zucc.), Iyo tangor (*Citrus iyo* hort. ex Tanaka), satsuma mandarin (*Citrus unshiu* Marc.), Japanese persimmon (*Diospyros kaki* Thunb.), apple (*Malus pumila* Mill.), peach (*Prunus persica* Batsch), Japanese plum (*Prunus salicina* Lindl.) and grape (*Vitis labrusca* L.) to survey mycorrhizal infection, spore populations, plant nutrition and soil fertility over one growing season. Soil and root samples were collected at a depth of 10 cm from within the tree drip line at about 0.5 m from the tree trunk. Leaf samples for tissue analysis were collected including the petiole from the middle of the current shoot.

Estimation of under-story plant cover

Under-story plants in four 1-m^2 units per orchard

Fig. 1. Monthly rainfall and mean temperature for the period between April and November, 2000.

were divided into three (small, medium and large) size-based groups. The span of a single plant from each size category was used to calculate the mean area covered by a plant from each of the three groups and the area of orchard floor under plant cover was estimated from the number of plants in each size category. The areas that were chosen for the evaluation were representative of the general under-story plant distribution in each orchard. Buxbaum's speedwell (*Veronica persica*), chickweed (*Stellaria media*), annual sow thistle (*Sonchus oleraceus*), annual blue grass (*Poa annua*), prince's feather (*Amaranthus viridis*), Italian rye grass (*Lolium multiflorum*), setaria (*Setaria spp*), Sumatra fleabane (*Conyza sumatrensis*), Japanese mugwort (*Artemisia princeps*) and wild oat (*Avena fatua*) were the common under-story plants in the orchards. Apple and peach orchards had a higher ratio of grass relative to broad-leaf weeds.

Quantification of fungal spore populations

Spores were separated from 5 g samples of air-dry soil by density gradient centrifugation with 50% sucrose. Spores were trapped in stacked sieves of 250 μm , 125 μm and 45 μm mesh size and rinsed into Petri dishes. The dishes were placed under a dissecting microscope against a gridded background; all spores from each sieve were counted.

Plant tissue and soil analysis

Leaf samples were washed in 15% acetic acid, rinsed in distilled water and heated at 90 °C for an hour to terminate enzyme activity. Samples were dried at 60 °C for three days and then pulverized with a pestle and mortar. Duplicate, 1-gram samples of each species were placed into crucibles and dry-ashed by heating for five hours at 550 °C in a muffle furnace. The ash was taken up in 1 ml of 20% HCl and the solution made up to 20 ml with distilled de-ionized water. Two hundred microliter aliquots from these solutions were further diluted to 10 ml before analysis for Ca, Mg, K and Zn

using an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto, Japan). Phosphorus, as molybdate-reactive P, was measured by blue colorimetry at 430 nm using a spectrophotometer (Hitachi U-2001, Tokyo, Japan). Leaf-N was analyzed using a CN coder (Sumitomo NC-80, Tokyo, Japan).

Soil-P was extracted with 0.5 M sodium bicarbonate, and molybdate reactive P was measured at 880 nm using a spectrophotometer (Hitachi U-2001, Tokyo, Japan). Soil Ca, Mg, K and Zn were extracted with a mixture of 0.05 M ammonium acetate and 0.0114 M strontium chloride by shaking. After filtration, the mineral content in samples was measured with an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto, Japan). Soil-N was analyzed as above.

Evaluation of root infection levels

Root pieces (1 ± 0.2 cm) were cleared by autoclaving in 10% KOH followed by staining with 0.03% Chlorazol Black E (CBE) solution (Brundrett, 1994). Infection levels were estimated under a compound microscope by the grid-intersect method (Giovannetti and Mosse, 1980). Root samples, not cleared immediately after collection, were stored in 50% ethanol until analyzed.

Statistical analysis

One-way analysis of variance was followed by the mean separation of spore number totals using Newman Keul's test. The principal component analysis was performed on a correlation matrix for soil, plant and mycorrhizal variables using the statistical package NCSS 97.

Results

Soil conditions

Major element levels in soils from the orchards varied, depending mostly on the management practices performed during the growing season (Table 1). At the beginning and end of the survey, N, P and K contents

Table 1. Soil concentrations of different mineral elements in the orchards at the start and end of the survey.

