

Replant Soil and Peach Detritus Inhibit Arbuscular Mycorrhizal Activity and Retard Peach Seedling Growth

Kipkoriony L. RUTTO^{1, 2)} and Fusao MIZUTANI¹⁾

Summary

Growth and arbuscular mycorrhizal (AM) activity were evaluated in peach seedlings transplanted in replant soils in the field, or in media supplemented with peach-root-bark under greenhouse conditions. Peach seedlings planted beside stumps of 15-year-old peach trees recorded lower AM infection and exhibited stunted growth typically associated with the peach replant problem. Mycorrhizal spore populations were also significantly lower around the tree stumps compared with locations 2.0m away. In the greenhouse experiment, incorporating root bark into nursery media retarded seedling growth and adversely affected AM activity. Decaying peach detritus seemed to inhibit mycorrhizal activity and probably delays the initiation of the mycorrhizal symbiosis between seedling hosts and indigenous AM. Such a delay may be one of the factors responsible for growth decline in replanted peach.

Introduction

Peach trees attain bearing age earlier than most fruit tree species but have much shorter productive lives¹¹⁾. Consequently, frequent orchard replanting is necessary but is complicated by the frequent occurrence of 'replant failure'. This disorder is prevalent in orchards previously planted with peach and manifests itself as a decline in tree vigor without clear etiology. This disorder has been studied and reported on extensively^{8, 12, 14)}, and some research projects have been designed based on the following possible causes of replant failure : i) depletion of common nutrients, either by the preceding crop or by soil organisms using root residues as a substrate, ii) minor element deficiencies, iii) diseases developed on the initial planting and carried over to the next crop, iv) direct toxicity of the roots or their decomposition products. The results of the above studies, though highly inconsistent, seem to relegate nutrition and disease to minor roles in the replant problem, with only the fourth hypothesis yielding interesting results¹⁴⁾. A study based on this hypothesis found no difference in growth when a comparison was made between peach seedlings grown in the greenhouse in 20L cans filled with either screened soil from a previous

1) The Experimental Farm, Faculty of Agriculture, Ehime University, Hattanji 498, Matsuyama, Ehime 799-2424, Japan

2) Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT), PO Box 62000, Nairobi 00200, Kenya

peach orchard or soil that had never been planted with peach before. However, when 500g of peach roots were added to virgin soil and the above planting conditions replicated, shoot length and weight was 481cm, 102.5g and 326cm, 53.7g for the control and treated pots respectively. Recently, it has been suggested that replant failure maybe among diseases with a complex etiology and probably involves multiple factors ²⁾.

The gradual accumulation of growth inhibitors in soil has been identified as a likely cause of the poor growth in replants. Root exudates and chemical compounds from decomposing plant parts probably constitute these inhibitors considering that cyanogenic glycosides and condensed tannins¹¹⁾, and hydrocyanic acid ⁸⁾ have been extracted from peach plant parts.

In spite of the documented benefits that plants derive from the mycorrhizal symbiosis, it has not been included in studies on replant failure. Of particular interest is the activity and health of mycorrhizae in replant soils, and the level of interaction with transplanted peach seedlings. The main benefit that plants derive from the mycorrhizal symbiosis is the increased uptake of P and to a lesser extent other immobile soil nutrients like Zn and Cu ^{6, 16)}. In addition, mycorrhizal infection also improves host fitness through improved tolerance to stresses like drought ^{1, 4)}, flooding¹⁵⁾, pest infestation ⁵⁾, and diseases¹³⁾.

The objective of this study was to investigate the relationship between arbuscular mycorrhizal (AM) fungi and peach root detritus and their role in the replant problem of peach.

Materials and Methods

Field experiment

Site

The experiment was carried out in the Ehime University Experimental Farm (Matsuyama City) in southwestern Japan (33° 57' N, 132° 47' E) at an elevation of 20m above sea level. The region has a mild temperate climate characterized by hot humid summers and cold dry winters, and the common soils are eutric fluvisols.

Seedling establishment and experimental setup

Stratified peach (*Prunus persica* Batsch var. 'Ohatsumomo') seed was germinated in sterile vermiculite at the end of March and seedlings were raised in a greenhouse after pricking into 15cm pots. In early May, the seedlings were transplanted in a field that was previously under peach to establish the following two experimental treatments : i) seedlings that were planted 30cm from stumps of 18-year-old peach trees that had been freshly cut (P1) , and ii) , seedlings planted in between rows outside the drip line of the freshly cut trees (P2). The first treatment was repeated by similarly planting seedlings beside the opposite row (P3) as shown in Fig. 1. Soil samples were collected from respective plots for mineral analysis.

