IDENTIFICATION OF RESISTANCE IN HYBRID RICE TO BACTERIAL BLIGHT

LIN SHANSHAN and ZENG XIAOPING

Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi, People's Republic of China

ABSTRACT

In order to select and utilize hybrid rice with resistance to diseases and significant heterosis, over four hundred rice cultivars from the Rice Research Institute of Jiangxi, PRC, including A-lines, B-lines, R-lines and hybridized combinations were tested by inoculation to study their resistance to bacterial blight during the years 1977-1985. The results indicate as follows: 1) The wild abortive type male-sterile line is susceptible or highly susceptible to the disease, while the BT japonica type male-sterile line appears to be resistant. 2) It is concluded that if the R-line is resistant, most of its hybridized combinations will turn out to be fairly resistant to the disease. Therefore, it is possible to develop rice hybrids with resistance to bacterial blight.

INTRODUCTION

Bacterial leaf blight (Xanthomonas campestris pv. oryzae) is one of the most important diseases of rice production in Southern China. It has been proved by scientific research and production practice that utilization of rice cultivars which are resistant to diseases is not only effective, but also economical in control of bacterial blight. In order to select and utilize hybrid rice with strong resistance to the disease and significant heterosis, more than four hundred rice cultivars from the Rice Research Institute of Jiangxi, including A-lines, B-lines, R-lines and hybridized combinations were tested by artificial inoculation to study their resistance to bacterial blight during the years 1977-1985.

MATERIALS AND METHODS

All hybridized combinations and lines tested were conventionally sowed in fields during the planting season. In 1977 and 1978, rice seedlings were transplanted into experimental plots till the end of the vegetative growth phase. When boot leaves came out, rice plants were once again transplanted with soil into earthen pots (30 cm in diameter), four pits of plants per pot and one cultivar per pot. During the years 1979-1982, seedlings were transplanted from the nursery directly into earthen pots (as above), while in the years 1983-1985, seedlings were transplanted to rectangular cement ponds (70 cm in width and 35 cm in depth with 20 cm deep soil). Plants in pots and ponds were irrigated with tap water. Other management techniques were almost the same as those used in production fields, e.g. applying urea 2-3 times before booting (although the amount was a little higher than that used in fields).

From 1977 to 1980, Gan S-1 blight strain was used as inoculum. In 1981 and 1982, Gan S-2 strain was chosen for inoculation. During the years 1983-1985, Gan S-3 strain was used. These three strains were isolates from Jinggangshan, Yichun and Yongxing district of Jiangxi province, respectively, which were all identified as strong pathotypes. After isolates were

cultured on "WF-P" medium at 28°C for 2-3 days, an aqueous bacterial suspension with a concentration of 3 x 10^7-10^8 cells per ml was prepared from the cultures. When the boot leaves were fully emerged, over forty boot leaves of each cultivar were inoculated by pin pricking the upper part of the leaves with multiple pins dipped in the aqueous blight suspension. About twenty days after inoculation, when lesion development had almost ceased, the reactions of rice cultivars to the disease were recorded leaf by leaf on the basis of the IRRI's 1965 0-9 rating scale. Based on the records, the combinations and lines were classified as resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS).

RESULTS

1. Among 334 combinations, 83 were resistant or moderately resistant; the remaining 251 being susceptible or highly susceptible.

2. Out of twenty-two A-lines, all the wild abortive type of male-sterile lines were susceptible or highly susceptible whilst three of the japonica type of male-sterile lines (Changbai 6A, Qiuguang A and Songqiang A) appeared to be resistant (Table 1).

3. A number of the indica type of R-lines (IR26, IR36, IR2085-78-1, IR2071-625-3, IR2153-159-4, IR42, IR52, IR54, CPT-76, TO498, 79-360 and their hybridized combinations) were resistant and two of the japonica type of R-lines (C57-80 and T20) also turned out to be moderately resistant.

4. When the trials were repeated, eleven of the wild abortive type combinations and four of the japonica type of combinations (and also IR36/Xiandang 1, Zao 36/Jiliang - two chemically emasculated combinations) were identified as being at least moderately resistant to bacterial blight (Table 2). They also had good economic characters.

DISCUSSION

In order to make an appropriate assessment for a cultivar, the variation from year to year in climate and cultural practice, the declining pathogenicity of the successively-used inoculum and the variation in reactions of a single combination over years are factors which have to be considered. In the experiment, we had a local cultivar and the hybrid rice, Shanyou 2, as susceptible controls. When planted as early-season rice, Shanyou 2 appeared to be susceptible or highly susceptible to the disease, although there were reduced reactions in some years (resulting from a decline in pathogenicity of the inoculum). While it grew in late seasons, its reaction to the disease was then generally lower. This phenomenon might be due to the falling temperature after inoculation (usually in late September), which would be unfavourable for lesion development. For example, when the combinations, Zhenshan 97A/Gu154 and Zhenshan 97/IR24 were grown as early-season rice, their mean reaction classes to the strain S-1 of *X. campestris* pv. *oryzae* were 7.2 and 7.0, respectively, but as late-season rice, their mean reaction classes were 5.7 and 6.2, respectively. Therefore, 1 was added to the mean class of the combination which was planted in the late season, in order to make an appropriate evaluation. Five combinations were grown in the late season of 1984 and 1985. The temperature in mid-September 1985 fell unexpectedly and remained low for 6-7 d with less than average rainfall; the mean reaction class of these five combinations was 1.5 less than that in 1984. For this reason, 1.5 was added to the mean reaction class to retain comparability.

TABLE 1

Reactions of A-lines, B-lines, R-lines and their combinations to bacterial blight (R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible)

A-lines	Reaction	B-lines	Reaction	R-lines	Reaction	Combinations	Reaction
Zhenshan 97A	HS	Zhenshan 97B	HS	Zaowanbao 24	MR	Zhenshan A/Zaowanbao 24	S
				IR24	S	Zhenshan A/IR24	HS
				IR26	R	Zhenshan A/IR26	MR
				IR36	R	Zhenshan A/IR36	MR
				Gu 154	S	Zhenshan A/Gu 154	S
				Kezhen 145	S	Zhenshan A/Kezhen 145	S
				CPI-76	R	Zhenshan A/CPI-76	R
V20 A	HS	V20 B	HS	Fen 225	S	V20 A/Fen 225	HS
				IR26	R	V20 A/IR26	MR
				T0498	R	V20 A/T0498	R
				0082	S	V20 A/0082	HS
				T0494	S	V20 A/T0494	S
75785 A	HS	75785 B	HS	IR24	S	75785 A/IR24	S
				IR26	R	75785 A/IR26	MR
Gannanwan A	S	Gannanwan B	S	Guixuan 7	S	Gannanwan A/Guixuan 7	S
Zhaoyangzao A	HS	Zhaoyangzao B	HS	IR24	S	Zhaoyangzao A/IR24	S
Zhongmaoyouyi 74A	HS	Zhongmaoyouyi 74B	HS	IR24	S	Zhongmaoyouyi 74A/IR24	S
CA	S	CB	HS	90208	S	CA/90208	HS
Hunanzao A	HS	Hunanzao B	HS	Gui630	S	Hunanzao A/Gui630	S
D Shan A	HS	D Shan B	HS	2224	S	D Shan A/2224	S
(Gang) Zhao A	HS	(Gang) Zhao B	HS	75001	S	(Gang) Zhao A/75001	S
Changbai 6A	R	Changbai 6B	R	C57-80	R	Changbai 6A/C57-80	R
Songqiang A	MR	Songqiang B	S	C57-80	R	Songqiang A/C57-80	MR
Qiuguang A	R	Qiuguang B	R	T20	MR	Qiuguang A/T20	MR

TABLE 2

Reactions of lines and hybridized combinations to bacterial blight (repeat test) (R = resistant, MR = moderately resistant, S = susceptible, HS = highly susceptible)

A-lines	Reaction	R-lines	Reaction	Combinations	Reaction
V20 A	S	IR26	R	Weiyou 6	MR
Zhenshan 97A	S	IR26	R	Shanyou 6	MR
29 nan A	S	IR26	R	Nanyou 6	MR
V2 A	S	IR28	R	V20 A/ IR28	MR
Zhenshan 97A	S	IR36	R	Zhan A/ IR36	MR
77ABA	S	IR26	R	77ABA/ IR26	MR
Junxie A	S	Ce 64	R	Xieyou 64	R
IR36	R	Xiandang 1	S	IR36/ Xiandang(C)*	R
				Zao 36/Jiliang(C)*	R
Gannanwan 8A	S	IR26	R	Gan A/ IR26	MR
75785 A	HS	IR26	R	75785 A/ IR26	MR
Zhenshan 97A	HS	CPI-76	R	Shan A/ CPI-76	MR
V20 A	HS	TO498	R	V20 A/ TO498	MR
Huangjin A	HS	300		Huangjin A/ 300	R
Qiuguang A	MR	T20	MR	Qiuguang A/ T20	MR
Changbai 6A	R	C57-80	R	Chang A/ C57-80	R
Songqiang A	MR	C57-80	R	Song A/ C57-80	R

(C)* = chemically induced sterility

The blight isolate used as inoculum in the trials was chosen because it was a strong pathotype. The isolate was inoculated on the susceptible cultivar 7055 in October of every year. Diseased leaves were then collected and stored at 0-5°C for next year's inoculum. It turned out that the pathogenicity of isolate Gan S-1 (which was originally a strong pathotype) declined obviously after being used for four years. Consequently, isolate Gan S-2 was used instead. Two years later the isolate Gan S-3 was used for the same reason. It has been reported that the pathogenicity of isolates cultured and stored on artificial media would gradually reduce after inoculation on susceptible cultivars, but increase after inoculation on resistant ones. Therefore, it is important to measure and stabilize the pathogenicity of the inoculum for use in identifying the resistance of cultivars by inoculation.

It was found that, if the R-line is resistant, most of the combinations with it turn out to be at least moderately resistant (Table 1). This indicates that it is certainly possible to select and develop hybridized combinations with resistance to bacterial blight. It is generally accepted that the resistance of rice to bacterial blight is controlled by a few major genes, and the resistance seems to be dominant and is dominated by the nuclear gene but not affected by the plasmagene. Therefore, when either the A-line or the R-line is resistant, the probability of obtaining resistant offsprings is large.

INFLUENCE OF AIR POLLUTANTS ON BIOLOGICAL CONTROL OF BROWN LEAF SPOT OF RICE BY PHYLLOPLANE FUNGI

BHARAT RAI, R.S. SOLANKI AND R.S. UPADHYAY

Department of Botany, Banaras Hindu University, Varanasi 221 005, India

ABSTRACT

The influence of some air pollutants, viz., ammonia, sulphur dioxide and cement dust, on biological control of brown leaf spot of rice (caused by Helminthosporium oryzae) by phylloplane fungi was studied. The pattern of colony interaction, in vitro and in vivo, was greatly influenced by the air pollutants, consequently altering the incidence of the disease. It was aggravated by Aspergillus flavus, Alternaria alternata, Aspergillus niger, Drechslera australiensis and Fusarium oxysporum under NH_3 exposure. The disease was, however, significantly suppressed due to increased antagonism by A. alternata, A. niger, Cladosporium cladosporioides, Curvularia lunata, D. australiensis, F. oxysporum and Penicillium citrinum in response to SO_2 exposure. Cement dust also effectively modified the antagonism between the pathogen and the saprophytes on the phylloplane. The disease was favoured by A. niger and P. citrinum when inoculated along with the pathogen. However, A. alternata, C. cladosporioides, C. lunata, D. australiensis and F. oxysporum caused more antagonism to suppress the disease under cement dust pollution.

INTRODUCTION

Antagonism of phylloplane micro-organisms against several leaf pathogens has been studied in view of their possible biological control (Fokkema 1973, 1978, Leben 1965). It has been suggested that a leaf which is able to support a microflora antagonistic to a pathogen may have greater resistance than those which cannot (Last & Deighton 1965, Last & Warren 1972). Studies by various workers have revealed that air pollutants play an important role in pathogenesis and incidence and severity of various foliar diseases (Heagle 1973, Saunders 1973, Bevan & Greenhalgh 1976, Laurence et al. 1979, Rist & Lorbeer 1985). One cause of the altered pathogenesis has been attributed to the role of air pollutants in altering microbial interactions on the phylloplane (Rai & Upadhyay 1988). Ecological disturbances causing reduced activity of antagonists on the phylloplane has been offered as an explanation for the increased disease incidence. The air pollutants which directly affect the aerial plant surfaces may also cause favourable or unfavourable ecological imbalances in microbial interactions. The present study was undertaken on the influence of air pollutants viz., ammonia (NH_3), sulphur dioxide (SO_2) and cement dust, on the biological control of Helminthosporium oryzae (causing brown leaf spot disease of rice) by host phylloplane fungi.

MATERIALS AND METHODS

Effect of air pollutants on in vitro colony interactions between H. oryzae and phylloplane fungi

The effects of air pollutants on colony interactions was studied by inoculating the selected phylloplane fungi individually with H. oryzae in dual cultures (Skidmore & Dickinson 1976) on PDA medium (pH 5.5) and exposing them to the pollutants. Control sets were also prepared as above but without treatment. The plates containing exposed cultures were incubated at 25 ±1°C for 6 days and the positions of the colony margins were recorded. The inhibition in the radical growth of H. oryzae was calculated by the formula of Fokkema (1973).

The NH_3 treatment was given in a pollutant exposure chamber especially designed for this purpose by Standard Appliances, Varanasi. NH_3 was generated by heating a dilute solution of ammonium hydroxide at 47°C. Estimation and standardization of NH_3 concentration was carried out by the indophenol method (Katz 1976). Dually-inoculated Petri dishes with cultures of the test pathogen and phylloplane fungi on PDA were exposed to NH_3 at 6.1×10^3 $\mu g/m^3$ concentration for up to one hour in the pollutant exposure chamber and then incubated at 25 ± 1°C. The SO_2 treatment was given in the pollutant exposure chamber. SO_2 was produced from a Standard Sulphur Dioxide Gas Generator designed and manufactured by Standard Appliances, Varanasi. Dually-inoculated cultures were exposed to SO_2 at 26.7×10^2 $\mu g/m^3$ in the exposure chamber for 1 h. Cement dust was mixed in PDA at a concentration of 2×10^7 $\mu g/l$. Blocks from colonies of H. oryzae and from the phylloplane fungi were inoculated opposite each other on the medium and growth recorded after 6 days.

Effects of air pollutants on in vivo interactions on the phylloplane and on biological control of H. oryzae

Rice (Oryza sativa L.) var. Mussorie was grown in earthenware pots, irrigated every three days to maintain a high soil moisture level. Any naturally infected plants were removed from the experimental pots. Healthy leaves were rubbed gently with a wet cotton swab, then inoculated with a spore suspension (1×10^3 spores/ml) of H. oryzae at three sites on each of three young leaves on three 45-day-old plants for each saprophyte-pathogen combination. The total number of combinations (polluted treatments plus controls) was 63. For inoculation, leaves were wounded with a needle and a standard-sized drop (c. 0.025 cm^3) containing spore suspension was placed over the wound. The plants were then covered with sterilized, wet polythene bags to maintain high humidity (Rai & Singh 1980). Older leaves were avoided because they tended to senesce during the course of the experiment. After three days the polythene bags were removed and the size of lesions on treated leaves measured. The plants were then inoculated with spores of phylloplane fungi (2.5×10^3/ml) (one species per plant) and covered with polythene bags again for 24 h.

The inoculated test plants were then exposed to NH_3, as described above, at 7.0×10^4 $\mu g/m^3$ for 1 h or to SO_2 at 2.7×10^4 $\mu g/m^3$ for 1 h or sprayed with cement dust using a hand sprayer (total dose 2×10^7 $\mu g/m^2$).

After 10 days of treatment the sizes of the lesions were measured and compared with the untreated controls. Percent inhibition or stimulation in size of the lesions was calculated.

