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Studies on the Use of Plant Growth-promoting Rhizobacteria and Effective Micro-organisms in Controlling White Root Disease of Rubber

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Plant growth-promoting rhizobacteria (PGPR) and 'effective microorganisms' (EM) were tested for biological control of white root disease of rubber caused by Rigidoporus lignosus. *In laboratory agar-plate interaction studies, specific PGPR strains and EM antagonised growth of* R. lignosus *as well as two other root disease fungi,* Ganoderma philippi *and* Phellinus noxious. *The PGPR strain 7NSK2 and EM were then used to treat rubber seedlings in separate field experiments on an Entisol. The treatments were PGPR (or EM), triadimefon, triadimefon and PGPR (or EM) and an uninoculated control, in five replications. Plants, diseased or dead, were verified weekly for up to 24 (PGPR experiments) and 40 (EM experiments) weeks. Application of PGPR or EM did not suppress the disease, and their integration with triadimefon were no better than control by the fungicide alone.*

White root disease (WRD) caused by *Rigidoporus lignosus* (Klotzsch) is a serious root disease of the rubber tree *Hevea brasiliensis*. In areas that previously had rubber, infected root debris remaining in soil following land clearing provide the source of inoculum that spreads to growing plants through root contact. The fungus forms profusely-branched rhizomorphs firmly attached to the infected roots, killing the wood, and developing fruitifications at the collar of trees. The classical method of control is through a collar protectant dressing with pentachloronitrobenzene (PCNB), after digging and removing infected roots. A less-laborious method, effective for immature trees with mild infections, is to drench trees with fungicides triadimefon (Bayleton® 25 WP) and propiconazole (Tilt® 250 EC), but is constrained by costs of multiple dressings, or severity of infections[1]. An alternative yet to be explored is the use of biological agents, or to integrate their application with chemical fungicides.

In laboratory trials, applying antagonistic *Trichoderma* species or triadimefon was effective against *R. lignosus*[2]. In the field, *Trichoderma* failed to enhance control of WRD by triadimefon[3]. *Trichoderma* had been used in Indonesia to control WRD on young plants[4].

Plant growth-promoting rhizobacteria (PGPR) have also been successfully used as

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biological control agents against important diseases caused by soil-borne fungi such as *Pythium ultimum*[5], *Phytophthora cinnamomi*[6], *Fusarium oxysporum* f.sp.lini[7,8], *Ceratocystis ulmi*[9], *Gaeumannomyces graminis*[10,11] and *Sclerotium rolfsii*[12]. Their suppression of root diseases in suppressive soils is *via* production of siderophores, antibiotics or bacterial HCN[8,13,14] that often resulted in large increases in growth and yield of some agricultural crops[15-19]. In plate culture studies, specific PGPR strains antagonise growth of *R. lignosus, Ganoderma philippi* and *Phellinus noxious*, inferring their potential for biological control of the three major rubber root diseases[2]. Strains of PGPR have been identified as *Pseudomonas fluorescens, Pseudomonas putida, Arthrobacter citreus* and *Serratia liquefaciens*[20] but the list of species involved, and the ionophores characterised, may in reality be longer.

In a biologically-active soil, the complex community of beneficial microorganisms and soil fauna decompose organic wastes, recycle plant nutrients and protect plants from pests. Conventional agriculture contributes to biological imbalance by replacing biological diversity with a single plant genotype, often in monoculture. A school of organic agriculture (Kyusei Nature Farming, Japan) advocates developing a more stable, productive and sustainable nature farming system that excludes chemical fertilisers and pesticides by using 'effective microorganisms' (EM)[21,22]. The mixed cultures of beneficial soil microorganisms increase the microbial diversity of soils which in turn enhance the growth, health and yield of crops. There has been some evidence to indicate that EM suppress the activity of pathogenic microorganisms and control pests when sprayed on plants[22].

The purpose of this study was to examine the biocontrol potential of PGPR and EM against WRD in laboratory media, and to evaluate their use and integration with chemical fungicides in the field.

MATERIALS AND METHODS

Strain and Inoculum Preparation

PGPR. All strains were prepared by culturing on King's Medium B (KB) agar[23] for 72h at 28°C. The widely-known PGPR strains used were *Pseudomonas aeruginosa* 7NSK2 supplied by W. Verstraete, University of Gent (Belgium)[24], *Pseudomonas fluorescens* TL3 and *Pseudomonas putida* BK1 from T.J. Burr, Cornell University (USA)[15], *P. fluorescens* strains 34-13 and GR12-2, *Serratia proteamaculans* 1-102 from Esso Ag Biologicals (Canada)[20], and the unknown isolates Hi, Ro, WG18NF, CR30 and 1-4E1 from A.J. Caesar, University of California, Berkeley (USA)[25]. In the field experiments, strain 7NSK2 was selected for its strong inhibition of root disease fungi on agar-plate interaction studies. It produces the fluorescent siderophore pyoverdin with inhibiting *in-vitro* effects of growth of various phytopathogenic fungi[26,27]. Inocula were prepared from 72 h agar-grown cultures by washing cells into a sterile buffer (0.1 M $MgSO_4.7H_2O$).