Plant growth and biomass data

Shoot length was measured weekly for ten weeks starting from two weeks after transplanting. The experiment was terminated three months after transplanting and the seedlings were carefully excavated from the soil and washed. Root tips were sampled from all the replicates and stored in

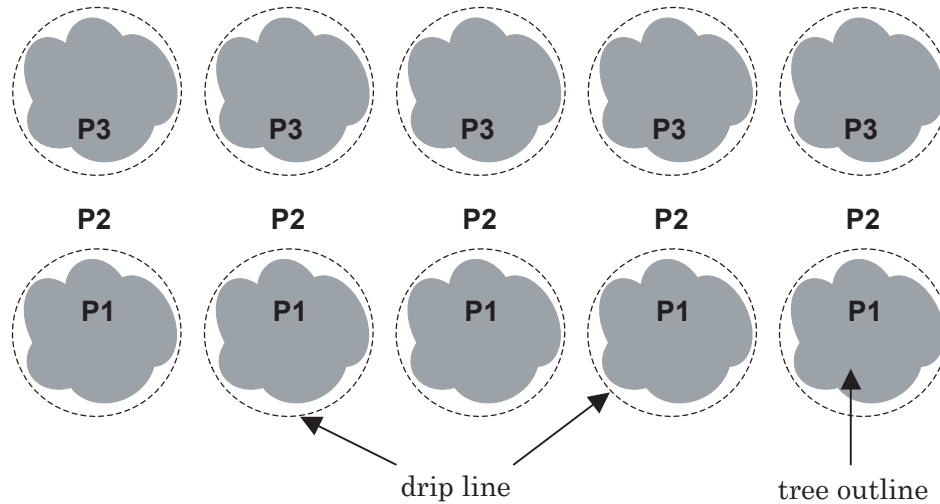


Fig. 1. Layout of the field experiment showing the plots within and outside the drip lines of old peach trees.

50% ethanol before analysis of mycorrhizal infection. Shoot and root fresh weights were measured immediately after harvesting and dry weights after drying for 72hrs at 80°C.

Soil and plant tissue mineral analysis

Soil-P was measured following the 'Bray No.2 method' by extracting 1g per sample soil in a 50ml mixture of ammonium fluoride (0.03M) and HCl (0.1M). Available P was measured by blue colorimetry at 730nm using a spectrophotometer (Hitachi U-2001, Tokyo). Soil Ca, Mg and K, were extracted with a mixture of 0.05M ammonium acetate and 0.0114M strontium chloride by shaking. After filtration, mineral content in samples was analyzed with an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto). Soil-N was analyzed by the dry combustion method using a NC analyzer (Sumigraph NC-80, Sumitomo, Tokyo) and pH (H₂O) was also measured. Oven dried shoots and leaves were ground with a pestle and mortar and a one-gram portion per seedling was weighed and dry-ashed by heating in a muffle furnace at 550°C for 5h. The ash was taken up in 1ml of 20% aqueous HCl and the solution made up to 20ml with distilled-deionized water. Two hundred microliter aliquots from these solutions were further diluted to 10ml before analysis for Ca, Mg and K using an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto). Phosphorus, as molybdate-reactive P, was measured by blue colorimetry at 730nm using a spectrophotometer (Hitachi U-2001, Tokyo), and N was measured by the dry combustion method using a NC analyzer (Sumigraph NC-80, Sumitomo, Tokyo).

Estimation of mycorrhizal infection and spore enumeration

The level of root infection by indigenous AM fungi was evaluated following established methods³⁾. Roots were cut into 1cm length and cleared in 10% KOH by autoclaving at 121°C and 1.2kg/cm² pressure for 15 minutes. After cooling, the roots were acidified with weak HCl and stained in 0.03% Chlorazol Black E solution for two days, followed by de-staining in 50% aqueous glycerol for two days. The root pieces were then mounted on glass slides and the root colonization was quantified under a compound microscope following the grid intersect method⁷⁾.