RESULTS

Influence of pollutants on in vitro colony interactions between H. oryzae and phylloplane fungi

The pattern of the colony interactions was greatly influenced by air pollutants (Table 1). The level of inhibition of H. oryzae by A. niger, C. cladosporioides, C. lunata, D. australiensis and P. citrinum was modified by NH_3 in that less inhibition was recorded compared with controls. However, in some cases viz. A. alternata, A. flavus and F. oxysporum the response was reversed. Interactions were also modified by SO_2 and cement dust (Table 1).

TABLE 1

In vitro colony interactions between H. oryzae and some selected phylloplane fungi under the influence of SO_2, NH_3 and cement dust.

Test fungi	Percent inhibition in H. oryzae colonies and types of interaction							
	Control (untreated)		SO_2 26.7×10^2 µg/m^3		NH_3 6.1×10^3 µg/m^3		Cement dust 20.0×10^6 µg/litre	
	*% I	Type	% I	Type	% I	Type	% I	Type
Alternaria alternata	5.2	D	8.2	D	10.5	B_2	4.9	C
Aspergillus flavus	5.7	C	22.7	D	24.1	D^2	9.7	D
A. niger	47.0	B_2	39.5	D	8.4	C	26.7	D
Cladosporium cladosporioides	25.4	D^2	28.1	C	4.1	C	42.1	D
Curvularia lunata	20.7	D	15.1	B_1	8.6	B_2	52.3	C
Drechslera australiensis	21.1	D	50.7	D^1	12.9	C^2	28.3	B_2
Fusarium oxysporum	6.9	A	9.1	C	10.3	A	2.4	C^2
Penicillium citrinum	21.3	B_1	39.7	B_1	2.1	C	8.3	D

* % I = % inhibition

A, B_1, B_2, C, D: Interaction score (Skidmore & Dickinson 1976): A, mutual intermingled growth; B_1, overgrowth by the antagonist; B_2, overgrowth by the pathogen; C, mutual slight inhibition; D, mutual inhibition at a distance

Effect of pollutants on in vivo biological control of H. oryzae by phylloplane fungi

H. oryzae lesion development was affected by air pollutants (Table 2). Ammonia enhanced disease severity (cf. untreated control) for the A. flavus, A. alternata, A. niger, D. australiensis and F. oxysporum treatments but reduced it in the case of C. cladosporioides, C. lunata and P. citrinum. SO_2 reduced disease severity in all treatments (except that of A. flavus) and the biocontrol potential of P. citrinum was highly activated. Lesions were greatly increased by A. flavus and P. citrinum in the presence of

cement dust, but this pollutant helped the other antagonist species to suppress the disease.

TABLE 2

Influence of air pollutants on interactions between some phylloplane fungi and H. oryzae on the leaf surface of rice.

Fungal species	Total doses of air pollutants		
	NH_3 70 806 µg/m^3	SO_2 26 690 µg/m^3	Cement dust 2x10^7 µg/m^2
Alternaria alternata	+ 42.85	- 20.00	- 54.28
Aspergillus flavus	+121.42	+ 90.47	+130.95
A. niger	+ 93.75	- 21.87	+ 9.37
Cladosporium cladosporioides	- 69.14	- 77.65	- 50.00
Curvularia lunata	- 64.40	- 74.57	- 87.28
Drechslera australiensis	+170.96	- 12.90	- 19.35
Fusarium oxysporum	+ 50.00	- 34.00	- 42.00
Penicillium citrinum	- 9.09	-745.45	+231.81
H. oryzae	+275.00	- 54.54	+163.63
LSD (P = 0.01)			
Species means	116.52		
Pollutants means	67.00		

Values represent % inhibition (-) or stimulation (+) in H. oryzae lesion size (cf. controls)

DISCUSSION

It is evident from the present study that air pollutants greatly influence the growth and activity of micro-organisms, altering their interactions on the phylloplane as well as their potentialities for causing beneficial or harmful effects. The antagonism of various leaf saprophytes against H. oryzae on rice leaves was markedly changed under the impact of these environmental pollutants. C. cladosporioides is a vigorous leaf saprophyte whose antagonistic role on the phylloplane has already been recognized (Rai & Singh 1980). In the present study this species was found to be more effective in reducing H. oryzae under the influence of various air pollutants, e.g. it effectively checked the growth of H. oryzae in the presence of cement dust (Table 1). Although this species was not very effective in checking the growth pattern of H. oryzae when exposed to NH_3 and SO_2 in vitro, when inoculated on the phylloplane these pollutants helped it reduce lesion development. This behaviour of C. cladosporioides may be attributed to its natural affinity with the phylloplane of rice (Singh et al. 1987) and several other plants (Dickinson 1976) and its ability to grow and sporulate under fluctuating climatical and nutritional conditions (Dickinson 1976).

Under exposure to SO_2 H. oryzae was suppressed by all the test fungi, except A. flavus. This may have been due to an adverse effect of SO_2 on H. oryzae and/or beneficial effect on the antagonistic fungi (Table 2).

The capacity of P. citrinum to control H. oryzae was greatly increased under exposure to SO_2. P. citrinum may be used as a biocontrol agent for foliar diseases in areas subject to SO_2 emissions. Disappearance of Rhytisma acerinum in SO_2-polluted localities has been reported (Bevan & Greenhalgh 1976) and this might be due to an effect on antagonists. It should be noted that P. citrinum stimulated H. oryzae under the influence of cement dust which emphasises the need for great care in the selection of biological control agents.

The results reported here could arise from a number of complex interactions; (i) the incidence and severity of the disease is changed by the air pollutants (ii) the pollutants may exert an unfavourable effect on the pathogen but not on the antagonists thereby reducing the disease (iii) the pollutants may have an unfavourable effect on the antagonists but not on the pathogen, thereby stimulating the disease (iv) the pollutants may have a favourable or unfavourable effect on both the pathogen and antagonists resulting in no significant effect on the disease and, finally (v) the pollutants may have an adverse effect on the host resulting in an increased disease level.

REFERENCES

Bevan, R.J.; Greenhalgh, G.N. (1976) Rhytisma acerinum as a biological indicator of pollution. Environmental Pollution 10, 271-285.

Dickinson, C.H. (1976) Fungi on the aerial surface of higher plants. In: Microbiology of Aerial Plant Surfaces, C.H. Dickinson and T.F. Preece (Eds), London: Academic Press, pp. 291-324.

Fokkema, N.J. (1973) The role of saprophytic fungi in antagonism against Drechslera sorokiniana (Helminthosporium sativum) on agar plates and on rye leaves with pollen. Physiological Plant Pathology 3, 195-205.

Fokkema, N.J. (1978) Fungal antagonism in the phyllosphere. Annals of Applied Biology 89, 115-119.

Heagle, A.S. (1973) Interactions between air pollutants and plant parasites. Annual Review of Phytopathology 11, 365-388.

Katz, M. (1976) Nitrogen compounds and oxidants. In: Air Pollution, Stern (Ed.), London: Academic Press, pp. 259-305.

Last, F.T.; Deighton, F.C. (1965) The non-parasitic microflora on the surfaces of living leaves. Transactions of the British Mycological Society 48, 83-99.

Last, F.T.; Warren, R.C. (1972) Non-parasitic microbes colonizing green leaves: Their form and functions. Endeavour 31, 143-150.

Laurence, J.A.; Weinstein, L.H.; McCune, D.C.; Aluisio, A.L. (1979) Effects of sulfur dioxide on southern corn leaf blight of maize and stem rust of wheat. Plant Disease Reporter 63, 975-978.

Leben, C. (1965) Epiphytic micro-organisms in relation to plant disease. Annual Review of Phytopathology 3, 209-230.

Rai, B.; Singh, D.B. (1980) Antagonistic activity of some leaf surface microfungi against Alternaria brassicae and Drechslera graminae. Transactions of the British Mycological Society 75, 363-369.

Rai, B.; Upadhyay, R.S. (1988) Impact of air pollutants on phylloplane microflora and disease development. New Botanist, New Delhi: Today and Tomorrow Publications (in press).

Rist, D.L.; Lorbeer, J.W. (1985) Air pollutants and foliar microbes. In: Frontiers in Applied Microbiology, K.G. Mukerji, N.C. Pathak and V.P. Singh (Eds), Lucknow: Print House (India), pp. 181-198.

Saunders, P.J.W. (1973) Effects of atmospheric pollution on leaf surface microflora. Pesticide Science 4, 589-595.

Singh, A.K.; Solanki, R.S.; Bharat Rai (1987) Effects of air pollutants on phylloplane mycoflora of Oryza sativa Linn. and development of leaf spot disease. In: Proceedings of the Symposium on Plant-Microbe Interaction (in press).

Skidmore, A.M.; Dickinson, C.H. (1976) Colony interactions and hyphae interference between Septoria nodorum and phylloplane fungi. Transactions of the British Mycological Society 66, 57-64.

BIOLOGICAL CONTROL OF SCLEROTIUM ROLFSII AND PYTHIUM APHANIDERMATUM DAMPING-OFF ON TOBACCO WITH TRICHODERMA HARZIANUM CULTURE

H.X. TRUONG; M.D. SALINAS; A.S. OBIEN AND R.C. CARASI

Crop Protection Division, National Tobacco Administration-Research and Training Center, Batac, Ilocos Norte, Philippines

ABSTRACT

Trichoderma harzianum Rifai waas found in most soil planted to tobacco in Northwestern and Eastern Luzon, Philippines. The fungus was also isolated from parasitized sclerotial bodies. It was easily mass-produced in a mixture of rice bran:rice hull: water (3:1:1.2 v/v/v). The culture incorporated into baked and unbaked silt/loam soil (6.7% sand, 69.2% silt and 24.1% clay, pH 7.8-8.1, 1.7% organic matter and 12-30% moisture) at 50 cc per litre of soil, effectively controlled *S. rolfsii* Sacc. and *P. aphanidermatum* Fitz. causing damping-off on tobacco. The total number of transplantable seedlings produced from soil treated with *T. harzianum* no. 1 was 2- to 3-fold higher than untreated soil.

Both *T. harzianum* and *S. rolfsii* were recovered from soil at 70 days after sowing. About 41 to 76% of the sclerotial bodies were parasitized with *T. harzianum*.

INTRODUCTION

Tobacco is an important cash crop in the northern parts of Luzon island in the Philippines, with an estimated total area of 45,000 ha devoted to it in 1987-88.

Damping-off poses a serious problem to the production of healthy tobacco seedlings. It often delays planting, thereby depriving growers of the best market price for their produce. It also causes a non-uniform crop stand. The disease is induced by complex soil-borne fungi consisting of *Pythium aphanidermatum* Fitzpatrick, *Sclerotium rolfsii* Sacc, *Fusarium oxysporum* Schlecht and *Rhizoctonia solani* Kuhn (Dipon 1988). *Pythium* damping-off is the most destructive and prevalent in the tobacco growing areas. *S. rolfsii* retards seedling growth and is the major pathogen causing wilt of tobacco at maturity.

Soil fumigation, although effective against many pests, is not practical because the farmers cannot afford the cost and lack the technical knowledge in handling it safely. The use of fungicides as a drench is recommended, but no single fungicide can control the pathogen complex. Most farmers are reluctant to adopt the recommendation and still rely on burning crop residues on the top of the seedbed to control the disease.

This paper presents results on the use of *Trichoderma harzianum* no. 1 as a promising biological control agent against *Pythium* and *Sclerotium* damping-off on tobacco.

METHODOLOGY

Isolation of Trichoderma species

From soil

A total of 90 samples were taken from soil planted to tobacco in Isabela (Northeastern Luzon) and in Ilocos Norte, Ilocos Sur and La Union (Northwestern Luzon). Each sample (200 g) was a composite of 10 cores randomly taken from a 500–1000 sq m area, to a depth of between 2.5 and 10 cm. It was air-dried for 24 to 72 h, depending on soil moisture. Isolation was done using the soil dilution method on a Trichoderma-selective medium (TSM) (Elad et al. 1981b), except that dexon (p-dimethylamino-benzenediano sodium sulfonate) was replaced by 10 ppm manzate. The culture was incubated at 28–30°C. Colonies of Trichoderma were singled out and transferred onto malt extract agar (MEA) for identification. The species identification followed the description of Rifai (1969). Colonies of Trichoderma were described together with other fungi that appeared in the medium.

From sclerotial bodies

About 100 g of soil was taken from around the root system of each wilted tobacco infected by S. rolfsii. Sclerotial bodies were collected by a flotation-sieving technique (Rodriguez-Kabana et al. 1974). These were surface disinfested with 2% sodium hypochloride for 1–2 min, rinsed with sterilized distilled water and plated on a potato dextrose agar (PDA) block. Conidia of Trichoderma that grew out from the bodies were picked up and produced in MEA.

Mass culture of Trichoderma

T. harzianum no. 1 and T. hamatum no. 1 were isolated directly from sclerotial bodies whereas T. harzianum no. 5, 10 and 11 isolated from soil were allowed to grow in a mixture of autoclaved rice bran:rice hull:water (3-5:1:1.2 v/v/v). Two agar discs (10 mm in diameter) of Trichoderma on MEA were introduced into 200 cc of mixture contained in a 1 litre jar and incubated at 30°C for 10–14 days before use. Other species such as T. aureoviridae no. 103, T. harzianum no. 118, T. hamatum no. 230, T. longibrachiatum no. 60 and T. piluliferum no. 233, which were given by Dr T.W. Mew, Department of Plant Pathology, IRRI, were also included for evaluating the culture. Their conidial suspension was prepared by shaking 10 g of culture in 90 ml of distilled water and the conidia count was determined with the aid of an haemocytometer. The survival of each species in the medium at room temperature was noted.

Isolation and mass culture of Pythium and Sclerotium

Isolation of P. aphanidermatum from soil followed the baiting method using cucumber seedlings (Bouhot 1975). The fungus was produced in oatmeal agar at 28°C and harvested at 7 days after the start of incubation (DAI). Two agar discs of Pythium were grown in a mixture of sand (50 cc), oatmeal (20 cc) and 2% dextrose (20 ml) in a jar for 14 days. S. rolfsii was cultured on PDA and mass-produced following the same method as for Pythium.

Evaluation of Trichoderma culture against P. aphanidermatum and S. rolfsii damping-off

Silty loam soil (6.7% sand, 69.2% silt and 24.1% clay, pH 7.8–8.1, and 1.7% organic matter) was first disinfested by baking it for 4 h. Pythium

culture was mixed thoroughly with soil at the rate of 25 cc/l. The mixture was divided into 2 l/clay basin and watered with 0.5 l each. The basins were arranged on a wooden bench in the greenhouse and covered with corrugated plastic sheets for three days. About 100 cc of Trichoderma culture (T. harzianum no. 1 and no. 118, T. hamatum no. 1 and no. 230, T. aureoviridae no. 103) was thoroughly incorporated into the soil in each basin. Soil with Pythium added served as a control. Four days later, seeds of tobacco cv. NCBY were sown at 25 mg or about 550 seeds/basin. Soil moisture was maintained from 27-31% by watering the soil twice a day. Treatments were arranged in a Randomised Complete Block Design with four replications.

The experimental procedure for evaluating Trichoderma culture against S. rolfsii followed the procedure described above, except that soil artificially infested with a culture of S. rolfsii at 50 cc/l, as well as naturally infested soil, was used. The soil moisture was 12-14%. Seeding rate was 12.5 mg/basin.

In both trials, the number of surviving seedlings were recorded at 14, 21, 30 and 60 days after sowing (DAS). The introduced Trichoderma species in soil were recovered at 70 DAS by the soil dilution method and with the modified TSM described earlier. Colony forming unit (CFU) were determined from 5-7 DAI. Parasitism by Trichoderma species on the recovered sclerotial bodies was demonstrated by seeding the bodies 10 mm apart and on the modified TSM contained in the petri dishes. Percent parasitized bodies were recorded from 5-7 DAI.

RESULTS

Isolation of Trichoderma species

Trichoderma colonies on the modified TSM were flat and appeared white from 3-5 DAI. Later, the advancing zone of the colony became thick and green, while the center remained white or turned greenish. They could be distinguished from the thick, white to yellow and concentric colonies characteristic of Aspergillus. T. harzianum was isolated from 30-40% of the soil samples, while both T. harzianum an T. hamatum were occasionally isolated from sclerotial bodies collected from the rhizosphere of the infected tobacco.