Effective microorganisms. The stock EM-4 solution was supplied by Prof Sharifuddin Hj Abd Hamid and Dr Anuar Rahim, Universiti Putra Malaysia, Serdang. The formulation was regenerated by mixing 8 ml of original stock and 8 ml of molasses with 4 litres of water in an airtight plastic container and incubating for 72 h[28]. The EM-4 mixture is predominantly lactobacilli with smaller numbers of

photosynthetic bacteria, yeasts and actinomycetes.

Plate Culture Studies

Eleven PGPR strains were interacted against 40 isolates representing 25 fungal genera that were kept in the RRIM culture collection. Large (5 mm) squares or circles were cut from the edges of actively-growing colonies of the various fungi growing on malt-extract agar (MEA; Oxoid CM 59, Unipath Ltd., Basingstoke, UK) and placed on plates of KB seeded with PGPR. The PGPR strains were prepared as 48h cultures in KB broth, and seeded to a final cell density of c.10^4 cells/ml agar. Depending on the growth rates of the fungi, three colonies, spaced approximately 5 cm apart, could be placed on a plate. Controls were plates unseeded with PGPR. For some fungi that failed to grow on KB agar *e.g. Rigidoporus, Ganoderma* and *Guignardia*, a mixture of both MEA and KB at equal rates was used. The prepared plates were incubated at 28°C, and all reactions examined at 3 days – 7 days (7 days – 10 days for the slower-growing fungi) for possible interactions (inhibition, neutral or stimulation). Interaction ratings were made by comparing the growth radius of the test fungi on PGPR plates against the controls, and scored as 0 – (no interation), 1– (weak inhibition *e.g.* slight but definite growth reduction) and 2– (strong inhibition). Two replicate plates were prepared for each combination, and the experiment was repeated to confirm the ratings. The EM mixture was interacted against four representative fungal genera shown (*Table 2*) but using nutrient agar (Oxoid CM 67, Unipath Ltd., Basingstoke, UK) and tryptic soy agar (Gibco BRL, Life Technologies Ltd., Paisley, UK), mixed at equal rates with MEA, to seed the organisms. Controls were plates unseeded with EM-4 and include a set of plates with triadimefon incorporated at 125, 250 and 500 p.p.m. final concentrations. Interaction ratings were based on 5 replicate plates.

Field Experiments

Experimental design. All experiments were laid out in a randomised block design with 5 replications on a low-fertility Entisol (Sg Buloh series; Orthoxic Quartzipsamment) at the RRIM Sungai Buloh Experiment Station. The basic layout was a centrally-placed infected rubber stump as the WRD inoculum source in each replicate plot surrounded by four rubber seedlings placed 15 cm away. Each plot was spaced 1 m apart from its neighbouring plot. Clone RRIM 901 at the two-whorl leaf stage was used as the planting material. The presence of collar rhizomorphs in all plants were used to indicate the success of WRD infection. Plants diseased or dead were verified weekly for 28 weeks in the PGPR experiments (*Experiment 1*, 24 weeks; *Experiment 2*, 28 weeks) and up to 40 weeks (*Experiment 3*, 40 weeks; *Experiment 4*, 28 weeks) in the EM experiments.

In the PGPR experiments, the treatments were PGPR and an uninoculated control (*Experiment 1*) and PGPR, triadimefon, triadimefon + PGPR and uninoculated control (*Experiment 2*). In the EM experiments, the treatments were EM, triadimefon, triadimefon + EM and an uninoculated control (*Experiments 3* and *4*). Since *Experiment 4* was simply a repeat of *Experiment 3* but with a modified inoculum, the same field plots were used after 16 weeks to benefit from ecosystem stability.

Microbial inoculation. In Experiment 1, the seedling roots in polybags were drenched with a turbid 100 ml suspension of 7NSK2 one week prior to planting. The uninoculated control treatments received similar amounts of the buffer solution. In Experiment 2, the treatments were applied around the infected stumps in the field after 4 weeks when seedlings were already infected by WRD. In Experiment 3, the roots of polybag seedlings were also drenched with the undiluted EM-4 suspension prepared from stock cultures (100 ml/plant) one week prior to transplanting in the field. A second application of EM-4 solution was applied in the field at 11 weeks after planting. Because the EM-4 liquid inoculum in Experiment 3 did not suppress the disease, the inoculum was modified in Experiment 4 by incorporating the organisms in a chicken dung-rice bran formulation ('Bokashi'[26]) and applied to roots at planting. After positioning the seedlings in the planting hole, 200 g/plant of the solid inoculant was placed around roots in layers and successively covered with soil. Thereafter a weekly drench of freshly-prepared EM-4 liquid inoculum was applied (100 ml/plant) for up to 24 weeks.

Fungicide. Triadimefon (Bayleton® 25WP; 25% a.i.) was applied at 5 g in 500 ml water to each plant. For the mixed treatments, triadimefon and microbial antagonists were separately prepared but poured together during root drenching. The rate of triadimefon application was reduced to 2.5 g in 500 ml of water/plant in the EM experiments.

Statistics

Analyses of variance to compare means of individual treatment at the P=0.05 probability level were based on the $\sqrt{(X + 1)}$ transformed data.