Mycorrhizal spores were counted in 10g soil samples. Soil samples were repeatedly washed into

stacked sieves (250 μ m, 125 μ m and 45 μ m mesh sizes) and trapped spores were decanted into long test tubes and extracted from excess soil by density gradient separation in 50% sucrose solution. The process was repeated twice per sample before spores were rinsed into Petri dishes and counted under a dissecting microscope.

Greenhouse experiment

Twenty-four uniform seedlings from the batch germinated for the field experiment were transplanted in 15cm ϕ pots filled with a perlite/vermiculite mixture (1 : 2). A two-factor experiment consisting of three (0, 10 and 20g) levels of peach root bark added to media, and inoculation or non-inoculation with AM was set up. Inoculation consisted of spreading 10g (approx. 500 spores) per pot of soil-based *Gigaspora margarita* Becker & Hall (Central Glass, Tokyo) inoculum on the media surface followed by light watering. The seedlings were maintained under greenhouse conditions and each seedling was fertilized weekly with 200ml of full strength Hoagland's nutrient solution¹⁰. Plant height was recorded weekly for ten weeks after transplanting. Shoot and root fresh and dry weight biomass and mycorrhizal root infection data were collected using procedures already described under field experiment.

Lab experiment

One-gram of peach root bark powder was extracted in 100ml of 80% aqueous ethanol for 48hrs at 5 $^{\circ}$ C . The extract was filtered and the ethanol fraction removed by evaporation *in vacuo*. The solution was made up to 100ml (10000mg root bark equivalent L⁻¹) with distilled water and used to design a lab experiment involving the axenic germination of mycorrhizal spores. The treatments were 0, 0.1, 1, 10 and 100mg L⁻¹ root bark equivalent taken up in gellan gum (5gL⁻¹) media and poured into 7cm ϕ Petri dishes before autoclaving at 121 $^{\circ}$ C and 1.2kg/cm² pressure for 15mins. *Gigaspora margarita* spores were isolated from a soil based commercial inoculum (Central Glass, Tokyo), sterilized in a mixture of 5% Chloramine T and 0.04% streptomycin (with a few drops of Tween 20) rinsed repeatedly with sterile distilled water and aseptically transferred onto the gellan gum media described previously (4 spores per dish). The dishes were incubated at 25 $^{\circ}$ C for two weeks, and spore germination (%) was determined and hyphal length per germinated spore estimated under a binocular microscope.

Results and Discussion

Field experiment

The content of major nutrients in the soil and peach shoots and leaves is shown in Tables 1 and 2. Soil N and K are significantly higher in samples collected from the middle of the row, 2.0m away from the tree stumps. However, there was no difference in plant nutrition between plots implying that soil mineral content did not limit seedling growth, although, there was a significant difference in seedling growth between the two treatments. Stunted growth, characteristic of peach replants growing in sick soil was observed in seedlings transplanted beside peach tree stumps. Growth in seedlings planted between rows, 2.0m away from the stumps was more vigorous and there was a significant difference in height between the two groups of seedlings starting from three weeks after

transplanting (Fig. 2). The long-term accumulation in soil of growth inhibitors contained in chemical compounds released after the decay of above and below-ground plant parts has been identified as a likely cause of the gradual decline in the performance of peach replants¹⁴. The difference in growth observed in this experiment suggests that inhibitor concentration decreases away from the tree trunk and may not exist outside the drip-lines of old trees.

Data on soil spore populations and seedling root infection by indigenous AM fungi is shown in Fig. 3. Soil samples collected from between tree rows yielded higher spore numbers than those from beside the trees stumps and seedling infection by AM fungi followed a similar trend. Root infection by AM fungi was significantly higher in peach seedlings outside than within the drip-lines of old trees. The greater difference in root infection between plots as compared with differences in spore numbers implies that a higher percentage of spores in the soil beside the trunks may have lost their viability.

These results demonstrate the likely adverse effect of peach detritus on mycorrhizal propagules and on the symbiosis itself. The importance of the mycorrhizal symbiosis in peach seedling growth has been demonstrated¹⁵. The likely negative interaction between AM fungi and the chemical compounds that accumulate over time in soils under peach introduces a second dimension to the peach replant problem; that of secondary seedling growth inhibition through the disruption of the mycorrhizal symbiosis.

Table 1 Soil pH and mineral content in a peach orchard replanted with peach seedlings.

