Pak.J.Bot., 31(2): 397-405, 1999.

MULTIPLICATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA FOR USE IN THE CONTROL OF ROOT ROT DISEASE OF CROP PLANTS

ISHRAT IZHAR, IMRAN ALI SIDDIQUI, SYED EHTESHAMUL-HAQUE AND ABDUL GHAFFAR

Soil-borne Diseases Research Laboratory,
Department of Botany,
University of Karachi, Karachi-75270, Pakistan.

Abstract

Efficacy of three strains of *Pseudomonas aeruginosa* multiplied on wheat bran, rice husk and saw dust was evaluated in the control of soil borne root infecting fungi under screen house and field conditions. Wheat bran was found as a good substrate for mass multiplication of the bacterium as compared to saw dust and rice husk. Wheat bran inoculum of *P. aeruginosa* strains significantly ($p < 0.05$) controlled infection of *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* on cotton under green house and also on sunflower, soybean, uridbean and cotton under field conditions with enhancement in plant growth. Bacterial antagonists showed better biocontrol and growth promoting effects in the screen house as compared to their use in the field.

Introduction

Several strains of *Pseudomonas fluorescens*, the plant growth promoting rhizobacteria (PGPR) have been reported to suppress soil-borne diseases caused by fungal pathogens (Sharma & Nowak, 1998; Weller, 1988). For large scale application of *P. fluorescens* for the control of soil-borne diseases there is need for the development of commercial formulations in which the bacteria can survive for a cosiderable length of time (Vidhyasekaran & Muthamilan, 1995). Wheat bran inoculum of microbial antagonists have been found more effective than conidial preparations in reducing the population of *R. solani* and incidence of damping-off of sugarbeet, cotton and radish (Lewis & Papavizas, 1985). There are reports that population of rhizobia established in soil by the application of rhizobial inoculant, can persist for many years following such application (Parker *et al.*, 1977). Rhizobia can thus multiply and persist at the expense of added substrates (Pena-Cabriales & Alexander, 1983; Gremida, 1988). Experiments were therefore carried out to see the effect of different strains of *P. aeruginosa* multiplied on different organic substrates on infection of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* in cotton, sunflower, soybean and uridbean under green house and field conditions.

Materials and Methods

Green house experiment: Soil used for the experiment was sandy loam, pH 8.1 with moisture holding capacity of 40% (Keen & Raczkowski, 1921). The soil had a natural population of 2-5 sclerotia g^{-1} of soil of *Macrophomina phaseolina* as found by wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 3-8% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2600 cfu g^{-1} of soil of *Fusarium solani* as assessed by using soil dilution technique (Nash & Snyder, 1962). *P. aeruginosa* strains multiplied on Nutrient Agar medium at room temperature for five days were scrapped from the surface with the help of a sterilized bent glass rod after adding 10 ml sterile distilled water. Bacterial suspension so obtained was collected in a beaker. Saw dust, rice husk and wheat bran were moistened with distilled water (25% w/w) and sterilized in 250 ml conical flask at 121°C for 20 minutes. After 24 hours 2 ml suspension of *P. aeruginosa* strains Pa-3 ($2.5x10^8$ cfu ml^{-1}), Pa-5 ($9.5x10^8$ cfu ml^{-1}) and Pa-7 ($2.1x10^8$ cfu ml^{-1}) were inoculated in each flask. The flasks were incubated for one week at a temperature range of 25-30°C. Saw dust, rice husk or wheat bran inoculum of *P. aeruginosa* was mixed thoroughly with soil @ 1% w/w and transferred in 8 cm diam., plastic pot, 250g/pot. After soil amendment seeds of cotton were sown @ 8 seeds/pot. Saw dust, rice husk or wheat bran without bacterial inoculum was also applied to the soil for comparison. In another set, seeds of cotton were treated with five day old cultures of microbial antagonists containing $9.5x10^8$ cfu ml^{-1} of Pa-3, $1.7x10^8$ cfu ml^{-1} of Pa-5 and $9.5x10^8$ cfu ml^{-1} of Pa-7 using 1% gum arabic as sticker. In another treatment, soil was drenched with 25 ml of water cell suspension of each test bacterium containing $9.5x10^7$ cfu ml^{-1} of Pa-3, $1.7x10^7$ cfu ml^{-1} Pa-5 and $2.5x10^7$ cfu ml^{-1} Pa-7. There were four replicates of each treatment and pots were kept randomized on the screen house bench of Soil-borne Diseses Research Laboratory, Department of Botany, University of Karachi where soil was kept at 50% moisture holding capacity.