RESULTS

Plate Culture Studies

All PGPR strains were capable of exhibiting some degree of *in-vitro* antagonisms against the fungi tested (*Table 1*). Among the PGPR, strain WG18NF was least effective and affected only one (*Pestalotia* sp.) fungal isolate. Strains 7NSK2 and Hi consistently showed strong inhibitions towards mycelial growth of *R. lignosus, G. philippi* and *P. noxious*. The EM-4 organisms, in either media combinations, also inhibited growth of these root parasites (*Table 2*; *Figure 1*). The fungal isolates were totally suppressed by the fungicide at all levels used.

Field Experiments

In *Experiment 1*, almost all plants displayed collar rhizomorphs within 2 weeks to 8 weeks. Although most plants were dead by 24 weeks, the plant deaths were slower in the PGPR treatments that were perhaps due to initial resistance to rhizomorph infection during the first 8 weeks (*Figure 2*). Treatment differences between PGPR and the controls in the number of plants dead were significant ($P<0.05$) at the 8, 12 and 16 week samplings. Inoculation with PGPR, however, did not suppress the disease by 24 weeks. In *Experiment 2*, all plants given PGPG were dead by 28 weeks and treatment differences between PGPR and the controls in the number of plants dead were not significant at all sampling stages. In this trial, only treatments containing triadimefon suppressed the disease. However, differences between the integrated triadimefon + PGPR and the triadimefon treatments were not significant at all sampling stages.

TABLE 1. THE EFFECT OF PGPR STRAINS ON GROWTH OF FUNGI[a]

Catalog No.	Fungi	Growth reactions with 7NSK2	1-102	TL3	BK1	34-13	GR12-2	CR30	1-4E1	WG18NF	Ro	Hi
1134	*Fusarium semitectum*	1-	1-	2-	1-	1-	2-	2-	2-	0	1-	2-
1133	*Fusarium oxysporum*	1-	1-	2-	2-	1-	1-	2-	2-	0	2-	2-
1160	*Fusarium moniliforme*	1-	1-	2-	1-	1-	1-	1-	2-	0	1-	1-
1194	*Fusarium decemcellulare*	1-	1-	1-	1-	1-	1-	1-	1-	0	0	1-
1195	*Fusarium solani*	1-	0	2-	1-	0	1-	1-	1-	0	0	2-
B8	*Trichoderma* sp. 1	2-	0	2-	2-	2-	2-	2-	2-	0	1-	2-
B9	*Trichoderma* sp. 2	2-	0	2-	2-	2-	2-	2-	2-	0	1-	2-
B10	*Trichoderma* sp. 3	2-	0	2-	2-	2-	2-	2-	2-	0	1-	2-
1	*Penicillium* sp.	2-	2-	2-	1-	2-	1-	2-	1-	0	0	1-
1184	*Ceratocystis fimbriata*	2-	1-	2-	2-	2-	1-	2-	2-	0	1-	2-
1262	*Guignardia heveae*	2-	2-	2-	0	0	0	0	0	0	0	0
1266	*Corticium salmonicolor*	2-	1-	2-	2-	2-	2-	2-	2-	0	2-	2-
1199	*Phoma* sp.	2-	2-	2-	2-	2-	2-	2-	2-	0	2-	2-
C8	*Colletotrichum gloeosporiodes*	1-	2-	2-	2-	2-	2-	2-	1-	0	1-	2-
1252	*Dreschlera heveae*	2-	1-	1-	1-	2-	2-	2-	1-	0	1-	2-
1248	*Corynespora cassiicola*	1-	1-	1-	1-	1-	1-	1-	1-	0	1-	2-
1154	*Curvularia* sp.	2-	1-	2-	1-	2-	2-	2-	2-	0	1-	2-
1198	*Botryodiplodia* sp.	2-	1-	2-	2-	2-	2-	2-	2-	0	2-	2-
1034	*Chaetomium* sp.	2-	2-	1-	2-	2-	2-	2-	2-	0	2-	2-
CY1	*Cylindrocladium quinqueseptatum*	2-	0	2-	2-	2-	2-	2-	2-	0	2-	2-
734	*Nigrospora* sp.	2-	2-	1-	2-	1-	1-	2-	2-	0	0	1-
1222	*Marasmius* sp.	0	0	0	0	0	0	1-	1-	0	0	1-
1234	*Ustulina* sp.	1-	1-	1-	2-	2-	2-	2-	2-	0	2-	2-
1146	*Xylaria* sp.	2-	1-	0	2-	2-	2-	2-	2-	0	2-	2-
1174	*Pestalotia* sp.	1-	1-	1-	1-	2-	2-	2-	2-	1-	2-	2-
1159	*Thielavia* sp.	1-	0	2-	1-	1-	2-	2-	2-	0	1-	2-

TABLE 1 THE EFFECT OF PGPR STRAINS ON GROWTH OF FUNGI[a] (CONTD)