Mass culture of Trichoderma species in rice and rice hull medium

T. harzianum no. 1, no. 5, no. 10 and no. 11 and T. piluliferum no. 233 grew fast in rice bran:rice hull medium. Their conidia count at 14 DAI was the highest at from 1.6 to 5 x 10^9/g of culture (fresh weight), followed by 1.8-2.1 x 10^9/g for T. hamatum no. 230 and 0.7-1 x 10^9/g for T. aureoviridae no. 130. All Trichoderma cultures remained viable for 66 days when kept at room temperature (28-33°C).

Evaluation of Trichoderma culture against P. aphanidermatum and S. rolfsii

Pythium damping-off occurred in the artificially infested soil with or without Trichoderma spp. but the soils with antagonists incorporated had more seedlings than untreated soils (Table 1). The greatest number of seedlings (42-46/basin) were obtained at 60 DAS from the treated soil infested with T. harzianum no. 1 and T. hamatum no. 1. Only 14 seedlings

were obtained from the infested soil without Trichoderma. Considering the number of transplantable seedlings (i.e., seedlings with a 4 mm diameter stem, 5-6 leaves and a root to shoot ratio of about 0.7-1); the number of such seedlings from soil with Trichoderma culture was four times more than from the infested control soil.

TABLE 1

Effect of Trichoderma culture on a number of surviving tobacco seedlings grown on soil artificially infested with P. aphanidermatum

Treatment*	No. of surviving seedlings per 2 l of soil at **			
	14 DAS	21 DAS	30 DAS	60 DAS
DS-Py-T. harzianum no. 1	266.8	170.5 bc	93.5 a	46.3 a
DS-Py-T. harzianum no. 118	265.0	255.5 a	68.5 ab	25.3 bc
DS-Py-T. hamatum no. 1	224.8	134.8 bc	70.0 ab	42.5 a
DS-Py-T. hamatum no. 230	279.8	253.5 a	99.5 a	31.3 ab
DS-Py-T. aureoviridae no. 103	277.3	238.3 ab	58.8 ab	30.8 ab
DS-Py	244.5	103.8 c	30.5 b	13.8 c

(*) Ds-Py = Silty loam soil was baked and inoculated with P. aphanidermatum culture at 25 cc/l of soil.
(**) Means in a column followed by the same letter are not significantly different at P < 0.05, Duncan's Multiple Range Test
DAS = Days after sowing

Similarly, the total number of seedlings obtained at 60 DAS from artificially and naturally Sclerotium-infested soil treated with different Trichoderma species were about two times more than from the infested soil alone (Table 2). The number of sclerotial bodies recovered from both artificially and naturally infested soil treated with T. harzianum no. 1 was the lowest (60.5 and 19.5/basin), but percent parasitized bodies was the highest 76 and 41%, respectively (Table 3). Trichoderma propagules recovered from soil at 70 DAS ranged from 1.3 to 2.4 x 10^4 CFU/g of air-dried soil.

DISCUSSION

The effectiveness of T. harzianum in controlling soil-borne pathogens such as S. rolfsii, R. solani and Pythium species has been described by Wells et al. (1972), Backman and Rodriguez-Kabana (1975), Elad et al. (1980, 1981a, b, c). Our findings indicate that T. harzianum is common in soil planted to tobacco. In addition, a highly effective isolate of T. harzianum was also recovered from sclerotial bodies. The isolate protected tobacco seedlings from Pythium and Sclerotium damping-off and also parasitized and greatly reduced the density of sclerotial bodies in the soil. The isolation of Trichoderma from the pathogen may, therefore, prove to be a superior source of isolates of antagonists against the pathogen.

TABLE 2

Effect of Trichoderma culture on the number of surviving tobacco seedlings grown on soil artificially infested with S. rolfsii, and on soil naturally infested with S. rolfsii

Treatment*	No. of surviving seedlings per 2 l of soil at **			
	14 DAS	21 DAS	30 DAS	60 DAS
DS-Sr-T. harzianum no. 1	87.0 abc	85.5 ab	72.3 abc	33.5 ab
DS-Sr-T. harzianum no. 118	106.8 ab	100.0 ab	76.0 ab	31.8 bc
DS-Sr-T. hamatum no. 1	95.8 ab	95.0 ab	64.8 abc	30.0 bc
DS-Sr-T. hamatum no.230	91.8 ab	91.8 ab	67.5 abc	37.8 ab
DS-Sr-T. aureoviridae no. 103	109.0 ab	109.0 ab	78.8 ab	42.5 a
DS-Sr	56.8 bc	56.3 bc	38.0 cd	16.8 de
NIS-T. harzianum no. 1	92.3 ab	86.3 ab	68.8 ab	29.5 bc
NIS-T. harzianum no. 118	64.8 bc	64.0 bc	51.5 bc	22.0 cd
NIS-T. hamatum no. 1	119.8 a	119.0 a	96.0 a	32.0 ab
NIS-T. hamatum no. 230	127.5 a	120.5 a	76.8 ab	30.3 bc
NIS-T. aureoviridae no. 103	88.3 abc	80.5 ab	62.3 abc	27.8 bc
NIS alone	39.3 c	25.8 c	15.3 d	12.3 e

(*) Ds-Sr = Silty loam was baked and inoculated with S. rolfsii culture at 50 cc/l of soil; NIS = soil naturally infested with S. rolfsii.

(**) Means in a column followed by the same letter are not significantly different at $P < 0.05$, Duncan's Multiple Range Test

DAS = Days after sowing

TABLE 3

Parasitism of Trichoderma species on sclerotial bodies recovered from silty loam soil naturally and artificially infested with S. rolfsii

Treatment*	No. of sclerotial bodies recovered from 2 l of soil**	Percent sclerotial bodies parasitized by Trichoderma
Ds-Sr-T. harzianum no. 1	60.50 a	76.0
Ds-Sr-T. harzianum no. 118	168.75	43.9
Ds-Sr-T. hamatum no. 1	47.25 a	82.0
Ds-Sr-T. hamatum no. 230	180.00	63.4
Ds-Sr-T. aureoviridae no. 103	138.75	18.5
Ds-Sr	143.00	0
NIS-T. harzianum no. 1	19.50 b	41.0
NIS-T. harzianum no. 118	19.50 b	5.6
NIS-T. hamatum no. 1	37.50 b	7.5
NIS-T. hamatum no. 230	31.25 b	0
NIS-T. aureoviridae no. 103	31.00 b	0
NIS alone	64.75	0

(*) Ds-Sr = Silty loam soil was disinfested by baking for 4 h, and artificially inoculated with S. rolfsii at 500 cc/l of soil; NIS = naturally Sclerotium-infested soil.

(**) Sclerotial bodies were recovered from soil at 70 DAS. Means followed by the letter a or b are significantly lower than those from the DS-Sr and NIS controls, respectively.

Culture of Trichoderma in wheat bran was previously reported as a simple way to mass produce these antagonists (Hadar et al. 1979, Elad et al. 1981). Its application successfully protected tomato, eggplant, beans and carnation from infection by soil-borne pathogens. The findings of this study show that a mixture of rice bran and rice hull (which are inexpensive in The Philippines) with water can be used as a food base in mass culture. The fungus grown in this substrate produced from 1.6 to 5 x 10^9 conidia/g of fresh culture at 10-14 DAI. This density is equivalent to that required to achieve an effective control of S. rolfsii, R. solani and Pythium spp. (Hadar et al. 1979). When the culture was applied to a silty loam soil, it protected the seedlings from damping-off. After harvesting the seedlings, Trichoderma spp. were recovered from soil. This suggests that the antagonists can be established in the soil. In the soil, the highly specific parasitism of T. harzianum no. 1 to sclerotial bodies may be an important factor in reducing inoculum density of this major soil-borne pathogen. The parasitism was easily determined by a simple method of plating sclerotial bodies onto modified TSM. This method was found to be reliable for screening the antagonistic Trichoderma species and isolates.

REFERENCES

Backman, P.A.; Rodriguez-Kabana, R. (1975) A system for the growth and delivery of biological control agents to the soil. Phytopathology 65, 819-821.

Bouhot, D. (1975) Research on ecology of soilborne pathogens. VII. Quantification of the techniques for estimating the inoculum potential of soils, composts and substrates infested by Pythium sp. Annals of Phytopathology 7, 155-158.

Dipon, P.D. (1988) Cultural and Epidemiological Studies of Four Soilborne Pathogens Causing Damping-Off of Tobacco (Nicotiana tabacum L.). Ph.D. Dissertation, University of the Philippines at Los Banos, College, Laguna, pp. 120.

Elad, Y.; Chet, I.; Ktan, J. (1980) Trichoderma harzianum: a biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology 70, 119-121.

Elad, Y.; Chet, I.; Henis, Y. (1981a) Biological control of Rhizoctonia solani in strawberry fields by Trichoderma harzianum. Plant Soil 60, 245-254.

Elad, Y.; Chet, I.; Henis, Y. (1981b) A selective medium for improving quantitative isolation of Trichoderma spp. from soil. Phytoparasitica 9, 59-67.

Elad, Y.; Hadar, Y.; Hadar, E.; Chet, I.; Henis, Y. (1981c) Biological control of Rhizoctonia solani by Trichoderma harzianum in carnation. Plant Disease 65, 675-677.

Hadar, Y.; Chet, I.; Henis, Y. (1979) Biological control of Rhizoctonia solani damping-off with wheat bran culture of Trichoderma harzianum. Phytopathology 69, 64-68.

Rifai, M. (1969) A revision of the genus Trichoderma. Commonwealth Mycological Institute, Kew, Surrey, England. Mycological Paper 116, 1-56.

Rodrigeuz-Kabana, R.; Backman, P.A.; Wiggins, E.A. (1974) Determination of sclerotial populations of Sclerotium rolfsii in soil by a rapid flotation-sieving technique. Phytopathology 65, 610-615.

Wells, H.D.; Bell, D.K.; Jawarski, C.A. (1972) Efficacy of Trichoderma harzianum as a biological control for Sclerotium rolfsii. Phytopathology 62, 442-447.

SCREENING POTENTIAL BIOANTAGONISTS AGAINST PATHOGENS OF TURF

S.H. WOODHEAD[1], A.L. O'LEARY[1]. D.J. O'LEARY[1], AND R.D. BATTERSHELL[2]

[1]Agricultural and Chemistry [2]Research, Ricerca, Inc., 7528 Auburn Road, Painesville, OH 44077 and [2]Fermenta Plant Protection Co., P.O. Box 8000, Mentor, OH 44061.

ABSTRACT

A technique was developed to evaluate potential bioantagonists against Pythium and Rhizoctonia diseases on turf. 'Penncross' bentgrass was grown in 288-plug plastic trays. Fourteen grass plugs were planted, evenly-spaced, in 13 X 17.5 X 6 cm plastic pans of pasteurized soil. Grain infested with Rhizoctonia solani or Pythium aphanidermatum was placed in a center hole in the soil, a grass plug was planted in the hole, and infested grain was placed on the plug. Potential bioantagonists were either incorporated in the soil prior to planting the plugs, or were sprayed on the plugs after planting. Treatments were evaluated by counting the number of dead and symptomatic grass plugs. For example, in pans inoculated with R. solani, 80% of the plugs survived when sprayed with conidia of a Trichoderma sp. compared to none of the unsprayed plugs.

INTRODUCTION

Biological control, the suppression of one organism by another, is a natural phenomenon occurring in the environment. Several of these organisms, or bioantagonists, have been successfully exploited for control of plant diseases. Innumerable examples remain to be developed.

One of the key components of any research effort aimed at developing biocontrol agents is locating the potential bioantagonists. There are two basic ways in which such a search can be approached, either systematically or nonsystematically. In a systematic search, microorganisms selected for some reason, such as their association with a particular host plant, are screened against a targeted pathogen or pathogens. In this case the target has been selected in advance. In a non-systematic search serendipity plays a large part. A biologist may observe, by chance, that a contaminant in a petri dish culture is antagonistic to a plant pathogen. In a directed biological control research program, the systematic approach offers the greater likelihood of identifying bioantagonists.

This paper describes an in vivo technique developed to select potential bioantagonists of two important pathogens of turf grasses, Pythium and Rhizoctonia. The objectives were to screen a large number of bacteria and fungi against these pathogens using a technique that was rapid, relatively simple to perform and reproducible.

MATERIALS AND METHODS

Over 2000 indigenous isolates of fungi and bacteria were first tested for antagonistic activity against six isolates of each target genus in an in vitro screen on agar medium. Those isolates showing activity in vitro advanced to the in vivo screen. For this screen, bacteria were grown in nutrient broth, or nutrient yeast dextrose broth, on a rotary shaker at 200 RPM for 24 h at 26° C. Bacterial cells were harvested by centrifugation

and resuspended in 0.05 M phosphate buffer. Sporulating fungi were grown on potato-carrot agar at 25° C. Spores were harvested by flooding plates with deionized water plus 0.1% Tween 20® and filtering the suspension through cheesecloth. Non-sporulating fungi and those forming submerged spores were grown in potato dextrose broth on a rotary shaker for 1 wk at 26° C. The broth cultures were filtered, rinsed with deionized water, and ground in a blender in 100 ml deionized water.

The pathogens used in the in vivo screen were isolates of P. aphanidermatum and R. solani originally isolated from turf. Inoculating media for the pathogens was prepared by growing P. aphanidermatum on sterilized oat grains and R. solani was grown on sterilized rye grains. The inoculum was incubated for 6 d at 28° C.

The host used in the screen was Agrostis palustris (creeping bentgrass cv. 'Penncross'). The grass was planted in pasteurized soil in plastic plug trays which produced circular "plugs" of grass 1.8 cm in diameter. Initially, each antagonist was applied to the bentgrass by two separate methods. In the first method, a suspension of propagules of each antagonist was incorporated into 600 g of pasteurized soil mix, the soil was placed in a 13 X 17.5 X 6 cm plastic pan, and 14 grass plugs were planted, evenly-spaced, in the infested soil. In the second method, the grass plugs were first planted in uninfested soil and then sprayed with a suspension of the antagonist. The treated pans were incubated for 24 h prior to inoculation with the pathogens to allow the antagonists to become established. Each pan was then inoculated by placing grain infested with one of the pathogens in a central hole in the soil, a grass plug was placed in the hole, and more infested grain was placed on the plug. The two pathogens were tested in separate pans in replicate.

After inoculation, the pans were incubated in a mist chamber (90-100% r.h.) at 30° C for one week. Treatments were evaluated by counting the number of dead plugs in each pan. A treatment was considered effective if more than 50% of the plugs remained healthy.

RESULTS

Of 2 200 isolates tested in the in vitro screen, 368 showed antagonistic activity against one or both of the target pathogens. In the in vivo screen, 104 of these controlled one or both of the pathogens in a single test and 20 of these showed good activity when repeated. For example, in pans inoculated with R. solani, 80% of the plugs survived when sprayed with a conidial suspension of a Trichoderma sp. as compared to none of the plugs in the control (no antagonist applied) pans. In pans inoculated with P. aphanidermatum, 82% of the plugs survived when sprayed with a suspension of cells of an unidentified bacterium compared with none of the unsprayed control plugs. Many of the isolates which failed in the second test had shown only marginal activity in the first test.

Tests were easily and quickly rated. No estimation of percentage disease was necessary as turf plugs were rated as healthy or dead.

DISCUSSION

The screening method described was useful in selecting the most promising of many candidates for biocontrol of Pythium and Rhizoctonia diseases of turf. Independent turf units (plugs) allowed easy rating of the effectiveness of the antagonists. The results were achieved quickly and

could be repeated to verify the effect. The method facilitated testing of numerous candidate antagonists and reduced the number of isolates going into advanced testing.

The conditions under which the antagonists were tested were severe. The incubation chambers were kept at 30° C and 90-100% r.h. continuously for 1 wk. These conditions provided a very challenging test for the antagonistic capabilities of the isolates. However, the extreme environmental conditions could also potentially exclude useful isolates.