Field experiment: Experiment was carried out in 2x1 meter microplots in randomized complete block design with four replicates. The soil had similar characteristics and population of the pathogen as described in the pot experiment. *P. aeruginosa* strains multiplied on wheat bran as described earlier was added to soil in ridges @ 18 g per meter row. The wheat bran inoculum contained $2.6x10^9$ cfu of Pa-3, $3.1x10^9$ cfu of Pa-5 and $4.2x10^9$ cfu of Pa-7 per gram. Rows applied with wheat bran without PGPR served as control. After application of biocontrol agents in soil, seeds of test crops like sunflower, cotton, soybean and uridbean were sown @ 30 seeds per row. Plants were watered as needed. Observations on plant height and fresh weight of shoot were recorded after 30 and 60 days of seedling emergence. To determine the incidence of fungi on root, five 1 cm long root pieces after surface sterilization in 1% $Ca(OCl)_2$ were transferred onto PDA plates containing penicillin (100,000 units/L) and streptomycin (0.2g/L). Plates were incubated at 28°C for 5 days and incidence of root infecting fungi was recorded. Data were analysed and subjected to Factorial ANOVA (FANOVA) followed by least significance difference (LSD) according to Gomez & Gomez (1984).

Results

Pseudomonas aeruginosa strain Pa-5 showed better growth when multiplied on wheat bran than rice husk and saw dust as recovery of Pa-5 per gram of substrate was greater on wheat bran giving a population of $6x10^{11}$ cfu ml^{-1} of Pa-5, $1x10^{9}$ cfu ml^{-1} of Pa-3 and $2x10^{10}$ cfu ml^{-1} of Pa-7. Rice husk contained $1.65x10^{9}$ cfu ml^{-1} of Pa-5, $2.4x10^{8}$ cfu ml^{-1} of Pa-3 and $1x10^{8}$ cfu ml^{-1} of Pa-7 per gram of substrate. Saw dust contained $2.3x10^{9}$ cfu/ml of Pa-5, $1.9x10^{9}$ ml^{-1} of Pa-3 and $8x10^{9}$ cfu ml^{-1} of Pa-7 per g of substrate.

Green house experiment: *M. phaseolina* infection on cotton roots was completely reduced by *P. aeruginosa* strain Pa-5 where inoculum multiplied on wheat bran or rice husk was mixed with soil. Pa-3 and Pa-7 also completely prevented infection of *M. phaseolina* where inoculum multiplied on saw dust and rice husk was used or Pa-7 used as soil drench. Infection of *F. solani* was completely reduced by Pa-5 when used as seed dressing and soil drench or when inoculum multiplied on saw dust, rice husk and wheat bran. Pa-3 and Pa-7 also showed complete control of *F. solani* infection when used as soil drench or where inoculum multiplied on wheat bran and saw dust was used. *R. solani* infection was completely reduced by Pa-5 when used as seed dressing and soil drench or when inoculum multiplied on saw dust, rice husk and wheat bran was used. Pa-7 completely prevented infection of R. solani when used as soil drench or where inoculum multiplied on saw dust and rice husk and by Pa-3 inoculum multiplied on wheat bran, sawdust and rice husk was used. Soil amendment with wheat bran, saw dust and rice husk with out bacterial inoculum also reduced more than 50% infection of *M. phaseolina, F. solani* and *R. solani* (Fig.1). Maximum plant height and fresh weight of shoot was produced by strain Pa-7 multiplied on wheat bran (Fig.2).

Field experiments: After 30 days, infection of *M. phaseolina* was significantly ($p<0.05$) reduced in cotton, sunflower, soybean and uridbean by wheat bran inoculum of *P. aeruginosa* strains Pa-3 and Pa-5, while Pa-7 was effective on cotton and sunflower. Soil amendment with wheat bran also showed effective control of *M. phaseolina* infection in cotton and soybean. In 60 day old plants, *M. phaseolina* infection was significantly ($p<0.05$) controlled in cotton and sunflower by strain Pa-3 and Pa-7 and in uridbean by Pa-3, Pa-5 and Pa-7. In 30 day old plants, *F. solani* infection was significantly suppressed where wheat bran inoculum of Pa-7 was used in cotton. In cotton and sunflower wheat bran used alone also significantly ($p<0.05$) suppressed *F. solani* infection. After 60 days, *F. solani* infection was significantly ($p<0.05$) suppressed where Pa-5 multiplied on wheat bran was used in sunflower and uridbean. Strain Pa-7 on cotton and sunflower and Pa-5 on uridbean showed complete inhibition of *R. solani* infection in 30 day old plants whereas in 60 day old plants, a complete suppression of *R. solani* infection was recorded where wheat bran was used alone and strain Pa-5 on soybean and strain Pa-7 on sunflower was used (Table 1).