Catalog No	Fungi	Growth reactions with 7NSK2	1-102	TL3	BK1	34-13	GR12-2	CR30	1-4E1	WG18NF	Ro	Hi
4	*Gliocladium* sp	2-	0	2-	1-	1-	1-	2-	1-	0	1-	0
1462	*Phytophthora botryosa*	2-	0	0	2-	2-	2-	2-	2-	0	2-	2-
1375	*Phytophthora palmivora*	2-	2-	2-	1-	2-	2-	2-	1-	0	2-	2-
1216	*Rigidosporus lignosus*	2-	1-	1-	0	0	0	1-	1-	0	0	2-
1189	*R lignosus*	2-	1-	1-	1-	0	1-	2-	1-	0	0	2-
1215	*R lignosus*	2-	1-	1-	1-	0	1-	0	1-	0	1-	2-
1161	*R lignosus*	2-	1-	0	1-	0	1-	2-	1-	0	0	2-
1037	*R lignosus*	2-	2-	2-	2-	2-	0	2-	2-	0	2-	2-
1060	*Phellinus noxious*	2-	0	2-	2-	1-	2-	2-	2-	0	2-	2-
1187	*Ganoderma philippi*	2-	0	2-	2-	2-	2-	2-	0	0	2-	2-
1122	*G philippi*	2-	0	1-	1-	2-	1-	2-	1-	0	2-	1-
1123	*G philippi*	2-	0	2-	2-	2-	1-	2-	2-	0	2-	2-
1066	*G philippi*	2-	2-	2-	2-	2-	2-	2-	2-	0	1-	2-
1099	*G philippi*	2-	2-	2-	2-	1-	1-	2-	2-	0	2-	2-

[a]Means of duplicate plates, 3 fungal colonies/plate, at 7d observations. Interaction rating 0, neutral, 1- , weak inhibition; 2- , strong inhibition

TABLE 2. THE EFFECT OF 'EFFECTIVE MICROORGANISMS' (EM) ON GROWTH OF ROOT DISEASE FUNGI[a]

Catalog No.	Fungi	Growth reactions with EM-4
NRL	*R. lignosus*	2-
1161	*R. lignosus*	2-
1187	*G. philippi*	2-
1060	*P. noxious*	2-

[a]Means of 5 replications; 3 fungal colonies/plate, at 7d observations. Interaction rating as in *Table 1*.

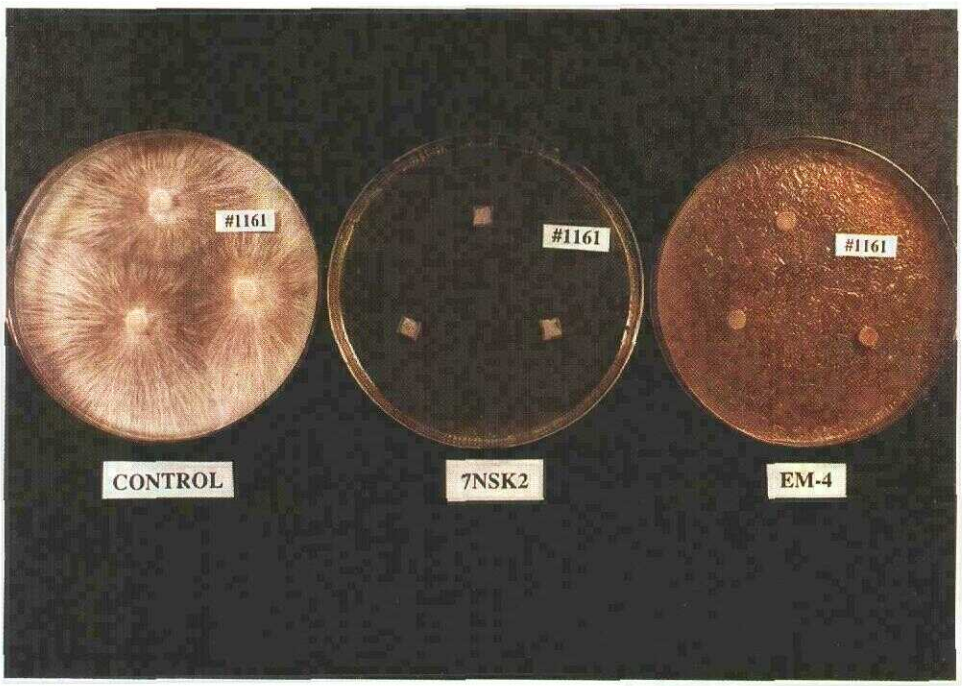

Figure 1. The inhibition of R. lignosus *(#1161) by* P. aeruginosa *(7NSK2) and EM-4.*