The *in vivo* screen has been a valuable screening method allowing efficient time and space utilization. Techniques such as this must be developed to make screening of biological control agents more efficient and routine.

STIMULATION OF FUNGAL CONTROL OF MYCOPHAGOUS NEMATODES AND WEED MOULDS IN MUSHROOM COMPOST

P.S. GREWAL*

Nematology Section, National Centre for Mushroom Research and Training, Solan - 173213, India

ABSTRACT

In an attempt to develop an integrated pest and disease management system, dried leaves of Azadirachta indica, Cannabis sativa, Eucalyptus hybrid (E. tereticornis) and Ricinus communis were incorporated (at 3 kg/100 kg of dry wheat straw) in mushroom compost before composting. Leaf-matter incorporation reduced and/or eliminated the populations of dominant pathogenic/competitor moulds including, Fusarium solani, Sepedonium sp. and Verticillium fungicola from compost and increased the populations of antibiotic-producing fungi including Paecilomyces sp. and Penicillium spp. Populations of a mycophagous nematode, Aphelenchoides composticola were reduced below economic injury levels in the treated composts. Addition of leaf-matter significantly increased the yield of the mushroom, Agaricus bisporus when compared to controls.

INTRODUCTION

In India, mushroom compost used for the cultivation of Agaricus bisporus is largely prepared by the 'long' method (Mental et al. 1972) and spawned unpasteurized. Under such conditions, mycophagous nematodes and fungal pathogens cause heavy losses in mushroom yield. Due to the low efficacy of contact nematicides and residue problems (in the produce) encountered with the use of systemic pesticides, alternative nematode control methods are needed. With this in mind a large number of plants were screened for their leaf-extract toxicity to a myceliophagous nematode, Aphelenchoides composticola (Grewal & Sohi 1988).

In a preliminary experiment Sohi et al. (1987) observed that the incorporation of dried leaves of some plants in pasteurized compost at spawning, decreased the populations of pathogenic/competitor moulds and increased that of some antibiotic-producing fungi. The present investigation was made to elaborate these findings and develop an integrated pest and disease management system in unpasteurized composts. In this paper, the data on changes in fungal and nematode populations during composting are given, the remaining results are being published elsewhere (Grewal, in preparation).

MATERIALS AND METHODS

Five compost stacks were prepared with common ingredients; wheat straw (100 kg), chicken manure (30 kg), urea (2 kg) and wheat bran (5 kg). Dried leaves (3 kg) of each plant species, one plant species per stack) of Azadirachta indica, Cannabis sativa, Eucalyptus hybrid (E. tereticornis) and

*Present address: Entomology & Insect Pathology Section, IHR, Littlehampton, West Sussex, BN17 6LP

Ricinus communis were added to four different compost stacks before stacking (day 0). The fifth stack served as a control. On the 14th day after stacking, 10 kg gypsum (calcium sulphate) was added to all five stacks.

The chicken manure used in all treatments contained a natural fungal flora (Grewal & Grewal 1988a). The manure was also used to introduce nematodes from a laboratory-reared population of A. composticola (cultured on wheat-grain spawn of A. bisporus strain S11) to the stack. Nematode-infested spawn (100 g per stack) was added to the chicken manure before it was mixed with the other ingredients.

The substrate was prepared by the 'long' method; eight compost turnings were given, the first on the seventh day after stacking, the second on day 11 and the others at three day intervals. Nematodes were extracted from compost using a modified Cobb's decanting and sieving technique (Hooper 1986) and fungi were isolated by a standard serial dilution method. Detailed crop management practices used are described elsewhere (Grewal, in preparation).

RESULTS

Fungal populations

The changes observed in the populations of the dominant fungi during composting are presented in Fig 1. In general, the total fungal populations in all the five compost stacks increased initially (until 7th day after stacking) and then declined (lowest on 14th day after stacking) and thereafter increased again. Incorporation of dried leaves of A. indica to the compost resulted in maximum total colony-forming units (CFU) of all the fungi by the end of the composting process (44.3×10^5 CFU/g compost). A. indica was followed by E. tereticornis and control treatments (30.6×10^5 each), C. sativa (25.7×10^5) and R. communis (20.0×10^5).

Selective 'fungicidal' effects of leaf incorporation were evident. The populations of three major pathogenic/competitor moulds viz., Fusarium solani, Sepedonium sp. and Verticillium fungicola were considerably reduced in leaf-matter-treated composts when compared to controls. Dried leaves of A. indica and R. communis were most effective (when compared to any other treatment or controls) in eliminating populations of F. solani and V. fungicola from the compost by the end of the composting process and in reducing Sepedonium sp. to a very low level. At the end of the composting process (on 29th day after stacking) the total population of F. solani, Sepedonium sp. and V. fungicola represented 70.3% of the total fungal population in the control treatment, whereas they constituted only 9% in the R. communis, 9.6% in the C. sativa, 10.7% in the A. indica and 34.3% in the E. tereticornis treatments.

Interestingly, the addition of leaf-matter increased the population of antibiotic-producing fungi, including Paecilomyces sp. and Penicillium spp. At the end of the composting process, Penicillium spp. represented 59.0% of the total fungal population in the R. communis treatment, 52.3% in the A. indica, 45.3% in the E. tereticornis and 42.4% in the C. sativa treatments as compared to that of 10.7% in controls. Paecilomyces sp. also represented 19% of the total fungal population in the R. communis and 18.9% in the A. indica treatments but only 5% in controls.

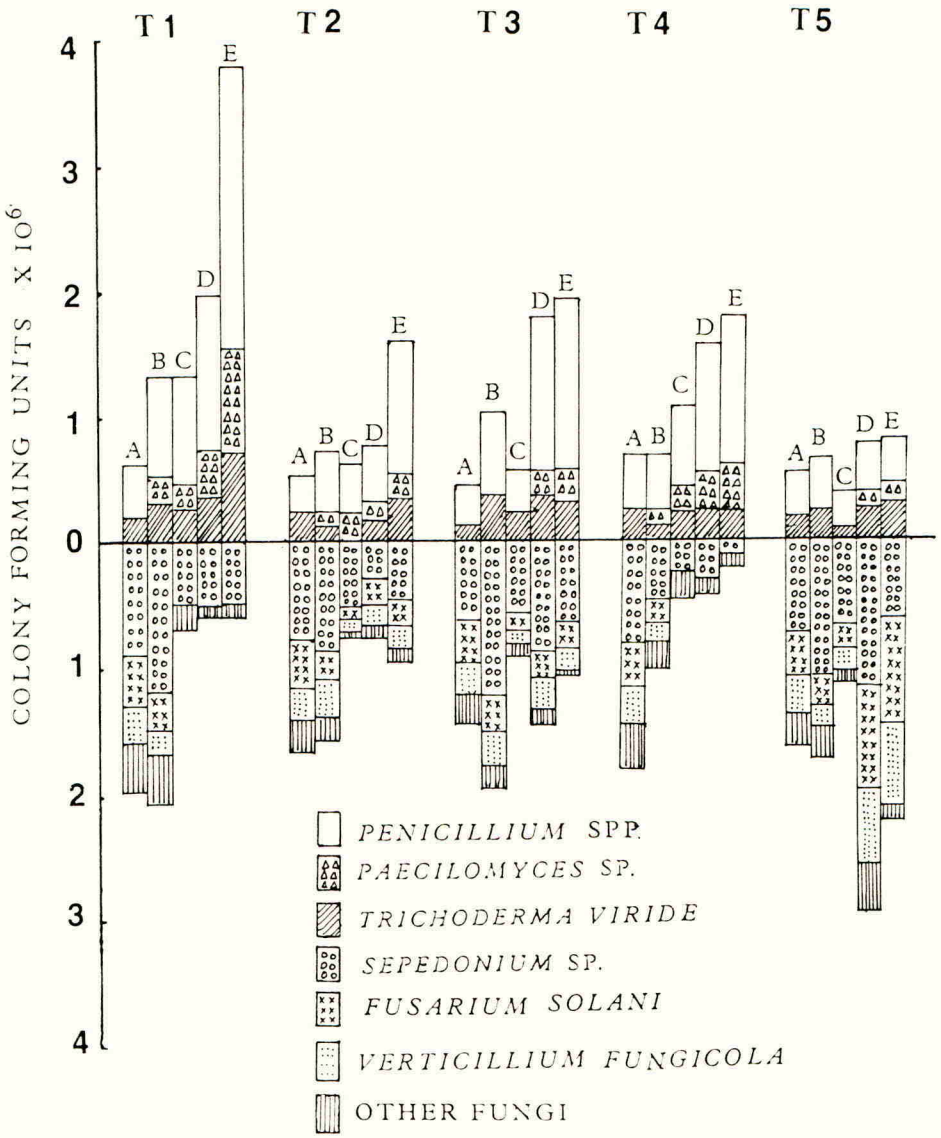


Fig. 1. Fungal populations isolated on 0, 7, 14, 20 and 29th day (A-E) post-stacking from compost stacks incorporated with dried leaves of Azadirachta indica (T1), Cannabis sativa (T2), Eucalyptus tereticornis (T3), Ricinus communis (T4) and control stack (T5).

Nematode populations

Incorporation of dried leaves of all four plant species in compost considerably reduced the A. composticola populations when compared to controls (Fig 2). The compost treated with R. communis leaves showed the lowest nematode populations by the end of the composting process when compared to any other treatment or controls. The 'nematicidal/nematostatic' effects of leaf incorporation were also evident during cropping (especially in the 40 d post-spawning period). However, by the end of cropping, the nematode populations in the controls also declined and the differences among various treatments were not very obvious.

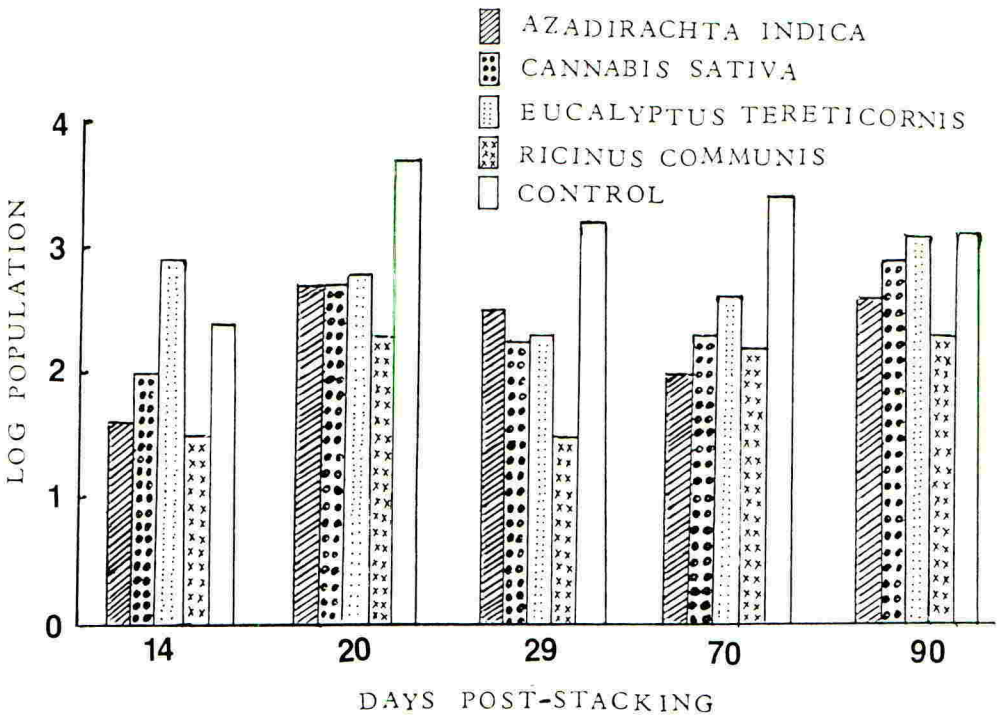


Fig. 2. Population of A. composticola in (200 g) compost at days post-stacking.

Mushroom yield

The yield of A. bisporus obtained from different composts is presented in Table 1. Leaf-incorporated composts produced significantly higher yields when compared to controls; the dried leaves of R. communis, C. sativa, A. indica and E. tereticornis increased the yields by 108.5, 88.7, 85.9 and 74.8 per cent respectively when compared to controls.

TABLE 1

Yield of *Agaricus bisporus* in various treatments

Treatment	Mean numbers of sporophores	Mean wt (g) per sporophore	Yield kg/bag	Yield kg/100 kg compost
A. indica	124.8	10.6	1.3	13.2
C. sativa	160.4	8.7	1.3	13.9
E. tereticornis	90.6	10.4	0.9	9.5
R. communis	164.8	9.2	1.5	14.8
Control	47.2	12.8	0.7	7.1
L.S.D. ($p < 0.05$)	23.4	1.7	0.2	

DISCUSSION

Addition of dried plant leaves in compost exhibited both 'fungi-suppressive' and 'fungi-stimulatory' effects. Leaf-matter-incorporated composts were dominated by antibiotic-producing fungi (*Penicillium* spp. and *Paecilomyces* sp.) whereas controls were represented mainly by pathogenic/competitor moulds (*F. solani*, *Sepedonium* sp. and *V. fungicola*). Such myco-floral changes exhibited by leaf-matter incorporation might be either due to (I) selective fungicidal effect of leaf-extracts and/or their decomposition products by which some fungi were suppressed and others got a free substrate to multiply or (II) to a changed nutritional environment created by leaf incorporation which favoured some fungi but disfavoured others. The favoured fungi (especially those producing antibiotics) then reduced and/or eliminated other fungi by way of antibiosis (competitive elimination).

Both the above possibilities have been supported by evidence from the literature. For instance, Grewal & Grewal (1988b) reported selective fungicidal effects of leaf- and seed-extracts of *A. indica* and leaf-extracts of *Chrysanthemum indicum* and *Tagetes erecta* to most of the mushroom weed moulds. Singh *et al.* (1986) found that in a soil amended with the oil-seed cakes of castor, mustard and neem, the frequency of saprophytic fungi increased and that of parasitic fungi decreased. Sohi *et al.* (1987) suggested that the addition of dried leaves of some plants to pasteurised compost at spawning encouraged the growth of some antibiotic-producing fungi and that the latter suppressed the growth of weed moulds. It therefore seems probable that both the above factors might have played a considerable and collective role in defining the ultimate fungal composition in compost.

Leaf-matter incorporation in compost reduced the populations of a mycophagous nematode, *A. composticola* below economic injury levels. These 'nematicidal' effects can be attributed to three factors including, leaf-extract toxicity, toxic leaf-decomposition products and 'fungal-antibiosis'. The toxicity of leaf-extracts of many plants to *A. composticola* have been studied (Grewal & Sohi 1988). The second possibility is supported by evidence from Sayre *et al.* (1965) who reported that decomposition products (especially butyric acid) or rye (*Secale cereale*) and timothy (*Phleum pratens*) were toxic to plant parasitic nematodes, *Meloidogyne incognita* and *Pratylenchus penetrans*.

Evidence for 'fungal-antibiosis' came from Grewal and Sohi (1987) who reported that fungal metabolites extracted from the cultures of some antibiotic-producing fungi including Arthobotrys conoides, Gliocladium deliquescens, Paecilomyces sp., Penicillium sp., Trichoderma viride and Trichothecium roseum, were highly toxic to adults and juveniles of A. composticola. They further observed that the antinemic substances produced by these fungi were water soluble and required free water for their removal/release from fungal hyphae to be active against the nematodes. This requirement is easily met during composting and cropping.

Mushroom yields in the leaf-matter-treated composts were significantly higher when compared to controls. This is obviously due to a favourable change in mycofloral composition (suppression of pathogenic/competitor moulds) and reduction in the nematode populations. Besides these two factors, Grewal (in preparation) observed that leaf incorporation in compost, stimulated thermophilic fungi, the activities of which resulted in a better compost quality (higher N content and neutral pH) which might also have contributed towards higher mushroom yields.

From the foregoing results and discussion, it is concluded that the leaf-matter incorporation resulted in a sort of integrated bio-management system in which populations of antibiotic-producing fungi were encouraged and that of competitor/pathogenic moulds and of nematodes were reduced or eliminated.