Orchard species	Mineral content (May 2000)					Mineral content (October 2000)				
	N(%)	P(mg/g)	K(%)	Ca(%)	Mg(%)	N(%)	P(mg/g)	K(%)	Ca(%)	Mg(%)
<i>A. chinensis</i>	0.08 ± 0.01^z	0.51 ± 0.01	0.15 ± 0.07	0.63 ± 0.03	0.13 ± 0.01	0.17 ± 0.03	0.36 ± 0.03	0.11 ± 0.01	0.84 ± 0.08	0.16 ± 0.01
<i>C. crenata</i>	0.25 ± 0.02	0.54 ± 0.02	0.22 ± 0.04	0.67 ± 0.02	0.13 ± 0.00	0.18 ± 0.04	0.75 ± 0.02	0.20 ± 0.02	0.57 ± 0.06	0.14 ± 0.01
<i>C. iyo</i>	0.42 ± 0.08	0.92 ± 0.05	0.46 ± 0.09	0.65 ± 0.09	0.14 ± 0.01	0.21 ± 0.01	0.81 ± 0.10	0.31 ± 0.04	0.62 ± 0.05	0.13 ± 0.02
<i>C. unshiu</i>	0.16 ± 0.02	0.68 ± 0.03	0.29 ± 0.03	0.58 ± 0.03	0.17 ± 0.05	0.24 ± 0.03	0.52 ± 0.06	0.27 ± 0.04	0.88 ± 0.06	0.20 ± 0.00
<i>D. kaki</i>	0.18 ± 0.03	0.43 ± 0.10	0.13 ± 0.02	0.69 ± 0.06	0.14 ± 0.09	0.11 ± 0.01	0.43 ± 0.10	0.18 ± 0.02	0.82 ± 0.19	0.14 ± 0.02
<i>M. pumila</i>	0.17 ± 0.04	0.12 ± 0.01	0.07 ± 0.02	0.60 ± 0.04	0.14 ± 0.01	0.20 ± 0.01	0.12 ± 0.01	0.13 ± 0.03	0.84 ± 0.18	0.18 ± 0.02
<i>P. persica</i>	0.12 ± 0.05	0.30 ± 0.10	0.13 ± 0.01	0.62 ± 0.04	0.13 ± 0.01	0.15 ± 0.05	0.30 ± 0.10	0.16 ± 0.03	0.78 ± 0.07	0.14 ± 0.02
<i>P. salicina</i>	0.15 ± 0.01	0.44 ± 0.04	0.21 ± 0.01	0.58 ± 0.02	0.13 ± 0.01	0.25 ± 0.06	0.48 ± 0.10	0.24 ± 0.08	0.64 ± 0.01	0.14 ± 0.01
<i>V. labrusca</i>	0.11 ± 0.02	0.36 ± 0.06	0.14 ± 0.04	1.22 ± 0.07	0.19 ± 0.00	0.13 ± 0.01	0.36 ± 0.06	0.18 ± 0.00	1.31 ± 0.10	0.19 ± 0.01

^z Mean $\pm$ SE, n=5.

were lowest in the non-fertilized apple orchard and highest in the two citrus orchards, which were subjected to the most intensive management regime (Table 2). Nutrient levels were also low in the peach orchard, similar to the apple orchard; the average under-story vegetation was high.

Plant nutrition

Selected nutrients in leaves during the survey (Fig. 2) varied greatly among species. However, a general consistency was noted in the monthly fluctuations of Ca and Mg concentrations. Although most mineral elements measured were within the recommended range for normal growth and function (Jones Jr., 1998), samples collected from the apple orchard were a slightly deficient in K, Ca and to a lesser extent Mg (Fig. 2).

Mycorrhizal infection

Levels of root colonization by indigenous AM fungi in the different orchards ranged widely (Fig. 3). Infection was highest in apple, peach and Japanese plum; it was lowest in chestnut and Iyo tanger, and intermediate in the rest of the species. Orchards with more than 20% of the floor occupied by various weeds had relatively higher infection levels (Fig. 3, 4). Mean root colonization was highest in the apple orchard (Fig. 3).