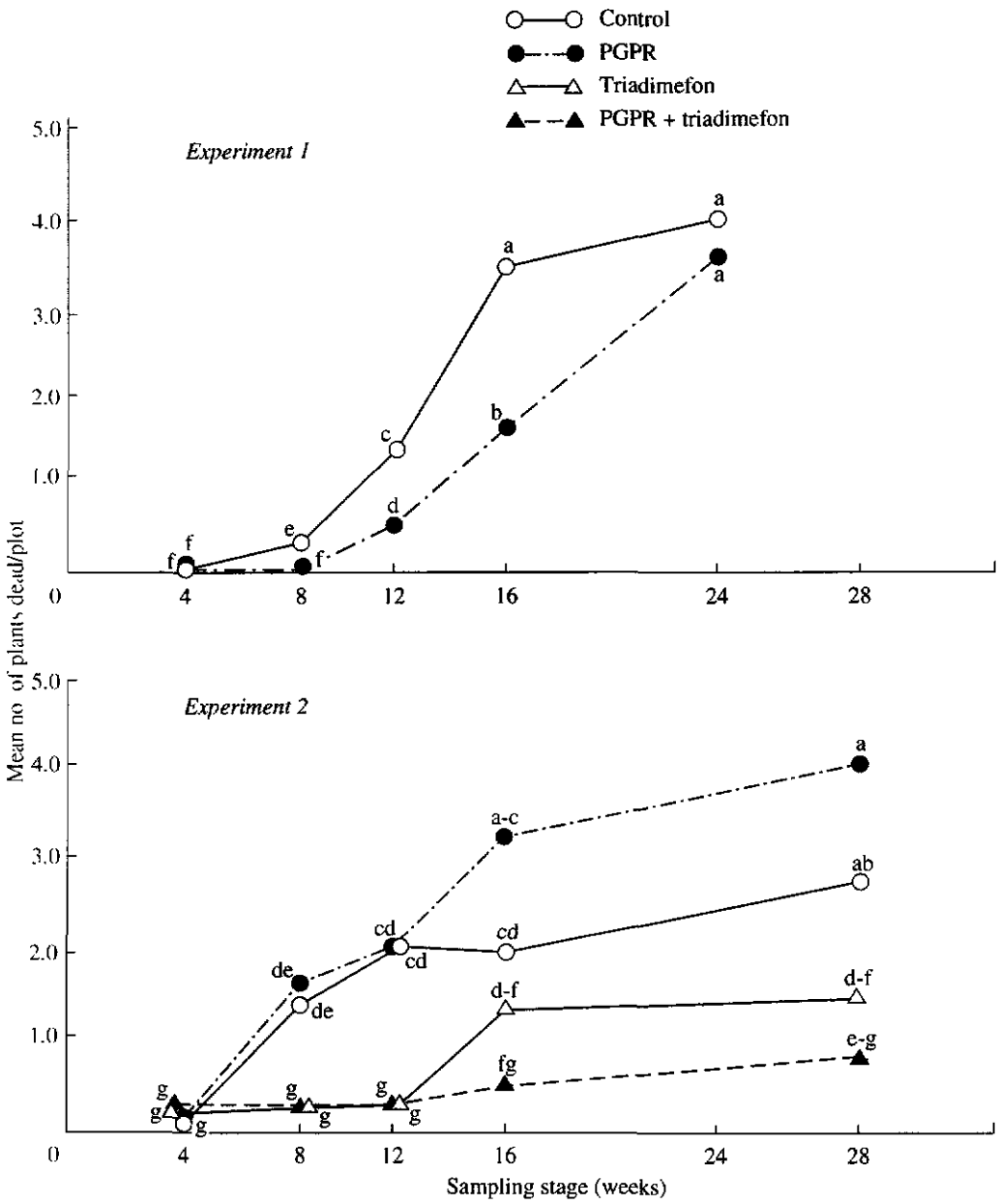

Figure 2. Effect of PGPR and triadimefon on the survival of seedlings infected with R. lignosus *(Field experiments). Data are means of 5 replicate plots; values not sharing common letters are significantly different (P<0.05).*

In *Experiment 3*, EM applied as a liquid inoculum did not suppress the disease and was not different from the untreated control in the number of trees dead by 40 weeks (*Figure 3*). Treatments containing triadimefon, with or without EM, were most effective in controlling the disease by 24 weeks. In *Experiment 4*, EM caused most plant deaths by 28 weeks but this was not significantly different from the controls. However, the loss of plants due to Bayleton + EM at 28 weeks was significantly higher than when Bayleton was applied alone.

DISCUSSION

Root diseases caused by *R. lignosus, G. philippi* and *P. noxious* has traditionally been a subject of utmost concern to rubber enthusiasts in Malaysia. These diseases cause a reduction in the planting stand thus lowering production of the rubber crop. In the plate culture studies, the PGPR organisms showed some evidence of antagonistic activity towards WRD. All except one PGPR strain were capable of varying degrees of antagonisms towards other parasitic fungi of economic importance (*Corticium salmonicolor, Colletotrichum gloeosporioides, Drechslera heveae, Corynespora cassiicola, Phytophthora botryosa* and *Phytophthora palmivora*) to *H. brasiliensis*. However, the findings on agar could not be reliably extrapolated into the natural soil environment since the field trials indicated that PGPR was not an effective protectant against WRD. PGPR inoculation before the onset of WRD may delay infection and death as in *Experiment 1* but failed to suppress the disease if applied after the onset of the disease (*Experiment 2*). A similar trend was observed in the EM experiments. There was no statistical evidence to indicate that integrating PGPR with triadimefon was more effective in pathogen control than when using the fungicide alone. In fact, addition of EM reduced the effectiveness of triadimefon in *Experiment 4*. This could be due to the presence of organic matter in the inoculum since additional organic matter in a *Trichoderma* medium was also shown to reduce the effectiveness of WRD control by triadimefon[3]. In a previous experiment, a relatively better success in WRD biocontrol was achieved by using fungal hyperparasites[2]. Biological control works where chemical controls have been unacceptable or inadequate, as with *Fusarium* root rot of wheat, take-all of wheat, crown gall of fruit trees and *Phytophthora* root rot of avocado and pineapple[29]. Biological control alone rarely gives better control than a good fungicide and so an integrated system of a biocontrol agent and a compatible chemical fungicide is considered as possibly the most effective and economic method of control[29-31]. Another impediment to the widespread use of biocontrol aspects in commercial agriculture is its inconsistent performance[32].