Plot	Soil pH (H ₂ O)	Mineral content (%)				
		N	P	K	Ca	Mg
1	4.2a ^z	0.13a	0.50a	0.027a	0.71a	0.050a
2	5.5b	0.24b	0.69a	0.080b	0.91a	0.086b
3	4.6ab	0.10a	0.42a	0.024a	0.82a	0.059a

^zColumn values followed by different letters are significantly different at p<0.05 (Tukey's multiple comparison test).

Table 2 Mineral content in shoots and leaves of peach (*Prunus persica* Batsch) seedlings transplanted in different sites in an old peach orchard.

Plot	Mineral content (%)				
	N	P	K	Ca	Mg
1	3.9	0.047	1.5	0.77	0.49
2	5.3	0.050	1.8	0.67	0.46
3	4.1	0.046	1.9	0.66	0.41

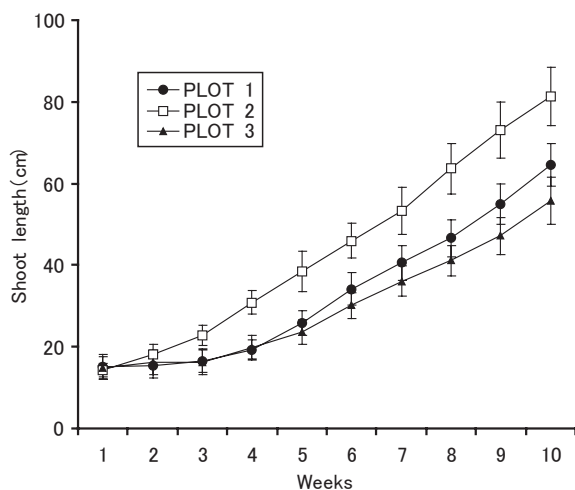


Fig. 2 Shoot growth in peach (*Prunus persica* Batsch) seedlings transplanted in field replant soil within or outside the drip-lines of 18 year-old trees. Bars represent SE (n=5).

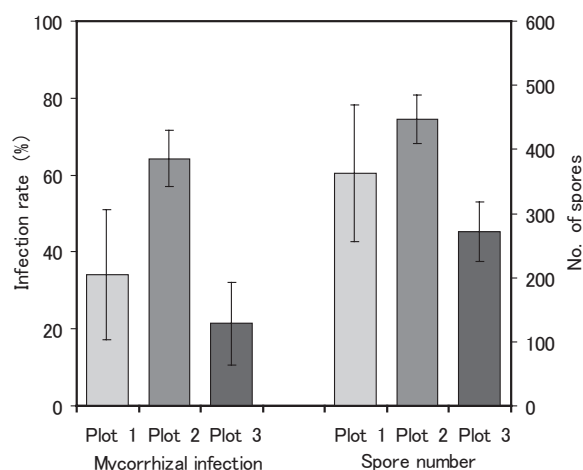


Fig. 3 Mycorrhizal infection, and spore populations in root and soil samples collected from peach (*Prunus persica* Batsch) seedlings transplanted in field replant soil within or outside the drip-lines of 18-year-old trees. Bars represent SE (n=5).

Greenhouse experiment

In the house experiment, seedling growth was relatively uniform up to week 7. The impact on seedling growth of AM inoculation is evident between week 8 and 9 and is expressed as a slight increase in mycorrhizal seedling growth as compared with the non-inoculated treatment (Fig. 4). There is, however, no difference in growth between the mycorrhizal control and mycorrhizal seedlings planted in media supplemented with peach root bark. This is probably due to the short duration of the study. The impact of the root bark supplement might be expressed cumulatively over a wider time span as the bark decomposes. Non-mycorrhizal seedlings planted in media supplemented with peach root bark powder performed slightly worse than the non-mycorrhizal controls planted in root bark-free media. This is evidence for the existence of a direct effect on plant growth by peach root detritus as already reported¹⁴). There is a gradual decline in mycorrhizal infection with increasing amounts of root bark amendment (Fig. 5). The effect of declining infection did not express itself in seedling performance because infection remained above 50% even in the treatment with the highest amount of root bark added to the media. However, assuming the decline in infection as a response to increasing proportions of root bark in media continues as projected in Fig. 5, it is clear that that at some point, it will have a negative impact on seedling growth. Future experiments should introduce root bark treatments over a wider range to test this hypothesis.

