Seasonal Fluctuations in Mycorrhizal Spore Populations and Infection Rates of Vineyard Soils Planted with Five Legume Cover Crops

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Summary

The spread of arbuscular mycorrhizal (AM) fungi and infection rates in five legume cover crops [Chinese milk vetch (*Astragalus sinicus* L.), hairy vetch (*Vicia villosa* Roth), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.) and narrow-leaf vetch (*Vicia angustifolia* L.)] were studied over one season. The legumes were sown between rows of five-year-old grapevines (4.0 m by 4.5 m). Mycorrhizal infection varied with the season and was highest in March when vegetative growth was most vigorous. Numerous arbuscules were observed in the intra-radical spaces of both legume and indigenous weed species. Spore populations were higher in May 2001 than in October 2000 in the cover crop plots as compared with clean cultivated control plots. Peach (*Prunus persica* Batsch) seedlings were readily infected when planted in soils collected from cover crop plots, but infection was significantly lower when seedlings were planted in soils from control plots. All surveyed legumes were found to be highly susceptible to mycorrhizal infection and potentially capable of maintaining high AM inoculum levels in the vineyard.

Key Words: arbuscular mycorrhiza, infection, infectivity, legumes, spore numbers.

Introduction

The mycorrhizal symbiosis is the normal status of most terrestrial plants. The extent to which a plant is colonized by AM fungi is a key determinant of a root system's ability to acquire nutrients from the soil matrix (Mosse, 1973). The plant-fungal interaction is characterized by a bi-directional flow of nutrients: inorganic minerals from the fungus to the host plant and photosynthates from the plant to the fungus (Sylvia et al., 1998). The fungus plays an important role in the plant uptake of P and other nutrients; it is particularly useful in low input, sustainable agriculture. The contribution of AM fungi to mineral nutrition and nitrogen fixation in legumes has been studied and reported extensively. Miller and Jastrow (1994) have summed up the possible roles of AM fungi in biogeochemical nutrient cycling over long periods as: (i) extending the residence time of mineral elements in plants by altering the nutrient content in the vegetation (ii) slowing down the natural nutrient cycling process by placing a greater proportion of minerals in plant biomass, and (iii) as a consequence of (i) and (ii), increasing the ratio of nutrients extracted by plants from mineralization processes instead of the

parent material pools.

Seasonal weeds and over-wintering cover crops are useful for stabilizing AM fungi populations in the soil (Boswell et al., 1998; Galvez et al., 1995; Kabir and Koide, 2000). This property is of particular importance in field crop agriculture where periodic cultivation followed by fallowing contributes to a gradual reduction in viable mycorrhizal propagules. Most legumes form associations with AM fungi. In particular, legumes growing in P deficient soils are dependent on this symbiotic relationship, owing to the high P requirement for nodulation and nitrogen fixation (Brown et al., 1988).

The contribution of orchard cover crops to insect pest management (Altieri and Schmidt, 1985), biomass production and nitrogen fixation (Goh et al., 1995) and tree growth and productivity (Anderson et al., 1992) have been studied. Research done in southwestern Japan has also shown that citrus orchards stand to benefit from sod culture systems. That bur clover sod improved plant growth and fruit yield in satsuma orange trees, and ladino clover improved soil structure, organic matter content and moisture retention capacity; which resulted in improved growth in experimental citrus trees (Sakamoto et al., 1958, 1965). The alleviation of soil erosion and the prevention of the release of nitrate fertilizers into groundwater reserves are other benefits

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from cover crops (Sakamoto et al., 1958; 1960). Ishii et al. (1996) reported that a Bahia grass-based sod culture system is capable of improving the development of AM fungi in citrus orchards.

The dual benefit of biological nitrogen fixation and stabilization of inoculum populations, derived from legumes used as orchard cover crops, has not received much attention. In most citrus orchards in southwestern Japan, intensive cultivation and aggressive chemical weed control ensure that the orchard floor is free of plant cover for most of the season. It has been observed that mycorrhizal propagules are virtually non-existent in such orchards with correspondingly low tree infection (Ishii and Kadoya, 1994, 1996; Rutto et al., 2002). There are no reports on the interaction between mycorrhiza and legume cover crops for the region where our experiment was conducted.