REFERENCES

Grewal, P.S.; Grewal, S.K. (1988a) Solar heat pasteurization of casing soil and chicken manure for mushroom cultivation. Mushroom Journal for the Tropics 8, 35-45.

Grewal, P.S.; Grewal, S.K. (1988b) Selective fungicidal properties of some plant extracts to mushroom weed moulds. Phytopathologia mediterranea (in press).

Grewal, P.S.; Sohi, H.S. (1987) Effect of various fungal metabolites on Aphelenchoides composticola Franklin and its multiplication on some fungi. Mushroom Science (Abstracts) 12, 54.

Grewal, P.S.; Sohi, H.S. (1988) Toxicity of some plant extracts to Aphelenchoides composticola. Indian Journal of Nematology (in press).

Hooper, D.J. (1986) Extraction of free-living stages from soil. In: Laboratory Methods for Work with Plant and Soil Nematodes, J.F. Southey (Ed.), London: Her Majesty's Stationery Office, pp. 5-30.

Mental, E.F.K.; Agarwala, R.K.; Seth, P.K. (1972) A Guide to Mushroom Cultivation. Farm Bulletin No. 2. Published by Farm Information Unit, Directorate of Extension, Ministry of Agriculture, New Delhi, India.

Sayre, R.M.; Patric, L.A.; Thorpe, H.J. (1965) Identification of a selective nematicidal compound in extracts of plant residues decomposing in soil. Nematologica 11, 263-268.

Singh, S.P.; Pant, V.; Khan, A.M.; Saxena, S.K. (1986) Changes in phenolic contents, related rhizosphere mycoflora and nematode population in tomato inoculated with Meloidogyne incognita as a result of soil amended with organic matters. Indian Journal of Nematology 15, 197-201.

Sohi, H.S.; Grewal, P.S.; Seth, A. (1987) Preliminary studies on the effects of plant extracts on mycelial growth and yield of Agaricus bisporus (Lange) Singer and fungal flora in the compost. In: Cultivating Edible Fungi, P.J. Wuest; D.J. Royse; R.B. Beelman (Eds), Amsterdam: Elsevier Science Publishers BV, pp. 121-128.

ROLE OF FUNGI IN BIOLOGICAL CONTROL OF PLANT-PARASITIC NEMATODES

K.D. UPADHYAY AND KUSUM DWIVEDI

Department of Entomology, C.S. Azad University of Agriculture & Technology, Kanpur-208002 (U.P.), India

ABSTRACT

Due to limitations inherent in the use of pesticides, their possible environmental hazards, difficulties encountered in developing resistant cultivars and economic pressures on land use, greater efforts are now being made to integrate biocontrol agents in plant-parasitic nematode management strategies. Fungi (ranging from predacious trap-forming species to obligate endoparasitic forms) play an important role in regulating nematode populations. There are more than a hundred species of predacious fungi attacking nematodes in agricultural soils. Endoparasitic fungi parasitize various developmental stages of nematodes. Egg parasites are known to be efficient biocontrol agents of nematodes. It can be concluded that the agents with the maximum potential for nematode control are likely to be those which are endemic in agricultural soils and can be exploited under conventional farming practices.

INTRODUCTION

Recent awareness of the possible danger of toxic pesticides, the time required for development of resistant cultivars, and limitation in use of cultural methods, are important factors increasing the need to determine the potential of biological management of plant-parasitic nematodes. In addition, many nematicides have been withdrawn from the market due to their persistence in soil or contamination of ground water. All these factors promote the need to concentrate on integration of biological control agents into overall nematode management strategies. Interaction between fungal antagonists and nematodes has been known for many years to occur in agricultural soils (Mankau 1980).

The nematode-destroying fungi play a vital role in keeping soil populations of nematodes under control, as well as in regulating the flow of important elements locked up in nematode biomass. The fungal antagonists of nematodes consist of a great variety of organisms which include nematode-trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs, parasites of nematode cysts, and other fungi that produce metabolites toxic to nematodes. Predacious, parasitic and biochemical relationships with nematodes have been evolved by all major groups of soil fungi, from Phycomycetes to Basidiomycetes (Table 1).

TABLE 1

Some fungi parasitizing important plant-parasitic nematodes

Fungus	Plant-parasitic nematode	Reference
Arthrobotrys robusta	Heterodera spp.	Hams & Wilkin 1961
A. arthrobotryoides	Meloidogyne incognita	Mankau 1961
Catenaria auxiliaris	H. avenae & G. rostochiensis	Kerry 1980
Dactylaria candida & D. thaumasia	Heterodera spp.	Hams & Wilkin 1961
D. thaumasia	Meloidogyne incognita	Mankau 1961
Dactylella oviparasitica	Meloidogyne spp.	Stirlin & Mankau 1978
Cylindrocarpon destructans	G. rostochiensis	Morgan-Jones et al. 1986
Nematophthora gynophila	H. carotae, H. cruciferae, H. schachtii & H. trifolii	Kerry & Crump 1977
Paecilomyces lilacinus	M. incognita, G. pallida	Jatala et al. 1979
	Tylenchulus semipenetrans, Radopholus similis & G. rostochiensis	Jatala 1985
	Naccobus spp.	O'Hara & Jatala 1985
Verticillium chlamydosporium	H. avenae	Kerry 1980
	M. arenaria	Morgan-Jones et al. 1981
Torula heteroderae	H. schachtii	Korab 1929

PREDACIOUS FUNGI

The existence of fungi that trap and prey on nematodes was first reported by Zopf (1889). More than 100 species of these fungi trap and prey on nematodes in agricultural soils. In general, nematode-trapping fungi fall into two groups:

(i) Those having adhesive networks of hyphae, which are good saprophytes and grow rapidly.

(ii) Those which capture nematodes by adhesive knobs, branches, or constricting rings, are more predacious and grow slowly.

In addition, these fungi may also produce toxins that paralyse or kill nematodes prior to penetrating their cuticle (Olthof & Estey 1963). Activity of these fungi may be influenced by soil pH, moisture, temperature, and available nutrients (Mankau, 1968). Even where populations of these fungi are indigenous and common, there is no foolproof means of effectively increasing their biocontrol potential.

Two commercial products of predacious fungi were prepared by Cayrol and Frankowski (1979); 'Royal 300', a preparation of an isolate of Arthrobotrys robusta for control of Ditylenchus myceliophagus on mushrooms, and 'Royal 350', a different isolate of Arthrobotrys for control of Meloidogyne sp. on tomatoes. However, their non-specific nature in predation, slow growth and requirement for high doses of nutrients could be drawbacks to their success as candidates for commercial exploitation.

ENDOPARASITIC FUNGI

These consist of a great variety of organisms which parasitize various developmental stages of nematodes and are from a diverse group of fungi belonging to Chytridiomycetes (Catenaria sp.), Oomycetes (Myzocytium humicola), Zygomycetes (Meristacrum asterospermum), Deuteromycetes (Harposorium anquillulae), and Basidiomycetes (Nematoctonus sp.). The relationship between nematodes and these fungi may be governed by the number of infective fungal units and the number of nematodes present in the soil.

Endoparasitic fungi can be obligate or non-obligate. There are few species of the former; they are very specialised organisms and are capable of invading female nematodes within a few days of exposure on the root surface. The latter are also represented by a few spcies and in most cases are primarily egg parasites. Chaetomium cochloides, Exophiala pisciphila, Fusarium oxysporum, Phytophthora cinnamomi, Trichosperon beigelii, Pythium sp. and a sterile mycelium were found to be associated with females and precyst stages of Heterodera glycines (Gintis et al. 1983). However, difficulties in producing them on a large scale and establishing them as effective biocontrol agents in the soil environment are major constraints to the use of these organisms.

PARASITES OF EGGS

Egg parasites may be more effective in reducing nematode populations compared with other parasitic or predacious fungi. Nematode eggs of the group Heteroderidae and those deposited in a gelatinous matrix are more vulnerable to attack by these organisms than those of migratory parasites. This is because the oviposition behaviour of these nematodes (both in egg masses and cysts results in total exposure of the eggs to fungal attack. In all cases, eggs in the early embryonic developmental stage (prior to gastrulation) are more vulnerable to infection. Paecilomyces lilacinus were shown to infect eggs of M. incognita and destroy the embryos within five days. Percentage infection of the eggs was directly correlated to the length of time that they were exposed to the fungus. In the field, test plants grown in plots inoculated with fungus had a significantly lower root-galling index than did those grown in nematicide-treated plots (Jatala 1985).

P. lilacinus has been the subject of many recent investigations and the successful results obtained in The Philippines are the major factor behind its commercial production under the name of 'BIOCON' in this country (Jatala 1986).

CONCLUSIONS

Use of biocontrol agents for reducing nematode populations to levels below damage-thresholds should constitute an important aspect of the strategy of integrated nematode management. Considering the prevailing cultural and environmental factors, greater emphasis needs to be given to research on cyst and egg parasites on account of their great potential for nematode control.

REFERENCES

Cayrol, J.C.; Frankowski, J.P. (1979) Une methode de lutte biologique contre les nematodes a galles des racines appartenant au genre Meloidogyne. Review of Horticulture 193, 15-23.

Gintis, B.O.; Morgan-Jones, G.; Rodriguez-Kabana, R. (1983) Fungi associated with several developmental stages of Heterodera glycines from an Alabama soybean field soil. Nematropica 13, 181-200.

Hams, A.F.; Wilkin, A.F. (1961) Observations on the use of predacious fungi for control of Heterodera spp. Annals of Applied Biology 49, 515-523.

Jatala, P. (1985) Biological control of nematodes. In: An Advanced Treatise on Meloidogyne, Vol. 1, Biology and Control, J.N. Sasser and C.C. Carter (Eds), North Carolina State Graphics, 1, pp. 303-308.

Jatala, P. (1986) Biological control of plant-parasitic nematodes. Annual Review of Phytopathology 24, 453-489.

Jatala, P.; Kaltenbach, R.; Bocangel, M. (1979) Biological control of Meloidogyne incognita acrita and Globodera pallida on potatoes. Journal of Nematology 12, 226-227.

Kerry, B.R. (1980) Biocontrol: Fungal parasites of female cyst nematodes. Journal of Nematology 12, 253-259.

Kerry, B.R.; Crump, D.H. (1977) Observations of fungal parasites of females and eggs of the cereal cyst nematode, Heterodera avenae, and other cyst nematodes. Nematologica 23, 193-201.

Korab, J.J. (1929) Results of a study of the beet nematode Heterodera schachtii at the nematode laboratory of the Belaya Tserkov Research Station. Sbonnik Sortovog Semenovodches Kogo Upravleniya 8, 29-67.

Mankau, R. (1961) The use of nematode-trapping fungi to control root-knot nematodes. Nematologica 6, 326-332.

Mankau, R. (1968) Reduction of root-knot disease with organic amendments under semifield conditions. Plant Disease Reporter 52, 315-319.

Mankau, R. (1980) Biological control of nematode pests by natural enemies. Annual Review of Phytopathology 18, 415-440.

Morgan-Jones, G.; Godoy, G.; Rodriguez-Kabana, R. (1981) Verticillium chlamydosporium, fungal parasite of M. arenaria females. Nematropica 11, 115-119.

Morgan-Jones, G.; Rodriguez-Kabana, R.; Jatala, P. (1986) Fungi associated with cysts of potato cyst nematodes in Peru. Nematropica 16, 21-31.

O'Hara, C.M.; Jatala, P. (1985) Ultrastructure of Meloidogyne, Globodera and Nacobbus egg shells as related to the activities of biocontrol fungi. Journal of Nematology 17, 508.

Olthof, Th.H.A.; Estey, R.H. (1963) A nematoxin produced by the nematophagous fungus Arthrobotrys oligospora Fresenius. Nature 197, 514-515.

Stirling, G.R.; Mankau, R. (1978) Dactylella oviparasitica, a new fungal parasite of Meloidogyne eggs. Mycologia 70, 774-783.

Zopf, W. (1889) Zur Kenntnis der Infektions-Krankheiten nie derer Thiere Und Pflanzen. Nova Acta Leopoldina 52, 314-376.

PRELIMINARY STUDIES ON THE POTENTIAL OF *PASTEURIA PENETRANS* TO CONTROL *MELOIDOGYNE* SPECIES

A.G. CHANNER and S.R. GOWEN

Department of Agriculture, Reading University, Earley Gate, Reading, Berks, RG6 2AT

ABSTRACT

Pasteuria penetrans is an obligate bacterial parasite of nematodes with potential as a biocontrol agent. However, variation between *Meloidogyne* biotypes in their susceptibility to infection by particular strains of *P. penetrans* may lead to inconsistencies in control. Blending *P. penetrans* populations from a variety of sources increases the consistency of infection over a range of *Meloidogyne* biotypes and may circumvent the problem of nematode resistance in the field. The application of *P. penetrans* spore populations to soil resulted in significant nematode control, in both pot and field plot tests.

INTRODUCTION

Pasteuria penetrans (Thorne) Sayre and Starr has several of the attributes of a successful biocontrol agent: target specificity, resistance to heat and desiccation, tolerance of pesticides and safety to the user. These advantages are offset by its obligate parasitism. However, the development of a simple, if small-scale, *in vivo* method for producing spore inoculum (Strirling & Wachtel 1980) and reports that *P. penetrans* produced *in vivo* can give effective nematode control in microplots (Stirling 1984) prompted our realization that *P. penetrans* deserves investigation as a potential solution to the root knot nematode problems of small-scale cultivation.

Variation in host specificity between different *P. penetrans* populations and in susceptibility to infection between different *Meloidogyne* biotypes, detailed by Stirling (1985), has important implications for the use of *P. penetrans* as a consistent biocontrol agent.

The infection study described below had two aims. The first was to establish whether variation in susceptibility to infection within field populations of *Meloidogyne* has a genetic basis, and whether, therefore, selection for resistant biotypes could occur in the field. The second was to determine whether formulating blends of *P. penetrans* from different sources is likely to improve the consistency of nematode infection.

The second experiment tests the efficacy of biocontrol by P. penetrans in pots.

Finally, we report on the results of a field-trial undertaken on an atoll in the South Pacific state of Tuvalu, where home garden production is the only source of fresh vegetables, and where Meloidogyne species are a major yield constraint (Gowen, 1985).

MATERIALS AND METHODS

The following experiments employ one or more of five main P. penetrans populations: PP1 (from USA), PP2 (Australia), PP3 (South Africa), PP4 (Papua New Guinea) and PP5 (Ivory Coast).

Experiment 1:

A comparison of spore attachment levels obtained with three P. penetrans populations on five populations of Meloidogyne.

The three P. penetrans populations were as follows:
1. A blend of PP1, PP2, PP3, PP4 and PP5 plus smaller quantities of two other spore populations from Australia and the USA.
2. PP3, which has been cultured in Reading for 2 years.
3. PP5, obtained directly from a naturally-infected Meloidogyne population in the Ivory Coast.

All three populations were made up to 15000 spores/ml of water in watchglasses containing c. 20 juvenile nematodes from one of five Meloidogyne populations. The origins of these populations was as follows:

1. M. incognita from a field plot in Tuvalu.
2. The progeny of the nematodes in population 1 (which did not become infected when that population was exposed to a high concentration of PP3 spore suspension).
3. M. javanica from less than 5 egg masses, Sri Lanka.
4. Meloidogyne sp. from a field plot in Barbados.
5. M. incognita, from a single egg mass population at Rothamsted Experimental Station.

Two replicate watchglasses were used for each treatment combination and after 21 h incubation at 28°C, six nematodes were removed from each watchglass and the number of spores on each counted.

Experiment 2:

The effect of P. penetrans on reproduction of M. javanica in pots.

P. penetrans root powder inoculum,(a blend of PP1, PP2, PP3 and PP4), containing 41000 spores/mg was incorporated into 1.5 litres of soil (John Innes compost No. 2) at rates of 400 mg and 2000 mg per pot. Thorough mixing was achieved by shaking dry soil with inoculum in a plastic drum for 2 min. 48 h after P. penetrans incorporation, juveniles of the M. javanica Sri Lanka population were added at rates of 400 or 4200 per pot. One week later, 8cm-tall tomato plants, cv Tiny Tim, were planted. P. penetrans and nematode combinations were randomised within and replicated over three blocks. After 30 d plant tops were cut, dried, and weighed and the numbers of galls and egg masses per root system counted.