Spore populations

Mycorrhizal spore populations were highest at the start of the survey (May and June); the biggest increase occurred in some orchards in June (Table 3). There was a general reduction with time in spore populations for all

Table 2. The management practices performed on specific orchards.

Fruit species	May	Jun	Jul	Aug	Sep	Oct
<i>A. chinensis</i>	H _E	F _U ³ , P _E ³	F _U , P _E	H _E , F _U , I _R , P _E	—	F _U , P _E
<i>C. crenata</i>	P _L	—	P _L	—	—	—
<i>C. iyo</i>	F _E , F _U ³ , H _E , P _E ²	F _U , P _E ²	P _E	F _U , H _E , I _R , P _E	I _R , P _L	F _U , P _E ²
<i>C. unshiu</i>	F _U ³ , H _E , P _E ²	F _U , P _E ²	P _L , P _E	F _E , F _U , H _E , I _R , P _E	H _E , P _L	F _U , P _E
<i>D. kaki</i>	H _E	F _U ³ , P _E ²	F _U , P _E	P _E	—	—
<i>M. pumila</i>	M _O	—	M _O	—	—	—
<i>P. persica</i>	F _U , P _E	F _U ² , P _E ²	F _U , P _E	F _U , P _E	F _E , P _E , P _L	—
<i>P. salicina</i>	F _U , H _E , P _E	F _U , P _E	F _U , P _E	F _U , P _E	F _E , P _E , P _L	—
<i>V. labrusca</i>	H _E	F _U ² , P _E ³	F _U , P _E	F _U , I _R , P _E	P _E , P _L	—

F_E: fertilizer application; F_U: fungicide spray; H_E: herbicide spray; I_R: irrigation; M_O: mowing; P_E: pesticide spray; P_L: plowing
^{2,3} The number of times each operation was performed in one month.

Fig. 2. Seasonal changes in nutrient concentrations in the leaves of nine fruit species.

AC: *Actinidia chinensis*, CC: *Castanea crenata*, CI: *Citrus iyo*, CU: *Citrus unshiu*, DK: *Diospyros kaki*, MP: *Malus pumila*, PP: *Prunus persica*, PS: *Prunus salicina* and VL: *Vitis labrusca*.

Fig. 3. Mycorrhizal infection levels over a six-month period in nine fruit species. Bars represent SE (n=5).

AC: *Actinidia chinensis*, CC: *Castanea crenata*, CI: *Citrus iyo*, CU: *Citrus unshiu*, DK: *Diospyros kaki*, MP: *Malus pumila*, PP: *Prunus persica*, PS: *Prunus salicina* and VL: *Vitis labrusca*.

**Diospyros kaki* roots could not be sufficiently cleared in August or September.

Fig. 4. Changes in under-story plant cover density during the 2000 season in nine orchards.

AC: *Actinidia chinensis*, CC: *Castanea crenata*, CI: *Citrus iyo*, CU: *Citrus unshiu*, DK: *Diospyros kaki*, MP: *Malus pumila*, PP: *Prunus persica*, PS: *Prunus salicina* and VL: *Vitis labrusca*.

size categories in the surveyed orchard soils (Table 3). A distribution almost similar to the root colonization data occurred where spore populations are highest in the apple orchard. This was particularly true for the first month of the survey (May) when spore numbers were significantly higher in the apple than in all the other orchards. Species diversity, expressed as higher spore numbers within size categories, was also greatest in the apple orchard (Table 3).

Interaction between soil, plant and mycorrhizal variables

The relationships between variables after principal component analysis show that high soil nutrient content corresponds to lower root infection, spore number and leaf P content (Table 4). In particular, high soil P has an adverse effect on root colonization. It is also clear that high under-story plant cover goes hand in hand with high root infection and low spore numbers; low cover crop density is also related to high spore numbers.

Discussion

Symbiotic microorganisms, such as mycorrhiza and rhizobium, are useful in alleviating the limitations of phosphorus and nitrogen deficiency on plant growth in most agricultural systems (Jasper, 1994). However, research has shown that these microorganisms, particularly AM fungi, respond differently to various management practices and are most effective in low input agriculture (Read et al., 1992). Soil disturbance (Miller et al., 1995), crop rotation involving non-host crops (Karasawa et al., 2001), clean fallows for extended periods of time (Thompson, 1987), flooding (Ellis, 1998), some fungicides (Udaiyan et al., 1995), herbicides (Sieverding and Leihner, 1984) and some soil fumigants (Schreiner and Bethlenfalvay, 1997) adversely affect mycorrhizal activity. Generally, the degree of infection by AM fungi varies widely in response to the nutritional status of the host plant and the prevailing soil and climatic conditions.