This study compared the effect on soil mycorrhizal infectivity of five legumes that are suited to the mild temperate climate of the experimental site. The ultimate objective was to contribute information that could be useful in selecting legume cover crops for use in citrus and other tree orchards in southwestern Japan.

Materials and Methods

Site

The experiment was conducted at an elevation of about 20 m above sea level at the Ehime University Farm in southwestern Japan (33° 57' N, 132° 47' E). The region has a temperate climate with hot, humid summers and cold, dry winters. The soil is a eutric fluvisol (FAO/UNESCO) with a pH (1:5, soil:H₂O) of 5.7 and a bulk density of a 1.08 g · cm⁻³. Soil content of major elements at the start of the experiment (October 2000) was N, 0.16%; P, 0.48%; K, 0.08%; Ca, 1.10% and Mg, 0.14% DW.

Plant establishment

The row space between 5-year-old grapevines, planted at a spacing of 4.0 by 4.5 m was rotor-tilled in September 2000 and 0.25 m² plots with 0.5 m inter row spaces were prepared. In October, Chinese milk vetch (CMV), hairy vetch (HVE), white clover (WCL) and red clover (RCL) seeds obtained from Kaneko Seed and Seedling Co. (Maebashi, Japan), and narrow-leaf vetch (NLV) seeds harvested from the Ehime University Farm were sown by broadcasting to establish high-density stands. Two additional treatments, (i) plots under indigenous weeds minus any of the above legumes (IWE) and (ii) plant-free control plots (BSO) were also prepared as control. The plots were arranged in a completely randomized design with each treatment replicated six times. Control plots were kept vegetation-free by regular weeding.

Sample collection and processing

Soil samples for spore enumeration were collected from all plots in October 2000 and May 2001. Root samples for fungal infection analysis were collected from the legume and indigenous weed plots in January, March and May 2001. Root samples were washed and stored in 50% ethanol before they were analyzed.

Evaluation of root infection

Root tips (1 ± 0.2 cm) were excised and cleared by autoclaving in 10% KOH before staining in 0.03% Chlorazol Black E (CBE) solution (Brundrett, 1994a). The extent and nature of root colonization were quantified under a compound microscope (× 200) by employing a code for each infection category (McGonigle et al., 1990). The frequency of each type of infection was noted per field (10 grids) for ten fields, using the grid intersect method described by Giovannetti and Mosse (1980). To convert the data into percent infection per category, the frequency of each type of infection as a fraction of the total number of grids observed was multiplied by 100.

Spore enumeration

Arbuscular mycorrhizal spores in soil samples collected from all plots were counted at the start (October 2000) and after the termination of the experiment (May 2001). Spores were separated from 20 g of air-dry soil samples by density gradient centrifugation with 50% sucrose (Brundrett, 1994b). Spores were trapped in stacked sieves, rinsed, transferred into Petri dishes, and counted under a dissecting microscope.

Soil infectivity bioassay

The mycorrhizal infectivity of soil samples collected from the legume, indigenous weed and control plots at the start of the experiment (October 2000) and after the termination of the experiment (May 2001) were assayed on peach (*P. persica* Batsch) seedlings. Stratified peach embryos were sown in 500 ml pots, containing a mixture of vermiculite and 50 g each of the respective soil samples. Three replicates were established for each soil sample, and the plants were grown in a greenhouse. Five weeks after planting, root samples were collected from the seedlings and the frequency of mycorrhizal infection was evaluated using similar procedures as in the evaluation of legume infection.

Results

Legume infection

Infection of legumes by AM fungi was high throughout the duration of the experiment (Fig. 1). The level of infection in CMV and HVE remained relatively uniform; CMV consistently displayed higher infection levels relative to the others. All legume species were highly

Fig. 1. Infection levels by arbuscular mycorrhizal (AM) fungi on roots collected in 2001 from five field grown legume species (CMV, Chinese milk vetch; HVE, hairy vetch; NLV, narrow leaf vetch; RCL, red clover; WCL, white clover). Bars represent SE (n=6).