The current concern about environmental problems from a chemical-based conventional agriculture system has motivated researchers worldwide to seek alternatives in natural farming systems. In an EM-based Kyusei Nature farming system, EM formulations applied to soil enhance the availability of nutrients to plants, increase humus formation, suppress weeds and reduce diseases and insect infestation of crops. Indeed, EM is a beneficial microbial inoculant that increases the utility of soil organic matter and establishes a more favourable microbiological equilibrium in the rhizosphere[33]. In pot experiments with spinach grown in soils amended with compost or fertiliser, Imai and Higa[34] found that numbers of *Fusarium* spp. were suppressed in the

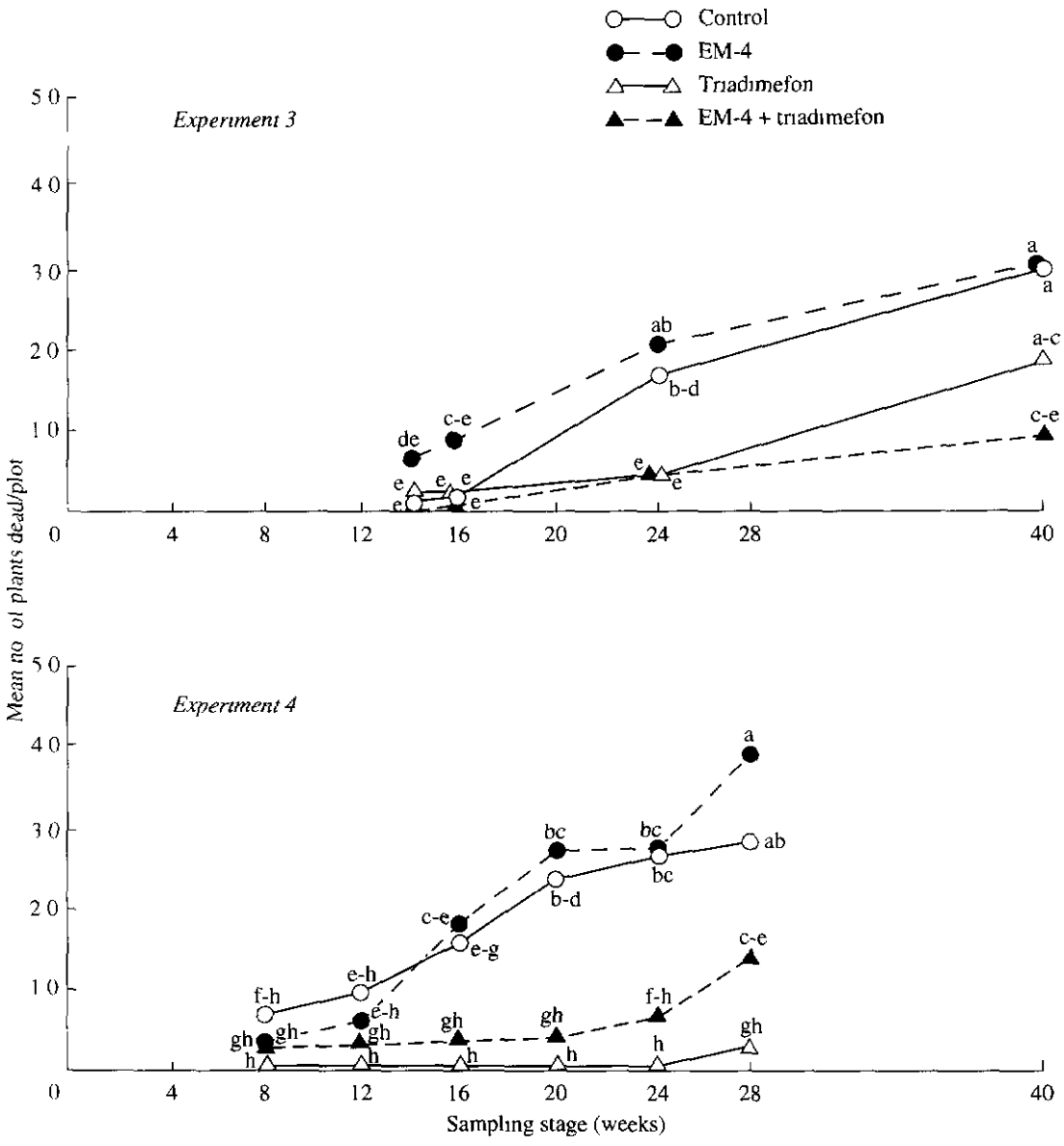

Figure 3 Effect of EM 4 and triadimefon on the survival of seedlings infected with R lignosus *(Field experiments) Data are means of 5 replicate plots, values not sharing common letters are significantly different (P<0 05)*