Experiment 3:

Effect of P. penetrans on reproduction of Meloidogyne sp. in a tomato field plot.

P. penetrans root powder inoculum, (a blend of PP1, PP2 and PP3) at a dose of 2 g per plant hole (at c. 11000 spores/g soil in a volume of 30 x 30 x 15cm) was incorporated into a nematode- infested plot in Tuvalu. The inoculum was mixed thoroughly with soil from the planting sites, and one week later tomatoes, cv Pixie, were planted in the treated soil. The experiment had a randomised block design with 3 blocks, each containing two plots of six plants, one with P. penetrans, the other without. After one month, tops were cut and weighed fresh. Roots were assessed as for Experiment 2. In addition, female nematodes were dissected out from the roots of plants in each treated plot and fifty examined for the presence of spores.

RESULTS

Experiment 1:

Both the nematode biotype and the P. penetrans source had a significant influence on the number of spores adhering per nematode (Table 1). The number of spores attached to the nematode population (Tuvalu 2) bred from those individuals which resisted infection, was significantly less than the number attached to the population (Tuvalu 1) from which those individuals were originally derived (t-test: $P < 0.001$).

TABLE 1

Comparison of the number of spores per nematode obtained with three P.penetrans populations on five populations of Meloidogyne

	Source of Meloidogyne population				
	Tuvalu(1)	Tuvalu(2)	SriLanka	Barbados	RES
Blend*	9.5(2.2)	1.2(0.3)	8.5(1.2)	2.9(0.8)	1.8(0.4)
PP3	10.2(2.9)	0.9(0.3)	16.6(2.9)	1.3(0.4)	0.8(0.3)
PP5	3.7(1.0)	0.1(0.1)	6.8(0.8)	0.0(0)	0.0(0)

ANOVA on square root transformed data: P.penetrans, P <0.001; Nematode, P <0.001; interaction P <0.01
*PP1,PP2,PP3,PP4,PP5 plus others.
( ) SE of original data.

While there was no significant difference overall in attachment levels obtained with the Blend and PP3, there was evidence of an interaction i.e., the Blend attached less well to the Sri Lankan nematodes than did PP3, but this was compensated for (if poorly) by relatively better attachment to the Barbados and Rothamsted nematodes.

Experiment 2:

The higher dose of P. penetrans achieved a significant reduction in initial invasion (Table 2) and an almost total curtailment of egg mass production (Table 3). The lower dose, while not burdening nematodes with enough spores to lower invasion, succeeded in causing parasitization and the infertility of about half the nematodes developing in the roots. (The presence of nematodes did not cause significant reduction in plant weight in comparison with nematode-free controls.)

TABLE 2

The effect of P. penetrans on the mean number of first generation galls of M. javanica per tomato root system.

	Dose of P. penetrans (mg)		
Nematode inoculum	0	400	2000
420	7.0	5.3	1.7
4200	53.0	52.3	22.0

Regression ANOVA on square root transformed data: P. penetrans dosage effect, P <0.05.

TABLE 3

The effect of P. penetrans on the mean number of first generation egg-masses of M. javanica per tomato root system.

Nematode-inoculum	Dose of P. penetrans (mg) 0	400	2000
420	5.3	2.0	0.0
4200	36.7	15.0	1.3

Regression ANOVA on square root tranformed data: P. penetrans dosage effect, P <0.001.

Experiment 3:

P.penetrans lessened nematode damage to plants in the field and thereby brought about a significant increase in plant health (Table 4).

TABLE 4

The effect of P. penetrans on galling, the percentage of Meloidogyne sp. females infected, egg mass levels, and plant weight in one-month-old field grown tomatoes

	Number per plant Galls	Egg masses	% females infected	Plant weight (g)
with P. penetrans	39.6	10.4	44	18.2
without P. penetrans	58.5	16.6	-	13.3

ANOVA: Effect of P. penetrans on galls, P <0.09; egg masses, N.S; Plant weight, P <0.05.

DISCUSSION

There is no doubt that P. penetrans can achieve dramatic control of root knot nematodes, but it is also clear that control may fail completely where a nematode population is resistant to infection by the P. penetrans strain(s) deployed.

Davies, et al. (1988) report that fifteen spores per nematode can reduce invasion by more than 70%. Our own (unpublished) data has shown that the

highest P. penetrans dose from soil in Experiment 2 achieved 100% attachment to M. javanica, with a mean of 15.1 (S.E. 1.3) spores per juvenile. However, this nematode population ("Sri Lanka") exhibited high and uniform susceptibility to the P. penetrans populations tested, which constrasts greatly with other nematode populations. Of particular concern, is the evidence that the field population of M. incognita from Tuvalu contains individuals which resist infection and which pass this resistance on from one generation to the next.

One possible way of avoiding this problem is to formulate and mass-produce blends of P. penetrans specific to all the Meloidogyne biotypes likely to be encountered. This would depend on a detailed assessment of the breadth of specificity and virulence of many P. penetrans populations from different sources. A successful blend would work less by achieving the high levels of infection required to reduce invasion than by infecting sufficient adult nematodes to perpetuate those spores most pathogenic to the particular Meloidogyne biotypes in the treated field.

If, after each crop cycle, roots can be dried (to kill uninfected nematodes) and then mixed back into the soil, we see reason to believe that P. penetrans could bring about consistent and economic control of Meloidogyne species within the medium-term.

ACKNOWLEDGEMENTS

This work was funded by the Overseas Development Administration. Thanks are given to the Agricultural department, Government of Tuvalu for their helpful collaboration, and also G. Stirling, V. Spaull, R. Sayre, Ramu Sugar Ltd. (PNG) and CIP St. Albans for isolates of P.penetrans and/or root knot nematodes. Meloidogyne spp. are used under MAFF licence number PHF1007/48(28).

REFERENCES

Davies, K.G.; Kerry, B.R; Flynn, C.A. (1988) Observations on the pathogencity of Pasteuria penetrans a parasite of root knot nematodes. Annals of Applied Biology 112, 491-501.

Gowen, S.R. (1985) Report on a survey of plant parasitic nematodes in Tuvalu. FAO & South Pacific Commission. Suva, Fiji, pp10.

Stirling, G.R; Wachtel, M.F. (1980) Mass production of Bacillus penetrans for the biological control of root knot nematodes. Nematologica, 26, 308-312.

Stirling, G.R., (1984) Biological control of Meloidogyne javanica with Bacillus penetrans. Phytopathology 74, 55-60.

Stirling, G.R. (1985) Host specificity of Pasteuria penetrans within the genus Meloidogyne. Nematologica 31, 203-209.

THE PRODUCTION OF *PASTEURIA PENETRANS* FOR CONTROL OF ROOT KNOT NEMATODES

S.R. GOWEN and A.G. CHANNER

Department of Agriculture, University of Reading, Earley Gate, Reading, Berks, RG6 2AT. UK.

ABSTRACT

Yields of *Pasteuria penetrans* spores from roots of tomato plants inoculated with different numbers of spore encumbered *Meloidogyne incognita* juveniles were compared. Production from roots of plants grown in 1 litre pots increased linearly with an increase of nematodes from 1000-5000. A similar, but less marked, trend occurred with spore yields from 0.3 litre pots inoculated with 500-3000 nematodes. Production was also improved when root systems were pruned before adding nematode inoculum. Spore production may be related to the pathogenicity of *P.penetrans* isolates for the particular *Meloidogyne* biotype used in the production system.

INTRODUCTION

Root knot nematodes (*Meloidogyne* spp) can be a major production constraint for many tropical and subtropical crops on land which is continuously cultivated with successions of nematode-susceptible hosts and where currently available nematode control practices are ineffective, uneconomic or otherwise inappropriate for low input agriculture.

Pasteuria penetrans has been known as an obligate bacterial parasite of nematodes for many years (Mankau, 1975). Most detailed studies have been done on those populations that parasitise *Meloidogyne* spp; the taxonomic status of the different *P. penetrans* populations parasitic on other plant parasitic nematodes is still under review (Starr and Sayre, 1988).

P. penetrans is considered to have potential as a biological control agent (Mankau 1975, Sayre 1980, Stirling 1984, Kerry 1987) but as no technique of *in vitro* mass production has been developed, the practical use of this parasite may be restricted to container-grown crops and to subsistence farmers and gardeners. These users may well be able to produce modest quantities of inoculum derived from *in vivo* production techniques (Stirling & Wachtel 1980).

To optimize production it is necessary to infect host plants with free-living juveniles which have been exposed to spores of *P. penetrans*. Host plants should be at a growth stage that will provide many invasion sites but

still be young and vigourous enough to sustain the burden of nematode parasites for a further 35-50 days after invasion, during which time the bacterium colonises the body contents of the developing female nematodes. After this period, spores of P. penetrans contained within the body of the nematode can be released by grinding the dried root system (Stirling and Wachtel 1980). The spore-containing powder can then be applied to soil as a powder or in a suspension.

In some glasshouse experiments at Reading, attempts have been made to determine an appropriate size of host plant and an optimum nematode inoculum density to obtain the highest yields of P. penetrans spores. These yields may then be related to the estimated spore concentrations necessary to control nematodes in the field.

MATERIALS AND METHODS

P. penetrans production on tomato plants

Six-week-old dwarf bush tomatoes cv Tiny Tim, grown in 0.3 litre pots in either a soil-less potting compost (Vermipeat) or a 2 : 1 loam Vermipeat mixture, were inoculated with juveniles of a M. incognita population originating from Bangladesh, at 500, 1000 and 3000 per pot. The juveniles had previously been exposed to a suspension of spores of P. penetrans (PP3) originating on M. javanica from South Africa. The plants were grown for 45 d at a temperature of 25-33° C after which the roots were washed, chopped, dried and powdered with a mortar and pestle, and spore estimates were made from a 100 mg sample of the powder suspended in 100 ml of water.

Under similar conditions, a second experiment was run concurrently using tomatoes of similar age, growing in 1 litre pots, containing a loam, sand, Vermipeat mixture (2 : 1 : 0.5). Plants were inoculated with nematodes at 1000, 2000 and 5000 per pot.

In a third experiment, 6-week-old tomato plants grown in 0.3 litre pots were repotted in 1 litre pots as follows:-

1. repotted with no root disturbance
2. repotted with no root disturbance: aerial shoot pruned above the second node
3. repotted with no root disturbance: drenched with 10ml of a 250 ppm solution of chlormequat chloride (CCC)
4. repotted after pruning the lower half of the root system

Two days after planting the pots were inoculated with 16000 eggs of M. incognita and drenched with a suspension of 10×10^6 spores of P. penetrans (PP3). The plants were grown for 6 weeks and estimates of spore concentration were made from the dried roots as previously described.

Spore production on tomato plants infected with different populations of M. incognita

Five-week-old-tomatoes cv Tiny Tim growing in 0.3 litre pots were inoculated with 1500 juveniles of M. incognita deriving either from a population from the South-West Pacific state of Vanuatu, or from a laboratory population of unknown overseas origin. In the 24 h before inoculation the nematodes were left in suspensions of spores of P. penetrans isolates PP1 and PP3 originating from Australia and South Africa respectively. Spore attachment on 15 randomly selected nematodes was recorded before inoculation .

The plants were maintained in a glasshouse at 25-35° C for 51 d after which roots were washed, dried, weighed and ground with a pestle and mortar. Spore concentrations were determined from 100 mg subsamples in 100 ml water suspensions.

RESULTS

Greater spore production was generally obtained from plants inoculated with the highest nematode inoculum (Tables 1a and 1b).

TABLE 1a 1b

Production of spores of P.penetrans (PP3) in tomato roots 45 days after inoculation with different numbers of spore-encumbered juveniles of M.incognita.

(a) in 0.3 litre pots with various growing media

Nematode innoculum	spores per root system x 10^6 Vermipeat	Loam:Vermipeat
500	23.9	31.9
1000	68.9	73.5
3000	47.5	126.3

ANOVA: Effect of nematodes P <0.05 Effect of medium N.S. Interaction N.S. (means of 8 replicates).

(b) In 1 litre pots in a soil: sand: vermipeat medium

Nematode innoculum	Spores per root system x 10^6
1000	480.1
2000	692.5
5000	1858.8

Linear trend significant at the 0.1% level. (means of 5 replicates)

Increased root growth in the peaty medium was offset by inconsistent invasion, and spore production in the two media was not significantly different. Plants grown in 1 litre pots were larger than those grown in 0.3 litre pots and spore production in these root systems was greater. Plants in which the root systems had been pruned before inoculation also yielded more spores (Table 2).

Production of PP3 was greater on the laboratory population of M. incognita than on that from Vanuatu despite the fact that spore attachment was greater on juveniles of the latter (Table 3). The isolate PP1 neither attached nor reproduced as effectively on these nematode populations.

TABLE 2

Production of spores of P. penetrans in roots of tomato plants treated in various ways before inoculation with eggs of M. incognita in 1 litre pots, drenched with spores of P. penetrans isolate PP3.

Treatment	spores per root system x 10^6 (Means of 5 replicates)
Plant re-potted	148.8
Shoot pruned	146.5
CCC[1] drench (10 ml 250 ppm)	156.2
Roots pruned	210.2*

*Spore production from root pruning-treatment significantly greater. (P = 0.05).

[1] CCC= chlormequat chloride.

TABLE 3

Spore production of different P. penetrans isolates on tomato plants 51 days after inoculation with juveniles of two populations of M.incognita

Source of M. incognita	Pasteuria isolate PP3 Spore attachment on juveniles	Spores per root system x10^6
Lab. (unknown origin)	14	354**
Vanuatu	20.5**	93
Source of M. incognita	**Pasteuria isolate PP1 Spore attachment on juveniles**	**Spores per root system x10^6**
Lab. (unknown origin)	0.36	9.6
Vanuatu	0.57	13.5

** Spore attachment and spores per root system with PP3 significantly greater (t test; P = 0.01) on Vanuatu and from laboratory populations respectively. No significant differences with PP1.

DISCUSSION

The most important requirement for producing P. penetrans is the provision of a host plant that has an actively growing root system with plenty of invasion sites for the root knot nematode. Overall, production is greater in root systems growing in larger volumes of soil, and under the conditions of these experiments the optimum size of nematode inoculum may not have been achieved. The nature of the life cycle of the bacterium requires that the host nematode develops to maturity, and so successful parasitism by both organisms depends on the continued vigour of the plant. The life cycle of the bacterium may not be completed if the host system is stressed and begins to senesce prematurely (Gowen, unpublished data).

Manipulation of the host plant to stimulate new root growth and thus create more invasion sites would be of benefit in improving spore production. In this respect root pruning was better than using the plant growth regulator CCC which has been used for improving tomato root development (Thomas 1976). Production may be influenced by the form of inoculation, and the numbers of infected juveniles entering the roots and the degree of host specificity of the Pasteuria isolates. It is possible

that the laboratory population of M. incognita is a better host of the PP3 isolate than is the M. incognita culture from Vanuatu, despite the differences in spore attachment.

Unfortunately, the total numbers of mature female nematodes in the root systems were not determined and it is possible that fewer encumbered juveniles of the Vanuatu population invaded the roots. It has been recorded elsewhere that heavy spore burdens may affect the ability of juveniles to locate and invade roots (Stirling 1984, Davies et al 1988).

A significant decrease in root knot nematode reproduction can occur over one crop generation when P. penetrans spores are mixed with nematode-infested soil at concentrations of 10^4 spores per g (Channer unpublished). To treat one square metre to a depth of 20 cm at this spore concentration, 2×10^9 spores would be required. With the results from the system described such a volume of soil would need the production of at least sixteen 0.3 litre pots or 1 to 3 litre pots. This production should be within the capabilities of growers wishing to treat seed beds, small vegetable plots or container-grown plants. Lesser dosages may be effective in situations where crop improvements over a longer time scale can be tolerated.