Fig. 2. Monthly variation in % mycorrhizal infections on roots sampled from five legume species (CMV, Chinese milk vetch; HVE, hairy vetch; NLV, narrow leaf vetch; RCL, red clover; WCL, white clover). Infection was separated into the following groups. Hy, hyphae only; Ar, arbuscules; Ve, vesicles; Sp, intra-radical spores; Un, not identified.

susceptible in March, a period of rapid vegetative growth. In May, there was a decline in WCL and RCL, but a slight increase in NLV infection (Fig. 1).

Legume infection properties

The proportion of different mycorrhizal structures in root samples varied with a greater proportion of arbuscules than vesicles occurring in the roots (Fig. 2). From

Fig. 3. Number of spores in 20 g soil samples collected from plots planted with five legumes (CMV, Chinese milk vetch; HVE, hairy vetch; NLV, narrow leaf vetch; RCL, red clover; WCL, white clover), under indigenous weeds (IWE) and a clean cultivated control plot (BSO) in October 2000 and May 2001. Bars represent SE (n=4).

January and March, there was a gradual increase in hyphal colonization of roots for most legumes. The increase in root area infected with only hyphae occurred at the expense of arbuscules and vesicles. With the exception of WCL, intra-radical sporulation was observed in all legumes in May with the highest incidence occurring in NLV (Fig. 2).

Spore population

Spore populations increased considerably in soil samples collected from the indigenous weed HVE and NLV plots after winter (Fig. 3). Generally, with the exception of BSO where there was a slight decline in spore population, an increase in spores was recorded in all treatments (Fig. 3).

Soil infectivity bioassay

Assays on peach (*P. persica*) seedlings of soils collected before the start of the experiment (October 2000) from all treatment plots revealed no significant differences in infectivity. Infectivity remained constant or improved slightly when soils collected in May, 2001 from plots with plant cover were assayed. A significant decline in infectivity was noted for BSO plots (Fig. 4).

Indigenous weed infection

There was constant variation in weed species composition during the experiment (Fig. 5). In addition to the persistent perennial weeds, there was an increase in species diversity in spring as compared with that of the winter months. There was a great variation in the mycorrhizal infection of weeds but the trends for individual species tended to parallel that of legumes: a

higher number of arbuscules appearing in the initial stages followed by a gradual increase in hyphal components (Fig. 5).

Discussion

Practically all legumes, as well as actinorrhizal plants, are mycorrhizal (Trappe, 1979). In this experiment, legume infection levels were high, reaching a maximum in March when plant growth was most vigorous. The

Fig. 4. Mycorrhizal infectivity of soil samples collected from plots planted with five legume cover crops: CMV, Chinese milk vetch; HVE, hairy vetch; NLW, narrow leaf vetch; RCL, red clover; WCL, white clover, under indigenous weeds (IWE) and from clean cultivated control plots (BSO). Peach (*Prunus persica*) seedlings were used for the bio-assay. Different letters denote significant differences (Tukey's multiple comparison test, $P < 0.05$).

high incidence of arbuscules in legume roots demonstrates their dependence on AM fungi for mineral acquisition. The transfer of mineral elements from the fungal partner to the plant host takes place at the arbuscule/cell interface. Polyphosphate is hydrolyzed in the arbuscules and transported as inorganic phosphate (Pi) across the plasma membrane of the host root cell (Smith and Gianninazzi-Pearson, 1988). There seems to be a close relationship between legume and mycorrhizal lifecycles. Maximum colonization occurs in March coinciding with a sudden and sharp increase in legume growth, while diminishing infection concurrent with intra-radical sporulation occurs in May prior to senescence. The increased hyphal infection as compared with arbuscule and vesicle occurrence in March is probably related to increased host metabolism and a concomitant abundance of fungal metabolites.