rhizospheres of plants grown in the compost-amended soil compared to numbers in the fertiliser-amended soils. They highlighted the importance of organic matter and suggested that EM enhanced the transformation of a disease-inducing soil to a disease-suppressive soil. It is also known that improved crop yields in the first test crop of an organically-farmed area derived from a conventionally-farmed system is rarely obtained since conventionally-farmed soils contain low levels of organic matter and that it may take years to develop a good quality soil from organic composted materials[34]. This would indicate that a stable agroecosystem with its harmonious balance of plant, animal and microbial biodiversity would be needed to preserve crop growth and health. Chemical fertilisers, herbicides and pesticides, although effective for crop production, may damage beneficial microorganisms and other intrinsic features of the soil if not correctly used, and causing soil to be disease-inducing. In our field experiments, EM-4 applied as a liquid inoculum or in a rice bran-chicken dung carrier were not able to suppress the disease. A better control by the fungicide in *Experiment 4* was probably achieved from its residual effects in soil.

The planting of rubber in modern agriculture with its dominant inputs of selected clones, chemo-synthetic fertilisers, agrochemicals, research skills, technology support and credit emphasises maximum crop yields with little emphasis on natural farming methods. Under these circumstances, it is unlikely for PGPR and EM to succeed on their own based on the stable agroecosystem concept discussed. Furthermore it is difficult to control *R. lignosus* which produces extensive rhizomorphs that spreads several metres on the roots. A microorganism that could parasitise *R. lignosus* rhizomorphs would have better potential than organisms which compete for nutrients and space since the pathogen is blessed with an ample foodbase. The potential of biocontrol agents in the approach to plant protection may perhaps be easier realised with diseases caused by Phycomycetes and the lower Ascomycetes. For the higher fungi, an integrated approach similar to that used for *Armillaria mellea* may be necessary[35].

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REFERENCES

1. TAN, A.M. AND ISMAIL HASHIM (1991) Control of White Root Disease of Rubber by Fungicide Dressing. *Proc. Rubb. Res. Inst. Malaysia Rubb. Grow. Conf. 1991*, 343–351.

2. ISMAIL HASHIM AND IKRAM, A. (1991) The Potential of *Trichoderma* and Plant Growth-promoting Rhizobacteria (PGPR) in the Control of Root Diseases of *Hevea* Rubber. *International Conference on Biological Control in Tropical Agriculture*, 27–30 August 1991, Genting Highlands, Malaysia.

3. ISMAIL HASHIM AND CHEW, B.H. (1996) Effects of Integrating *Trichoderma* and Fungicides on Control of White Root Disease of *Hevea* Rubber. *J. nat. Rubb. Res.*, **12(1)**, 43–57

4. SOEPENA, H. (1993) Pemberantasan Jamur Akar Putih Dengan *Trichoderma*. *Warta Perkaretan*, **12**, 17.

5 HOWELL, C R AND STIPANOVIC, R D (1980) Suppression of *Pythium ultimum* Induced Damping-off of Cotton Seedlings by *Pseudomonas fluorescens* and its Antibiotic Pyoluteorin *Phytopath* , **70**, 712 715

6 MALAJCZUK, N (1979) Biocontrol of *Phytophthora cinnamomi* in Eucalypts and Avocados in Australia, *Soil-Borne Plant Pathogens (Schippers, B and Gams, W, eds*), 635 London Academic Press

7 KLOEPPER, J W , SCHROTH, M N AND MILLER, T D (1980) Effects of Rhizosphere Colonization by Plant Growth-promoting Rhizobacteria on Potato Plant Development and Yield *Phytopath* , **70**, 1078–1082

8 SCHER, F M AND BAKER, R (1982) Effect of *Pseudomonas putida* and a Synthetic Iron Chelator on Induction of Soil Suppressiveness to *Fusarium* Wilt Pathogens *Phytopath* , **72**, 1567 1573

9 SHEFFER, R J (1983) Biological Control of Dutch Elm Disease by *Pseudomonas* species *Ann Appl Biol* , **103**, 21–30

10 COOK, R J AND ROVIRA, A D (1976) The Role of Bacteria in the Biological Control of *Gaeumannomyces graminis* by Suppressive Soils *Soil Biol Biochem* , **8**, 269–273

11 BRISBANE, P G AND ROVIRA, A D (1988) Mechanisms of Inhibition of *Gaeumannomyces graminis* var tritici by Fluorescent Pseudomonads *Plant Pathol* , **37**, 104–111

12 BRAITHWAITE, C W D AND CUNNINGHAM, H G A (1982) Inhibition of *Sclerotium rolfsii* by *Pseudomonas aeruginosa Can J Bot* , **60**, 237

13 MISAGHI, I J , STOWELL, I J , GROGAN, R G AND SPEARMAN, I C (1982) Fungistatic Activity of Water-soluble Fluorescent Pigments of Fluorescent Pseudomonads *Phytopath* , **72**, 33 36

14 SCHIPPERS, B , BAKKER, P A M M , BAKKER, A W , WEISBEEK, P J AND LUTGENBERG, B (1986) Plant Growth Inhibiting and Stimulating Rhizosphere Microorganisms, *Microbial Communities in Soil (Jensen V Kjoller A and Lorensen, L H , eds*), 35–49 London Elsevier