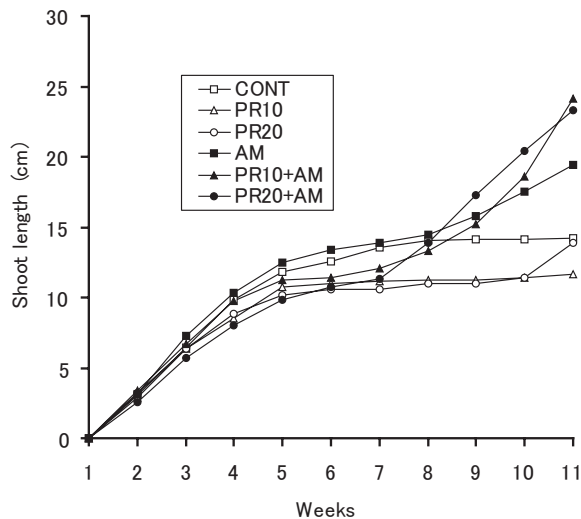


Fig. 4 Cumulative growth in mycorrhizal (AM) and non-mycorrhizal peach (*Prunus persica* Batsch) seedlings grown under greenhouse conditions in media amended with 0, 10 or 20g per pot of peach root bark (PR).

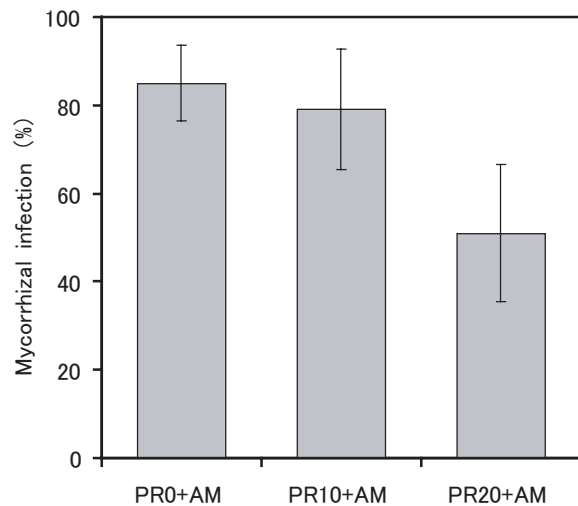


Fig. 5 Mycorrhizal infection in peach (*Prunus persica* Batsch) seedlings grown under greenhouse conditions in media amended with 0, 10 or 20g of peach root bark (PR) and inoculated with *Gigaspora margarita* Becker & Hall. Bars represent SE (n=4).

Lab experiment

The effects of peach root bark extracts on mycorrhizal spore germination and hyphal growth are shown in Table 3. Except at the highest extract concentration (100mg L^{-1}), spore germination was almost uniform, falling only slightly from 25% at 0 and 0.1mg L^{-1} to 17% at 1 and 10mg L^{-1} respectively. It appears root bark extracts do not affect spore germination at concentrations below 10mg L^{-1} , but spore germination along with microbial contaminants that are routinely encountered in such axenic culture conditions were completely inhibited at 100mg L^{-1} . However, root bark extract treatments had a significant effect on hyphal elongation. Hyphal growth was highest in the non-treated (water only) control experiment and significantly lower at 10mg L^{-1} . These results show that the extract has a negative effect on AM fungi and shows the likely effect that peach detritus may have on the mycorrhizal symbiosis under field conditions.

Table 3 Spore germination and hyphal elongation in *Gigaspora margarita* Becker & Hall cultured in media containing different levels peach root-bark extract.

Extract conc. (mg d.w. L^{-1})	Germination (%)	Hyphal length (mm)
0	25	105a ^z
0.1	25	70ab
1	17	35bc
10	17	25bc
100	0	0c

^zDifferent letters indicate significant difference at $p < 0.05$ (Tukey's multiple comparison test).

Conclusion

The results obtained in this experiment support the hypothesis that the interaction between decaying peach root detritus and the chemical compounds that accumulate over time in peach orchard soils with AM fungi contribute to the peach replant problem. We have demonstrated that seedlings planted near old peach tree trunks have lower levels of mycorrhizal infection than those growing further away, which in turn contributes to differences in seedling performance. When the possible active compounds in the old peach root bark were extracted and their effect on spore germination and hyphal elongation tested, it was found that at higher concentrations, the extracts are capable of completely inhibiting spore germination. Hyphal elongation was also observed to decline in direct proportion to the concentration of bark extract added to the media. These results are evidence of a likely link between the peach replant problem and the negative interaction between AM fungi and peach detritus.