ACKNOWLEDGEMENTS

This work was funded by the Overseas Development Administration. We thank Drs. G. Stirling and V. Spaull for providing isolates of Pasteuria. Meloidogyne spp. are used under MAFF licence number PHF 1007/48(28).

REFERENCES

Davies, K.G.; Kerry, B.R.; Flynn, C.A. (1988) Observations on the pathogenicity of Pasteuria penetrans a parasite of root knot nematodes. Annals of Applied Biology 112, 491-501.

Kerry, B.R (1987) Biological control. In: Principles and Practice of Nematode Control in Crops. R.H. Brown and B.R. Kerry, (Eds), Sidney, Academic Press, pp 447.

Mankau, R. (1975) Bacillus penetrans n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology. 26, 333-339.

Sayre, R.M.(1980) Biocontrol:Bacillus penetrans and related parasites of nematodes. Journal of Nematology 12, 260-270.

Starr,M.P.; Sayre, R.M. (1988) Pasteuria thornei sp. nov. and Pasteuria penetrans sensu stricto emend., mycelial and endospore-forming bacteria parasitic, respectively,on plant-parasitic nematodes of the genera Pratylenchus and Meloidogyne. Annales Institute Pasteur 139,11-31.

Stirling,G.R. (1984) Biological control of Meloidogyne javanica with Bacillus penetrans. Phytopathology 74, 55-60.

Stirling,G.R.,; Wachtel,M.F.(1980) Mass production of Bacillus penetrans for the biological control of root-knot nematodes. Nematologica 26, 308-312

Thomas, T.H.(1976) Growth regulation in vegetabe crops. Outlook on Agriculture 9, 62-68

THE LIFE CYCLE AND PATHOLOGY OF THE ROOT-KNOT NEMATODE PARASITE *PASTEURIA PENETRANS*

K.G. DAVIES, C.A. FLYNN and B.R. KERRY

AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ

ABSTRACT

The time taken for the completion of the life cycle of *P. penetrans* was found to be variable in a temperature-controlled glasshouse. *P. penetrans* spores varied in their compatibility to nematode hosts. The effect of *P. penetrans* and spore burden on nematode invasion and reproduction is reported. Although *P. penetrans* reduced juvenile invasion it was most effective in prohibiting reproduction.

INTRODUCTION

The *Pasteuria penetrans* group of bacteria are mycelial endospore-forming bacteria that are parasitic on plant-parasitic nematodes. Two species are currently recognised, *P. penetrans sensu stricto emend* which primarily parasitises root-knot nematodes such as *Meloidogyne incognita* and *Pasteuria thornei sp. nov.* which parasitises root-lesion nematodes such as *Pratylenchus brachyurus* (Starr and Sayre, 1988). The former has considerable potential as a biological control agent against *Meloidogyne* spp. of nematodes (Mankau, 1975; Stirling, 1984; Brown *et al.* 1985). *P. penetrans* spores adhere to the cuticle of second-stage juveniles of *Meloidogyne* as they move through the soil and most germination occurs when spore encumbered nematodes have entered the roots and started to feed. The cuticle is penetrated by a germ tube, forms a vegetative microcolony which then proliferates inside the body of the developing nematode. Eventually the microcolonies sporulate, filling the mature female with spores and prohibiting reproduction (Sayre and Wergin, 1977). Spores of *P. penetrans* adhere to the cuticle of many species of nematode (Spaull, 1981; Sturhan, 1985) but individual populations of the bacterium usually have narrow host ranges and are quite specific (Dutky and Sayre, 1978; Sayre *et al.* 1988). Before a nematode can be recognised as a host, the attachment of spores must lead to the completion of the life cycle within the nematode and culminate in the production of mature spores.

Although *P. penetrans* has not yet been successfully cultured *in vitro* studies are currently being pursued, and improved *in vitro* growth has been obtained by the addition of organic and mineral supplements to standard insect media formulations (Reise *et al.* 1988). The use of *P. penetrans* as a biological control agent will ultimately be determined by whether or not spore application rates to the soil can be economically produced and applied. The identification of bacterial populations which readily infect at low population densities will determine the amount of inoculum required in order to achieve control. The results reported here describe some aspects of the life cycle, host specificity and infection of *Meloidogyne* spp. of nematodes by *P. penetrans*.

MATERIALS AND METHODS

Life cycle of P. penetrans

Juveniles of M. incognita were encumbered with P. penetrans spores of population PP1 by agitating the juveniles in a suspension of 10^6 spores/ml until they had approximately 10 spores per juvenile (method; see below). Several hundred were then added around the roots of tomato plants (growing in 13 cm pots containing 3 : 1 peat/sand mixture), watered daily and maintained at 25°C with a 16 h day, 8 h night photoperiod in a glasshouse. Developing females were dissected from the roots on a regular basis over a 3 month period and examined by high power light microscopy to assess P. penetrans development. Several consecutive runs were undertaken.

Host specificity of P. penetrans

Spore suspensions of 10^6 spores/ml of 11 populations of P. penetrans were prepared and their attachment to root-knot and cyst nematodes was tested following a standard method (Davies et al. 1988). After 24 h gentle agitation at 25°C, 40 juveniles were microscopically examined and the number of spores adhering to the nematodes assessed.

The effect of spore burden and P. penetrans populations on invasion and infection of M. incognita

A suspension of spores of P. penetrans population PP1 was agitated with second-stage juveniles of M. incognita in order to obtain three levels of spore burden and a control (0, 1-5, 6-10 and 11-15 spores per individual juvenile). Approximately 5000 second-stage juveniles, at each spore range, was added around the roots of tomato plants, which were then grown in a glasshouse as previously described. After 35 d the roots were collected, washed and cut up before being digested in 25% Pectinex (Novo Enzyme Products Ltd). After 24 h at 25°C the slurry was homogenised and the number of adult females and second-stage juveniles were collected and quantified (Davies et al. 1988). Infection of adult females was assessed by examining a random sample of 20 squashed females under a microscope.

In a second experiment, second-stage juveniles of M. incognita were exposed to P. penetrans spores from either populations of PP1 or M1 (supplied by S.R. Gowen, University of Reading, U.K. and R.M. Sayre, U.S.D.A., Beltsville, Maryland, respectively). When the juveniles had between 1-5 spores each they were added around the roots of tomato plants and placed in a greenhouse as previously described. A control treatment was not exposed to either P. penetrans population. After 5 weeks the root systems were washed free of soil, digested in 25% Pectinex and the developing female nematodes extracted, counted and examined under a microscope for infection by P. penetrans.

RESULTS

The life cycle of P. penetrans

The rate of development of P. penetrans was highly variable. It was difficult to predict the stage of the life cycle that the infection had reached at any particular time (Table 1). Temperatures in the glasshouse varied; if the temperature dropped below 25°C this substantially slowed the

rate of development.

TABLE 1

The developmental stages of P. penetrans population PP1 in relation to the time of inoculation at 25°C (± 8°C)

Developmental stage	Number of days after inoculation
Stage 1 germination and formation of primary microcolonies	6 - 12
Stage 2 Poliferation of microcolonies	12 - 25
Stage 3 Fragmentation of microcolonies	21 - 36
Stage 4 Sporogenisis: mature doublets and immature single spores	28 - 48
Stage 5 Single spores retained within a sporangium	34 - 56
Stage 6 mature spores without sporangium	42 - 84

Host specificity

All P. penetrans populations tested had originally been isolated from species of Meloidogyne, and attachment (with only one exception) was limited to that genus. There was a certain amount of host specificity (Table 2); some populations of P. penetrans appeared to show a high degree of compatibility to one species of Meloidogyne and not to another.

TABLE 2

Level of attachment of individual populations of Pastueria to different species of plant-parasitic nematodes*

Nematode	Pasteuria population										
	PP1	PP2	PP3	PP4	M1	B7	PPd	PPe	PNG	CAL	EL48
M. acronea	-	na	-	+	-	-	na	na	-	na	na
M. arenaria	+	+	+	+	+	-	+	+	+	+	+
M. incognita	++	+	++	+	+	++	++	++	+++	++	++
M. javanica	++	+	++	+	+	++	++	na	+++	++	++
H. avenae	-	-	-	+	-	-	-	-	-	-	-
H. glycines	-	-	-	-	-	-	-	-	-	-	-
H. schachtii	-	-	-	-	-	-	-	-	-	-	-
G. rostochiensis	-	-	-	-	-	-	-	-	-	-	-

* -, no attachment; +, 1 to 10 spores/juvenile; ++, 11 to 50 spores juvenile; +++, above 50 spores/juvenile; na, not available

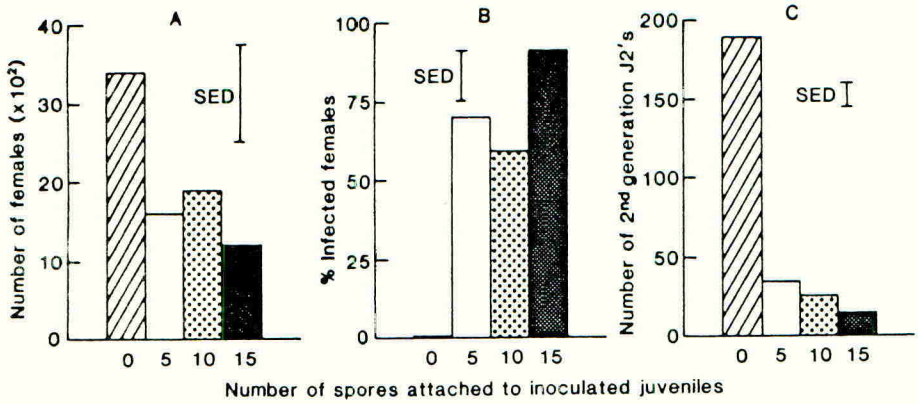


Fig. 1. The number (A) and percentage infection (B) of females and number of subsequent second stage generation juveniles (C) per root system produced from 5000 M. incognita juveniles encumbered with different levels of P. penetrans spores

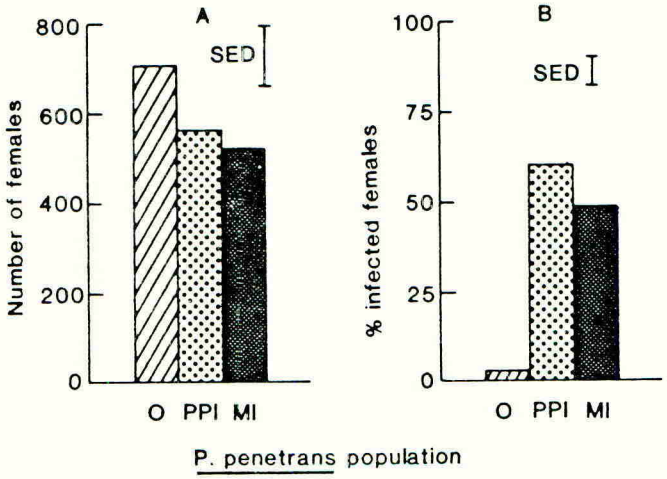


Fig. 2. The number of females per root system (A) and percentage infection (B) produced when 2500 M. incognita juveniles encumbered with between 1 to 5 spores of P. penetrans and a control were inoculated around the roots of tomato plants

Effects of spore burden and P. penetrans population on invasion and infection

Spores significantly reduced the number of adult females that developed, compared to the controls. However, there was no significant difference in numbers of females between the different levels of spore attachment. As spore burden increased there was a significant difference in the percentage of infected females and this had a considerable consequence for the second generation which was reduced by 93% (Fig. 1). A comparison of P. penetrans populations PP1 and M1 led to similar results. Both populations had a small effect on the number of females but again the greatest effect was on the proportion of females infected. PP1 resulted in a larger proportion of infected females than did M1 (Fig. 2).

DISCUSSION

The study of the life cycle shows that, once P. penetrans spores have attached, development through the life cycle is variable in glasshouse conditions. Glasshouses are susceptible to the external environment and temperatures can fluctuate widely. Stirling (1981) has shown that temperature can greatly influence the development of P. penetrans and this may account for the large discrepancies in the length of time taken for the life cycle to be completed. P. penetrans can greatly reduce the reproductive potential of root-knot nematodes. Even with only between 1 and 5 spores per individual juvenile the second generation was reduced by 82% and this increased to over 90% when juveniles were encumbered with between 11 and 15 spores. Individual populations of the parasite are highly specific. In only one case did any of the P. penetrans populations tested adhere to any of the cyst nematodes and as yet it has not been ascertained whether or not the cyst nematode was a true host enabling the P. penetrans population to complete its life cycle. Effective application in the field will be dependent on compatible spores being thoroughly mixed into the soil to ensure that migrating juveniles become encumbered with spores.

The results show that populations of P. penetrans differ in their compatibility to nematodes (Table 2); some strains were highly compatible to one or two species of nematode and not to others, whereas other populations had a low compatibility combined with wider host range. Therefore from a practical point of view, as nematode populations tend to be mixed, two extreme strategies of control are available. Either, P. penetrans strains that have a wide host range and low compatibility and can be applied at very high rates, or, mixed populations with high compatibility, can be applied at lower rates. So far a population with high compatibility on all Meloidogyne species has not been identified. However, with an understanding of the basis of attachment and host specificity at the biochemical level, it may be possible to improve the compatibility between P. penetrans and its host.

REFERENCES

Brown, S.M.; Kepner, J.L.; Smart, G.C. (1985) Increased crop yields following application of Bacillus penetrans to field plots infested with Meloidogyne incognita. Soil Biology and Biochemistry 17, 483-486.

Davies, K.G.; Flynn, C.A.; Kerry, B.R. (1988) Observations on the pathology of Pasteuria penetrans a parasite of root-knot nematodes. Annals of Applied Biology 112, 491-501.

Dutky, E.M.; Sayre, R.M. (1978) Some factors affecting infection of nematodes by the bacterial spore parasite Bacillus penetrans. Journal of Nematology 10, 285.

Mankau, R. (1975) Bacillus penetrans n.comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26, 333-339.

Reise, R.W.; Hackett, R.M.; Sayre, R.M.; Huettel, R.N. (1988) Factors affecting cultivation of three isolates of Pasteuria spp. (Abstract) Journal of Nematology 20, in press.

Sayre, R.M.; Wergin, W.P. (1977) Bacterial parasite of a plant nematode: morphology and ultrastructure. Journal of Bacteriology 129, 1091-1101.

Sayre, R.M.; Starr, M.P.; Golden, A.M.; Wergin, W.P.; Endo, B.Y. (1988) Comparison of Pastineura penetrans from Meloidogyne incognita with a related mycelial and endospore forming bacterial parasite from Pratylenchus brachyurus. Proceedings of the Helminthology Society of Washington 55, 28-49.

Spaull, V.W. (1981) Bacillus penetrans in South African plant-parasitic nematodes. Nematologica 27, 244-245.

Starr, M.P.; Sayre, R.M. (1988) Pasteuria thornei sp.nov. and endospore-forming bacteria parasitic, respectively, on plant parasitic nematodes of the genera Pratylenchus and Meloidogyne. Annals Institute Pasteur/Microbiology 139, 11-31.

Stirling, G.R. (1981) Effect of temperature on infection of Meloidogyne javanica by Bacillus penetrans. Nematologica 27, 458-462.

Stirling, G.R. (1984) Biological control of Meloidogyne javanica with Bacillus penetrans. Phytopathology 74, 55-60.

Sturhan, D. (1985) Untersuchungen über verbreitung und wirte des Nematodenparasiten Bacillus penetrans. Mitteleilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft, Berlin 226, 75-93.

MECHANISMS OF RICE VARIETAL RESISTANCE TO THE WHITEBACKED PLANTHOPPER SOGATELLA FURCIFERA

G. Liu and R. M. Wilkins

Department of Agricultural and Environmental Science,
University of Newcastle, Newcastle upon Tyne, NE1 7RU

ABSTRACT

A number of tropical rice cultivars are known to be resistant to the whitebacked planthopper, Sogatella furcifera (Horvath) (WBPH). The response ranges from the most susceptible TN1 through many varieties with Rathu Heenati being least preferred. The approach taken was to evaluate the potential role of plant chemicals by their effects on the insect behaviour in plant selection and settling by restricting access of the insect to cuticular compounds. Feeding responses have been monitored through electrical conductivity methods of the insect in situ on the plant surface. For a variety showing a high level of resistance the presence of compounds with deterrent activity and in a susceptible variety having feeding stimulation have been demonstrated.