The results obtained in this survey show a clear relationship between mycorrhizal function, soil fertility

Table 3. Mean spore populations in 5-g soil samples collected from around fruit trees under different management regimes.

Tree species	Spore size ^z	May	Jun	Jul	Aug	Sep	Oct
<i>A. chinensis</i>	>45 μm	248 $\pm$ 45 ^y	275 $\pm$ 88	129 $\pm$ 19	134 $\pm$ 8	118 $\pm$ 15	95 $\pm$ 21
	>125 μm	25 $\pm$ 8	8 $\pm$ 1	3 $\pm$ 1	5 $\pm$ 2	2 $\pm$ 2	1 $\pm$ 1
	>250 μm	7 $\pm$ 1	1 $\pm$ 1	0	1 $\pm$ 1	0	0
	Mean total	280bc ^x	284a	132ab	140a	120a	96a
<i>C. crenata</i>	>45 μm	365 $\pm$ 75	398 $\pm$ 74	293 $\pm$ 77	428 $\pm$ 76	356 $\pm$ 89	312 $\pm$ 66
	>125 μm	17 $\pm$ 7	19 $\pm$ 8	5 $\pm$ 2	17 $\pm$ 3	10 $\pm$ 1	8 $\pm$ 3
	>250 μm	2 $\pm$ 1	2 $\pm$ 1	0	2 $\pm$ 2	1 $\pm$ 1	1 $\pm$ 1
	Mean total	384c	419b	298e	447c	367c	321c
<i>C. iyo</i>	>45 μm	258 $\pm$ 37	463 $\pm$ 99	181 $\pm$ 46	137 $\pm$ 31	120 $\pm$ 23	99 $\pm$ 14
	>125 μm	23 $\pm$ 3	14 $\pm$ 3	7 $\pm$ 3	12 $\pm$ 5	9 $\pm$ 3	7 $\pm$ 2
	>250 μm	5 $\pm$ 3	4 $\pm$ 4	0	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1
	Mean total	286bc	481b	189bc	150a	130a	170a
<i>C. unshiu</i>	>45 μm	86 $\pm$ 27	166 $\pm$ 55	88 $\pm$ 42	280 $\pm$ 33	211 $\pm$ 45	194 $\pm$ 38
	>125 μm	10 $\pm$ 5	12 $\pm$ 7	7 $\pm$ 4	3 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2
	>250 μm	4 $\pm$ 2	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1
	Mean total	100a	179a	96a	284b	214b	197c
<i>D. kaki</i>	>45 μm	236 $\pm$ 88	272 $\pm$ 75	175 $\pm$ 44	136 $\pm$ 26	106 $\pm$ 31	94 $\pm$ 16
	>125 μm	27 $\pm$ 7	11 $\pm$ 7	6 $\pm$ 3	4 $\pm$ 3	3 $\pm$ 2	2 $\pm$ 1
	>250 μm	5 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	0	0
	Mean total	268bc	284a	182bc	141a	109a	96a
<i>M. pumila</i>	>45 μm	438 $\pm$ 99	410 $\pm$ 78	258 $\pm$ 42	205 $\pm$ 63	166 $\pm$ 27	142 $\pm$ 25
	>125 μm	43 $\pm$ 12	15 $\pm$ 4	6 $\pm$ 2	9 $\pm$ 2	6 $\pm$ 2	4 $\pm$ 1
	>250 μm	20 $\pm$ 6	3 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 1	1 $\pm$ 1	0
	Mean total	201d	428b	268cde	216a	173a	146b
<i>P. persica</i>	>45 μm	257 $\pm$ 73	151 $\pm$ 28	235 $\pm$ 17	206 $\pm$ 15	121 $\pm$ 26	104 $\pm$ 17
	>125 μm	18 $\pm$ 7	6 $\pm$ 1	10 $\pm$ 4	8 $\pm$ 4	5 $\pm$ 2	2 $\pm$ 2
	>250 μm	4 $\pm$ 2	2 $\pm$ 1	3 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1	1 $\pm$ 1
	Mean total	275bc	159a	248cde	216a	128a	107a
<i>P. salicina</i>	>45 μm	203 $\pm$ 42	436 $\pm$ 79	200 $\pm$ 37	160 $\pm$ 22	140 $\pm$ 32	116 $\pm$ 17
	>125 μm	8 $\pm$ 3	13 $\pm$ 4	7 $\pm$ 2	5 $\pm$ 1	3 $\pm$ 1	3 $\pm$ 1
	>250 μm	5 $\pm$ 2	6 $\pm$ 2	1 $\pm$ 1	0	1 $\pm$ 1	0
	Mean total	216b	455b	208bc	165a	144a	119ab
<i>V. labrusca</i>	>45 μm	208 $\pm$ 23	188 $\pm$ 68	178 $\pm$ 52	140 $\pm$ 32	108 $\pm$ 25	86 $\pm$ 20
	>125 μm	50 $\pm$ 7	9 $\pm$ 3	6 $\pm$ 4	9 $\pm$ 2	4 $\pm$ 3	4 $\pm$ 3
	>250 μm	13 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 2	1 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1
	Mean total	343c	199a	186bc	150a	113a	91a