An increase in sporulation after over-wintering was observed in all the treatments except in soils from control treatment where there was a slight reduction. Galvez et al. (1995) report a general increase in spring of spore populations in plots with plant cover as compared with those without. Except in bare plots where there was a considerable reduction in infectivity, soil infectivity improved or remained constant in all plots with over-wintering plant cover. The decrease in infectivity of bare plot soils after winter is probably due to a decline in spore viability and the absence of plant roots and hyphal fragments that would serve as additional sources of infection. The ability of cover crops to increase the mycorrhizal infectivity of soils has been reported for hairy vetch (Galvez et al., 1995), winter wheat (Boswell et al., 1998) and dandelion (Kabir and Koide, 2000). Contrarily, a significant reduction in

Fig. 5. Infection by arbuscular mycorrhizal (AM) fungi of roots collected from indigenous weed plots. Infection was separated into the following groups: Hy, hyphae only; Ar, arbuscules; Ve, vesicles; Sp, intra-radical spores; Un, not identified.

Av, *Amaranthus viridis*; Ap, *Artemisia princeps*; Af, *Avena fatua*; Bt, *Bothriospermum tenellum*; Cbp, *Capsella bursa-pastoris*; Ca, *Chenopodium album*; Cs, *Conyza sumatrensis*; Dc, *Digitaria ciliaris*; Gs, *Galium spurium*; Gc, *Geranium carolinianum*; La, *Lamium amplexicaule*; Lm, *Lolium multiflorum*; Ol, *Oenothera laciniata*; Pl, *Persicaria longiseta*; Pa, *Poa annua*; Se, *Setaria* spp.; Sa, *Solidago altissima*; So, *Sonchus oleraceus*; Sm, *Stellaria media*; To, *Taraxacum officinale*; Vp, *Veronica persica*.

mycorrhizal inoculum was observed in soils after an extended clean fallow (Thompson, 1987).

The legumes surveyed in this experiment are all capable of sustaining mycorrhizal populations but our data from indigenous weed plots show, however, that species diversity as in mixed stands of legumes or both legumes and non-legumes might prove more beneficial. Bahia grass (*Paspalum notatum* Flugge.) and rat's tail fescue [*Vulpia myuros* (L.) C. C. Gmel.] have been successful in citrus orchards for multiplication of AM and soil stabilization (Ishii et al., 1996; Kirino et al., 1998). A rat's tail fescue/legume combination when legume sowing is delayed would enable the legumes to attain maximum growth towards the end of spring after the fescue has died; this facilitates the establishment of permanent legume cover in orchards.

There are, however, possible disadvantages associated with legume cover crops. Under some circumstances, certain legumes, when used as cover crops, enhance pest infestation by providing alternative pest habitats. The limitations on the kinds of weed control methods that can be used with legume cover crops may be a problem that is often solved by breaking up weed growth cycles with the inclusion of a legume. These are among the many factors that need to be considered before choosing a cover crop system.

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5種類のマメ科作物草生果樹園における菌根菌感染性と胞子数の季節的消長

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摘 要

現在5年生のブドウが栽植されており、近い将来モモの樹を植えることになっている果樹園の空きスペースを利用して、5種類のマメ科被覆作物区と雑草区を作成し、それらの菌根菌感染性と土壤中の胞子数の推移を1年間にわたって調査した。マメ科植物における感染率は季節により異なり、マメ科植物が最も生育盛んになる3月において最も高かった。vesicular-arbuscularタイプに対してarbuscularタイプがマメ科植物と雑草のいずれにおいても優占種であった。胞子数

は2000年10月には裸地区と被覆区にあまり差はなかったが、2001年5月には被覆区で多かった。モモ実生を用いて土壌の菌根菌感染力を調べたところ、被覆区の土壌は調査期間中感染力は同じレベルに維持されるか改善されたが、裸地区では感染力が有意に低くなった。調査した全てのマメ科植物および雑草において高い菌根形成が見られたので、果樹園の被覆植物は窒素固定能を有するだけではなく、土壌の菌根菌感染力や胞子数の維持にも有用であると考えられる。