Future studies should attempt to identify the actual compounds contained in peach root bark that inhibit AM function. Experiments should also be designed to study the performance in replant situations of seedlings pre-inoculated with a viable AM strain.

Literature Cited

- (1) Augé, R. M., A. J. W. Stodola, M. S. Brown and G. J. Bethlenfalvay. 1992. Stomatal response of mycorrhiza cowpea and soybean to short-term osmotic stress. *New Phytol.* 120 : 117-125.
- (2) Benizri, E., S. Piutti, S. Verger, L. Pagès, G. Vercambre, J. L. Poessel and P. Michelot. 2005. Replant diseases : Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. *Soil Biol. Biochem.* 37 : 1738-1746.
- (3) Brundrett, M. 1994. Clearing and staining mycorrhizal roots. In: Brundrett, M., L. Melville and L. Peterson, (Eds.), *Practical methods in mycorrhiza research*. Mycologue Publications, Sidney, BC, Canada, pp. 42-46.
- (4) Drüge, U. and F. Schönbeck. 1992. Effect of vesicular-arbuscular mycorrhizal fungi infection on transpiration, photosynthesis of flax (*Linum usitatissimum* L.) in relation to cytokinin levels. *J. Plant Physiol.* 141 : 40-48.
- (5) Elsen, A., H. Baimey, R. Swennen and D. De Waele. 2003. Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. *Plant Soil* 256 : 303-313.
- (6) Gerdemann, J. W. 1968. Vesicular arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6 : 396-418.
- (7) Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytol.* 84 : 489-500.
- (8) Gur, A. and Y. Cohen. 1988. Causes of soil sickness in replanted peaches. 1. The role of cyanogenesis in peach soil sickness. *Acta Hort.* 233 : 25-31.
- (9) Gur, A. and Y. Cohen. 1989. The peach replant problem-some causal agents. *Soil Biol. Biochem.*

- 21 : 829-834.
- (10) Millner, P. D. and D. G. Kitt. 1992. The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza* 2 : 9-15.
 - (11) Mizutani, F. 1980. Studies of the replant problem and water tolerance of peach trees. *Mem. Coll. Agr., Ehime Univ.* 24 : 115-198.
 - (12) Mizutani, F., R. Hirota and K. Kadoya. 1988 Growth inhibiting substances from peach roots and their possible involvement in peach replant problems. *Acta Hort.* 233 : 37-43.
 - (13) Pozo, M. J., C. Cordier, E. Dumas-Gaudot, S. Gianinazzi, J. M. Barea and C. Azcón-Aguilar. 2002. Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *J. Exp. Bot.* 368 : 525-534.
 - (14) Proebsting, E. L. and A. E. Gilmore. 1941. The relation of peach root toxicity to the re-establishing of peach orchards. *Proc. Amer. Soc. Hort. Sci.* 38 : 21-26.
 - (15) Rutto, K. L., F. Mizutani, D-G. Moon and K. Kadoya. 2002. The relationship between cultural practices and arbuscular-mycorrhizal (AM) activity in orchards under different management systems. *J. Jap. Soc. Hort. Sci.* 71 : 601-609.
 - (16) Smith, G. S. 1987. Interactions of nematodes and mycorrhizal fungi. In : Veech, J. A. and D.W. Dickson (Eds.), *Vistas on nematology*. Society of Nematologists Inc., Hyattsville, MD, USA, pp. 292-300.

連作土壌およびモモの根の残渣は菌根菌の活動を抑制しモモ実生の生長を抑制する

キプオリオニ L. ルット・水谷房雄

摘 要

圃場における連作園土壌及び温室内でモモの根の残渣を加えたポットに植えられたモモ実生の生長と菌根菌の活動について調査を行った。15年生のモモの切り株の近くに植えたモモ実生は菌根菌の感染率が低く、典型的な連作障害と思われる生育抑制が観察された。菌根菌の孢子密度も2 m離れた場所に比べて、切り株の近くでは低かった。いっぽう、温室でのモモの根の残渣を加えた実験でも、根残渣の加用は実生の生長を抑制し、菌根菌の活動を抑制した。モモの根残渣の分解が、菌根菌の活動を抑制し、モモ実生と菌根菌の共生の開始を遅らせていると思われた。そのような共生の遅延もモモの改植樹の生長抑制の一因となっている可能性がある。