^z Spores in soil samples were counted in three size (45 μm , 125 μm and 250 μm) based categories.^y Mean $\pm$ SE, n=5.^x Values followed by different letters within columns are significantly different (Newman-Keul's test).

and under-story plant density. Available soil-P in orchards was generally higher than levels where plants are expected to derive maximum benefits from mycorrhizal infection. The critical P level that accounts for 95% of plant growth in cassava is 15 mg $\cdot$ kg⁻¹ in inoculated and 190 mg $\cdot$ kg⁻¹ in non-inoculated plants (Howeler et al., 1982). The high P concentrations in our orchard soils also impacted negatively on root infection (Table 4).

The relationships between soil, plant and mycorrhizal variables (Table 4) indicate that high soil fertility generally corresponds to lower spore numbers and a reduction in root infection, whereas a low weed population results in high spore numbers and a decrease in infection levels and vice versa. The high spore populations in orchards with low under-story vegetation are attributed to the absence of plant roots within a distance essential for spore germination and root infection.

Table 4. Eigenvectors and eigenvalues after principal component analysis of soil, plant and mycorrhizal variables. Factors 1, 2 and 3 accounted for 89.3% of the cumulative variance.

Variables ^z	Factor 1	Factor 2	Factor 3
Soil			
N	0.295518	0.242757	0.611970
P	0.416933	0.227339	0.000997
K	0.409226	0.066250	0.286957
Plant			
N	0.325629	0.211699	-0.035995
P	-0.266679	0.565203	-0.074015
K	0.292838	-0.442299	0.219912
Plant cover	-0.327582	-0.115829	0.554919
Root infection	-0.336974	-0.333271	0.288551
Spore number	-0.295979	0.447898	0.311152
Eigenvalues	5.122513	1.825174	1.085766
Individual percent	56.92	20.28	12.06

^z Mean monthly values for all fruit species and May and October means of soil mineral values were used for the analysis of principal components.

Furthermore, the absence of weeds indicates high soil fertility because the intensively managed orchards receive both fertilizers and repeated doses of herbicides. This inverse relationship was confirmed, as the apple orchard with the lowest P level among all the surveyed orchards had the highest AM colonization levels, whereas the intensively managed citrus orchards with relatively high soil P concentrations recorded the lowest infection levels.

The sudden and general increase in spore numbers in June is probably a response to the sharp fall in metabolite supply due to the elimination of weeds by the initial herbicide sprays and other weed control measures (Fig. 2). This was followed by a gradual decrease in spore numbers during the rest of the experiment. Schubert and Cravero (1990) report a similar trend in spore population dynamics for a kiwifruit vineyard where spore numbers were reportedly higher in spring and lower later in the growing season. Considering the repeated fertilizer and pesticide usage and clean culture histories of our orchards, the gradual reduction in spore populations might be predicted. A recent change in the cropping pattern e.g., a shift from sod to clean culture might explain the high spore numbers in the Japanese chestnut orchard without corresponding infection levels.