15 BURR, T J , SCHROTH, M N AND SUSLOW, T (1978) Increased Potato Yields by Treatment of Seedpieces with Specific Strains of *Pseudomonas fluorescens* and *P putida Phytopath* , **68**, 1377 1383

16 KLOEPPER, J W , LEONG, J , TEINTZE, M AND SCHROTH, M N (1980) *Pseudomonas* siderophores A Mechanism Explaining Disease-suppressive Soils *Curr Microbiol* , **4**, 317–320

17 KLOEPPER, J W , LEONG, J , TEINTZE, M AND SCHROTH, M N (1980) Enhanced Plant Growth by Siderophores Produced by Plant Growth-promoting Rhizobacteria *Nature*, **286**, 885–886

18 SUSLOW, T (1982) Role of Root-colonizing Bacteria in Plant Growth, *Phytopathogenic Prokaryotes* Vol 1 (*Mount, M S and Lacy G H , eds*), 187 London Academic Press

19 IKRAM, A , KARIM, M Z , SUDIN, M N AND NAPI, D (1994) Role of Plant Growth-promoting Rhizobacteria in Influencing the Early Growth of *Pueraria phaseoloides*– Strain and Soil Factors *J nat Rubb Res* , **9**, 48 55

20 KLOEPPER, J W , HUME, D J , SCHER, F M , SINGLETON, C , TIPPING, B , LALIBERTE, M , FRAULEY, K , KUTCHAW, T , SIMONSON, C , LIFSHITZ, R , ZALESKA, I AND LEE, L (1988) Plant Growth-promoting Rhizobacteria on Canola (rapeseed) *Pl Disease*, **72**, 42–46

21 HIGA, T (1991) Effective Microorganisms A Biotechnology for Mankind, *Proceedings of the First International Conference on Kyusei Nature Farming (Parr, J F*

Hornick, S.B. and Whitman, C.E., eds.), 8–14. Washington, D.C.: U.S. Department of Agriculture.

22. HIGA, T. (1994) Effective Microorganism: A New Dimension for Nature Farming, *Proceedings of the Second International Conference on Kyusei Nature Farming (Parr, J.F., Hornick, B. and Simpson, M.E., eds.)*, 20–22. Washington, D.C.: US Department of Agriculture.

23. KING, E.O., WARD, M.K. AND RANEY, D.E. (1954) Two Simple Media for the Demonstration of Pyocyanin and Fluorescein. *J. Lab. Clin. Med.*, **44**, 301–307.

24. ISWANDI, A., BOSSIER, P., VANDENABEELE, J. AND VERSTRAETE, W. (1987) Influence of the Inoculum Density of the Rhizopseudomonad Strain 7NSK2 on the Growth and the Composition of the Root Microbial Community of Maize (*Zea mays*). *Biol. Fertil. Soils*, **4**, 119–123.

25. CAESAR, A.J. AND BURR, T.J. (1987) Growth Promotion of Apple Seedlings and Rootstock by Specific Strains of Bacteria. *Phytopath.*, **77**, 1583–1588.

26. ISWANDI, A. (1986) Seed Inoculation with *Pseudomonas* spp. Ph.D. Thesis, State University of Gent, Faculty of Agricultural Science, Belgium.

27. BOSSIER, P., HOFTE, M. AND VERSTRAETE, W. (1988) Ecological Significance of Siderophores in Soil. *Advances in Microbial Ecology (Marshall, K.C. ed.)*, 385–414. New York: Plenum Press.

28. APNAN (1995) *EM Application Manual for APNAN Countries*. 1st Edition.

29. COOK, R.J. AND BAKER, K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens*. St.Paul, Minnesota, USA: APS Press.

30. LIFSHITZ, R., LIFSCHITZ, S. AND BAKER, R. (1985) Decrease in Incidence of *Rhizoctonia* Pre-emergence Damping-off by Use of Integrated Chemical and Biological Controls. *Pl. Disease*, **69**, 431–434.

31. COLE, R.J. AND ZVENYIKA, Z. (1988) Integrated Control of *Rhizoctonia solani* and *Fusarium solani* in Tobacco Transplants with *Trichoderma harzianum* and Triadimenol. *Plant Pathol.*, **37**, 271–277.

32. WELLER, D.M. (1988) Biological Control of Soilborne Plant Pathogens in the Rhizosphere with Bacteria. *Annu. Rev. Phytopathol.*, **26**, 397–407.

33. HIGA, T. (1988) Studies on the Application of Microorganisms in Nature Farming. II. Practical Application of Effective Microorganisms. *Proceedings of the 7th Conference of International Federation of Organic Agriculture Movements (IFOAM), Burkina, Faso.*

34. IMAI, S. AND HIGA, T. (1994) Kyusei Nature Farming in Japan: Effect of EM on the Growth and Yield of Spinach, *Proceedings of the Second International Conference on Kyusei Nature Farming (Parr, J.F., Hornick, S.B. and Simpson, M.E., eds.)*, 92–96. Washington, D.C.: U.S. Department of Agriculture.

35. MUNNECKE, D.E., KOLBEZEN, M.J., WILBUR, W.D. AND OHR, H.D. (1981) Interactions Involved in the Control of *Armillaria mellea*. *Pl. Disease*, **65**, 384–389.