INTRODUCTION

The whitebacked planthoppper, Sogatella furcifera (Horvath) (WBPH) is at present one of the most serious rice insect pests in the tropics (Khan and Saxena, 1985b). It attacks rice plants directly by sucking sap resulting in slow growth, prolonged tillering, reduction in grain formation, plant mortality and poor yields (Sogawa, 1973). No known disease-causing viruses are transmitted by this insect.

There are various behavioural and physiological responses to different rice cultivars by WBPH (Heinrichs and Rapusas, 1983; Khan and Saxena, 1985b; Gunathilagaraj and Chelliah, 1985a and b). Different levels of resistance have been identified within a large number of varieties (Khan and Saxena, 1985a; Singh and Rapusas, 1986). Steam distillate extracts of resistant rice varieties (Khan and Saxena, 1986) when applied to susceptible TN1 rice disrupted the normal feeding behaviour of WBPH, suggesting that the attack by the insect may be determined by volatiles.

The relationship between the plant and the insect may have several levels; the work described here was to test whether compounds present in the leaf surface were involved in insects settling and whether other compounds in the phloem regulated their feeding activity (Auclair and Baldos, 1982). Varieties with a range of susceptibilities to WBPH were selected for initial study and included N22 (Wbph1), ARC10239 (Wbph2), ADR52 (Wbph3), Podiwi A-8 (Wbph4), Rathu Heenati (Wbph?) and TN1 (none), resistant gene in parentheses. For further study, the most susceptible, TN1 and the most resistant of these, Rathu Heenati were chosen. The effect of surface chemicals was tested by covering the leaf with a film to prevent their access to the pest without interfering with feeding, and the response to phloem substances was compared using electrical recording of feeding behaviour (Khan and Saxena, 1984; Khan and Saxena, 1988).

MATERIALS AND METHODS

Orientational and settling responses

Free-choice test

The secondary tillers of 6-week-old potted resistant Rathu Heenati and susceptible TN1 rice plants were cut off and the main tillers were either exposed or totally wrapped with a 5 x 30cm piece of stretched parafilm, a water-proof, thermoplastic sealing film. Pairs of the exposed or wrapped tillers were individually inserted into a 15 x 30cm cylindrical mylar cage through small holes in a polystyrene disc which formed the base of the cage. 20 newly emerged macropterous WBPH females were anaesthetized with CO_2 and placed on the centre of the polystyrene disc. The females were allowed a free choice between: exposed Rathu Heenati and TN1; parafilm-wrapped and exposed TN1. Each treatment was replicated three times. The number of females on each tiller was recorded at 2,4,8 and 24 hours after release.

No-choice test

This was similar to the previous experiment but with a single tiller at each test. Fifteen macropterous WBPH females were introduced into each cage and the numbers of females on each tiller were recorded at 2,4,8 and 24 hours after release.

Electrically recorded feeding activities

The secondary tillers of 4-week-old potted Rathu Heenati and TN1, and of 6-week-old TN1 rice plants were cut off leaving the main tiller. Recently emerged (1-3 d) macropterous female WBPH were starved but water satiated for 6-8 hours. A silk-coated copper wire (50um diameter, 10cm long) was attached to the dorsum of a female by "Pritt" adhesive and then connected to the negative terminal of a chart recorder. The female was then placed on the leaf sheath. To complete the circuit the positive supply of 3V was connected to the leaf sheath substrate through the water saturated soil in the pot. The negative supply was connected directly to the positive input terminal of the chart recorder. The recorder pen was adjusted to chart baseline and insect feeding was monitored for approximately 60 minutes. A chart speed of 1.2cm/min and 100mV full-scale deflection was used. Observations were replicated 4 or 5 times for each age and cultivar using different plants and insects. Feeding activities were expressed as number of probes, and percentage of time spent in salivation, phloem ingestion and resting and walking categories, during each recording period.

RESULTS AND DISCUSSION

Orientational and settling responses

The numbers of macropterous WBPH females selecting with a free choice (Saxena and Khan, 1984; Singh, 1985) between and settling on wrapped and exposed Rathu Heenati and TN1 are given in Table 1. When given the choice between parafilm wrapped and exposed susceptible TN1 plants the insects initially oriented to both but by 24 hours clearly were preferring the exposed plants. In a choice between resistant Rathu Heenati and TH1 following an initial orientation to both plants there was a movement to

TN1 by 24 hours. It appeared that wrapped TN1 provoked a similar response in the insect to resistant Rathu Heenati. This could be explained on the basis that the parafilm precluded the insect's access to olfactory cues, in this case to attractants in the preferred TN1. Feeding behaviour was not significantly affected by the parafilm layer.

The orientational and settling behaviour where there was no choice of plants for feeding is shown in Table 2. In all cases, the number of females settled on the test plant significantly increased with time. Initially, the numbers on the exposed and wrapped TN1 were similar to Rathu Heenati, but following a state of change, at 24 hours those on wrapped TN1 and exposed Rathu Heenati were similar but significantly reduced compared to exposed TN1.

Table 1. Orientational and settling responses of macropterous S. furcifera females to the exposed Rathu Heenati (RH) and TN1, and the parafilm-wrapped TN1 rice plants (free-choice test).

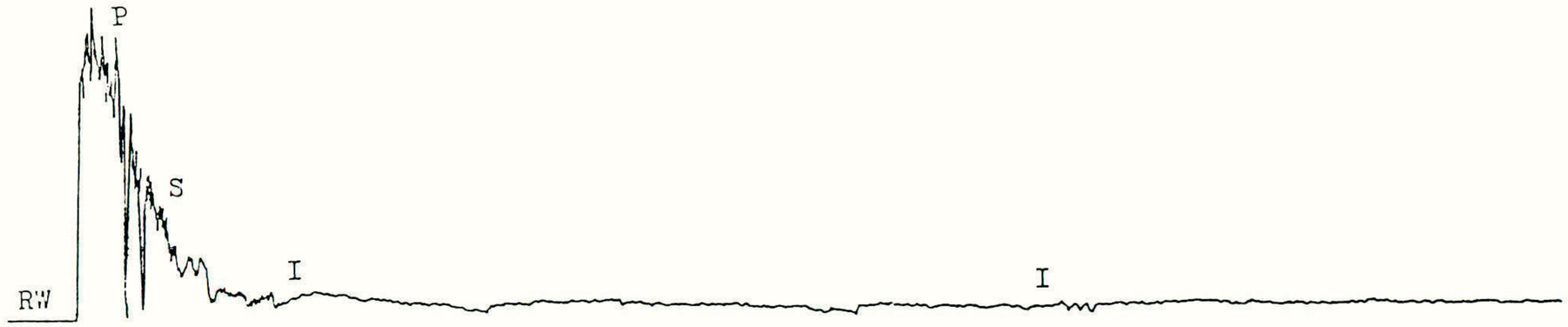
Treatment	Females oriented[1] (%) at hours after release			
	2	4	8	24
Wrapped TN1	14.8 A a	14.3 A a	22.6 A a	18.0 A a
	(6.7)[2]	(10.0)	(15.0)	(10.0)
Exposed TN1	28.8 A b	34.2 B b	45.9 C b	53.8 C b
	(23.3)	(31.7)	(51.7)	(65.0)
Exposed RH	19.9 A a	30.7 B a	24.8 AB a	10.4 C a
	(11.7)	(26.7)	(18.3)	(5.0)
Exposed TN1	36.2 A b	40.1 AB b	47.3 AB b	51.9 B b
	(35.0)	(41.7)	(53.3)	(61.7)

[1]Average of 3 replications. In arcsine percentage transformed value. In a column within a pair test, means followed by a common lowercase letter are not significantly different at P = 0.05 (Duncan's Multiple Range Test). Within a row, significance is designated by capital letters.
[2]Figures in parentheses represent the actual percentage of the females oriented.

Electrically recorded feeding activity

A typical waveform produced by recording the direct current voltage passed through a WBPH adult female while on the surface of a rice plant is shown in Figure 1. The three behavioural activity categories of the insect described above and also that of probing (P) can be identified by each characteristic waveform pattern. In the case shown in Figure 1, the time where steady ingestion of phloem content occurred has been reduced strongly from that for 4-week-old to that for 6-week-old plants of susceptible TN1. No current is conducted when the insect is resting or moving. Following a short probing action a sheath is formed within the leaf tissue around the stylet by salivation before feeding starts.

The feeding activity in 4- and 6-week-old plants of TN1 and 6-week-old Rathu Heenati is shown in Table 3. The number of probes and



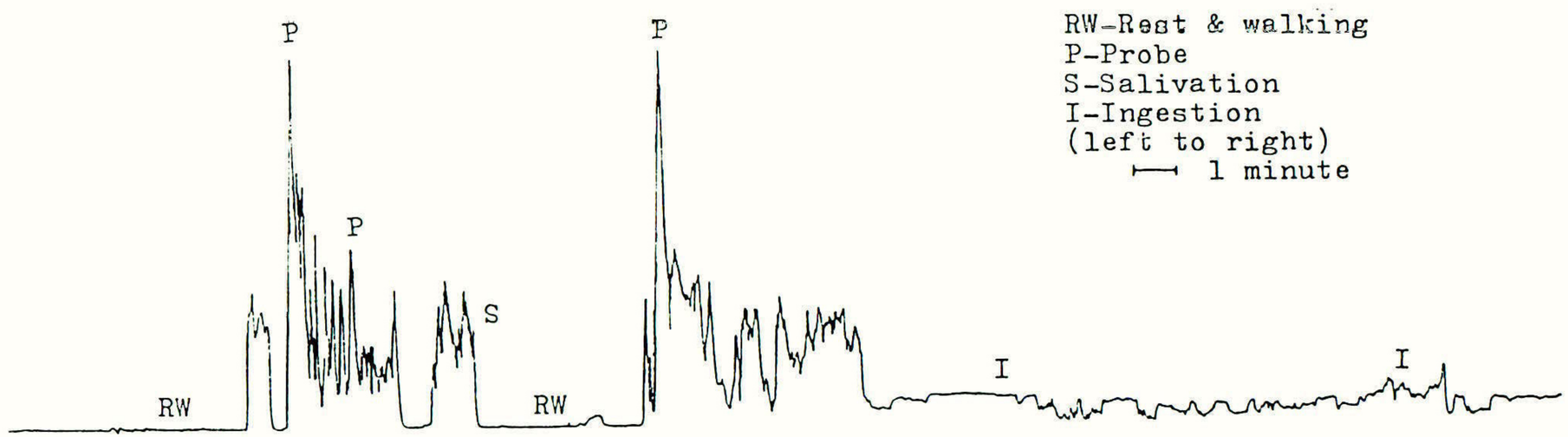


Fig 1. Waveforms recorded during S.furcifera feeding on 4-week-old (top) and 6-week-old (bottom) rice plants of susceptible TN1

the time spent not feeding are higher for Rathu Heenati, but salivation is similar. However, the time in ingestion is substantially greater for TN1 compared to Rathu Heenati. This comparison is somewhat reflected in the 6 week-old TN1, but salivation and ingestion are higher.

These studies indicate the presence of feeding stimulants in the phloem in the susceptible variety. The effect of nutritional factors, such as amino acids, sugars or other substances has been observed (Auclair and Baldos, 1982) which decline with age of the plant.

Table 2. Orientational and settling responses of macropterous S. furcifera females to the exposed Rathu Heenati (RH) and TN1, and the parafilm-wrapped TN1 rice plants (no-choice tests).

Treatment	Females oriented[1] (%) at hours after release			
	2	4	8	24
Wrapped TN1	5.0 A a (2.2)[2]	24.6 B a (17.8)	32.5 BC a (28.9)	49.6 C ab (57.8)
Exposed RH	15.0 A a (6.7)	27.9 B a (22.2)	33.7 B ab (31.1)	36.6 C b (35.5)
Exposed TN1	17.1 A a (8.9)	26.3 B a (20.0)	45.6 C b (51.1)	60.9 D a (75.6)

[1]Average of 3 replications. In arcsine percentage transformed value. In a column, means followed by a common lowercase letter are not significantly different at P = 0.05 (Duncan's Multiple Range Test). Within a row, significance is designated by capital letters.
[2]Figures in parentheses represent the actual percentage of the female oriented.

Table 3. Electronically recorded events during approximately 60 min feeding of macropterous S. furcifera females on 4-week-old resistant Rathu Heenati[1] (RH) and susceptible TN1[2], and on 6-week-old TN1[1] rice plants.

Plant age (week)	Electronically recorded events							
	Probe (no.)		Salivation (%)		Phloem Ingestion (%)		Resting & Walking (%)	
	RH	TN1	RH	TN1	RH	TN1	RH	TN1
4	14.0 a	7.2 bA	8.6 a	8.4 aA	21.3 a	68.8 bA	61.3 a	17.2 bA
6	-	14.0 B	-	17.5 B	-	44.8 B	-	33.1 B

[1]Average of 4 replications. [2]Average of 5 replications. Within an event, means followed by a common lowercase letter are not significantly different at P=0.05 (Duncan's Multiple Range Test). In a column, significance is designated by capital letters.

REFERENCES

Auclair, J. L.; Baldos, E. (1982) Feeding by the whitebacked planthopper, Sogatella furcifera, within susceptible and resistant rice varieties. Entomologia Experimentalis et Applicata 32, 200-203.

Gunathilagaraj, K.; Chelliah, S. (1985a) Feeding behaviour of whitebacked planthopper, Sogatella furcifera, on resistant and susceptible rice varieties. Crop Protection 4, 255-262.

Gunathilagaraj, K.; Chelliah, S. (1985b) Components of resistance to the whitebacked planthopper, Sogatella furcifera (Horv.), in some rice varieties. Tropical Pest Management 31, 38-46.

Heinrichs, E. A.; Rapusas, H. R. (1983) Levels of resistance to the whitebacked planthopper, Sogatella furcifera (Homoptera: Delphacidae) in rice varieties with the same major resistance genes. Environmental Entomology 12, 1793-1797.

Khan, Z. R.; Saxena, R. C. (1984) Electronically recorded waveforms associated with the feeding behaviour of Sogatella furcifera, (Homoptera: Delphacidae) on susceptible and resistant rice varieties. Journal of Economic Entomology 77, 1479-1482.

Khan, Z. R.; Saxena, R. C. (1985a) A selected bibliography of the whitebacked planthopper, Sogatella furcifera (Horv.) (Homoptera: Delphacidae) Insect Science Applications 6, 115-134.

Khan, Z. R.; Saxena, R. C. (1985b) Behavioral and physiological responses of Sogatella furcifera (Homoptera: Delphacidae) to selected resistant and susceptible rice cultivars. Journal of Economic Entomology 78, 1280-1286.

Khan, Z. R.; Saxena, R. C. (1986) Varietal resistance in rice against Sogatella furcifera (Horv.). Crop Protection 5, 12-25.

Khan, Z. R.; Saxena, R. C. (1988) Probing behaviour of three biotypes of Nilaparvata lugens (Homoptera: Delphacidae) on different resistant and susceptible rice varieties. Journal of Economic Entomology 81, in press.

Saxena, R. C.; Khan Z. R. (1984) Comparison between free-choice and no-choice seedling bulk tests for evaluating resistance of rice cultivars to the whitebacked planthopper. Crop Science 24, 1204-1206.

Singh, J. (1985) Reaction of rices to Sogatella furcifera in free-choice and no-choice seedling bulk tests. International Rice Research Newsletter 10, 14.

Singh, J.; Rapusas, H. R. (1986) Resistance in some selected rice varieties with different genes of resistance to the whitebacked planthopper Sogatella furcifera. Crop Improvement 13, 126-130.

Sogawa, K. (1973) Feeding of the rice plant- and leaf-hoppers. Review of Plant Protection Research 6, 31-43.