The negative effect of fungicides on AM activity is demonstrated by the marked reduction in infection levels in citrus and kiwifruit in July after repeated fungicidal sprays in May and June. Benlate (benomyl) in particular has been reported to strongly inhibit mycorrhizal activity (Schreiner and Bethlenfalvay, 1997). Herbicides also

affect AM activity directly through the depression of root infection and spore production, and indirectly by eliminating weeds that may be natural hosts of AM fungi. Sieverding and Leihner (1984) reported such a delay in spore build up causing a gradual decline in native mycorrhizal populations which would account for the high rates in pesticide-free, sod cultured apple orchard and in the peach and Japanese plum orchards. It is also possible that the weeds intercept a significant portion of the fungicide preventing its entry into the soil.

Kabir and Koide (2000) concluded that non-tillage contributes to an improvement in soil physical properties while Kurle and Pflieger (1994) found that it influences mycorrhizal spore populations by their effect on weed populations. Hence, the choice of weed control method could prove crucial to the survival and function of mycorrhizal fungi. Our data, relating mycorrhizal activity with production system and correlating weed populations with tillage, support their observations.

In addition to an improvement in rhizosphere microbial activity, there are other benefits that accrue from the use of cover crops in orchards. Ground covers enhance tree survival (Layne et al., 1994) and improve yield (Abdul-Baki et al., 1997). Altieri and Schmidt (1985) in their extensive review of cover crop manipulation in Northern California orchards pointed out that cover crops are generally associated with low densities of phytophagous insects, less fruit damage, large populations and more species of natural enemies, and an increase in their predatory activities. Mowed sodgrass or biomass mulch systems have also been reported to reduce the levels of nitrate-N and Benomyl fungicide both in surface run-off and in below ground leachate (Merwin et al., 1996).

However, in some instances, ground covers come with disadvantages: the main one being competition because the vegetation competes with young fruit trees for water and nutrients (Anderson et al., 1992). Hence, the deficiency of some mineral elements in our apple leaf samples could be related to such competition.

Our orchards have fairly consistent cropping histories, so that the short duration of our survey does not diminish the value of the data presented here. This report provides a valuable insight into the relationship between production practices and mycorrhizal activity in orchards under different managements. We conclude that a sustainable agro-ecosystem can be established with the judicious application of both fertilizers and pesticides, coupled with the manipulation of cover crop composition and density. The design of proper cover crop-orchard mixtures could also provide an opportunity for the enhanced biological control of specific pests.

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種々の果樹園における菌根菌 (AM) 活性と栽培管理との関係

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摘 要

土壌の耕起, 施肥, 農薬散布が菌根菌 (AM) の生存と作用に影響することが知られている。菌根菌の感染, 土壌中の胞子数, 土壌と植物の無機成分含量と栽培管理の間の関係を明らかにするために, 愛媛大学附属農場実験圃場において 2000 年の 5 月から 10 月の生育期にウンシュウミカン, イヨカン, リンゴ, ナシ, キウイフルーツ, カキ, スモモ, クリ, ブドウ園について 1 ヶ月間隔で調査した。集約的な管理がなされている土壌中の多量元素量は定期的な施肥のために一般的に高く, 葉内の多量無機成分含量は正常な生長と作用をはたす範囲内であった。調査期間中, 無施肥, 無農薬散布のリンゴの菌根菌感染率は年間を通じて高かった。殺菌剤の散布回数が 6 月に 3

回と集中したキウイフルーツでは 7, 8 月の感染率が減少し, 5, 6 月の殺菌剤の散布回数が 4 回のイヨカンでは 7 月の感染率が低かった。しかも, これらの園には被覆植物がなかった。しかし, モモは 5 月から 8 月の間, 5 回の殺菌剤を散布したものの, 感染率が高かったのは被覆植物の存在が関係していると思われた。また, 土壌の胞子数も年間の殺菌剤散布回数の多い園では少ない傾向が見られた。被覆植物の多いリンゴ園とモモ園で土壌中の胞子数が多かった。以上のことから, 多肥, 過剰の殺菌剤散布や清耕栽培は果樹の菌根菌の感染や土壌中の胞子数の維持に悪影響を与えていると考えられる。