In 30 day old plants, greater plant height was recorded in treatments with strain Pa-7 on cotton and uridbean and with strain Pa-3 on soybean and sunflower. Strain Pa-3 on cotton and uridbean, strain Pa-7 on sunflower and untreated control plants in soybean showed maximum plant height in 60 day old plants. After 30 days, highest fresh

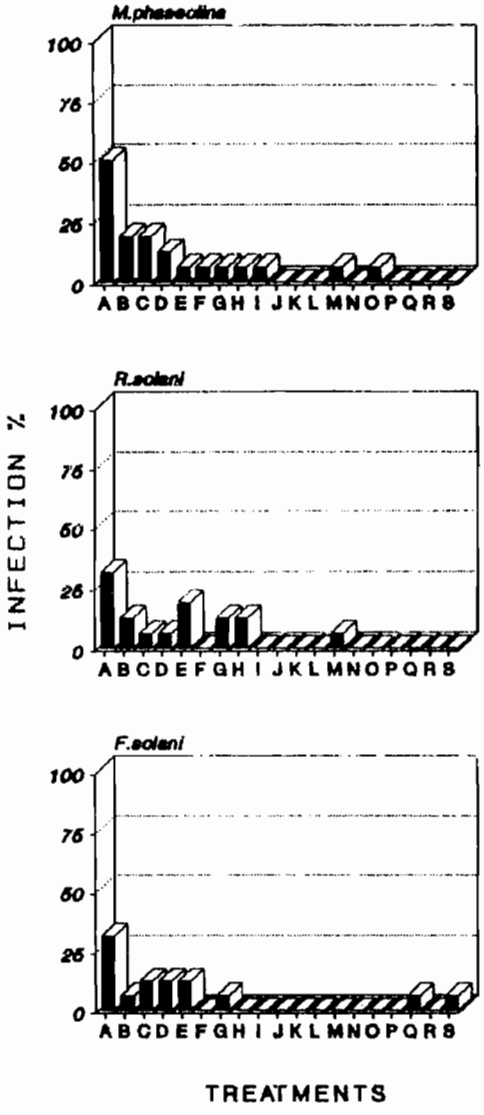

Fig.1. Effect of different sytrains of *Pseudomonas aeruginosa* multiplied on different substrates in the control of *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* infection on cotton roots:

A = Control (Un-amended), b=Control with Wheat bean alone, C = Control with Saw dust alone, D = Control with Rice husk alone.

PGPR Strains used as seed dressing: E = Pa-3, F=Pa-5, G= Pa-7.

PGPR Strains used as soil drench: H = Pa-3, I = Pa-5, J = Pa-7

PGPR strains multiplied on Wheat bran applied in soil: K = Pa-3, L = Pa-5, M = Pa-7

PGPR strains multiplied on saw dust applied in soil. N = Pa-3, 0 = Pa-5, P = Pa-7

PGPR strains multiplied on Rice husk applied in soil: Q = Pa-3, R = Pa-5, S = Pa-7.

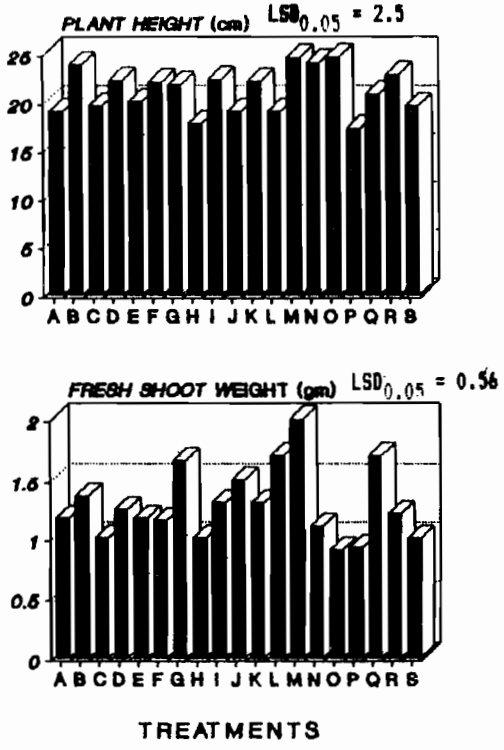

Fig.2. Effect of different strains of *Pseudomonas aeruginosa* multiplied on different substrates on the growth of cotton roots:

A = Control (Un-amended), b=Control with Wheat bean alone, C = Control with Saw dust alone, D = Control with Rice husk alone.

PGPR Strains used as seed dressing: E = Pa-3, F=Pa-5, G= Pa-7.

PGPR Strains used as soil drench: H = Pa-3, I = Pa-5, J = Pa-7

PGPR strains multiplied on Wheat bran applied in soil : K = Pa-3, L = Pa-5, M = Pa-7

PGPR strains multiplied on saw dust applied in soil: N = Pa-3, 0 = Pa-5, P = Pa-7

PGPR strains multiplied on Rice husk applied in soil: Q = Pa-3, R = Pa-5, S = Pa-7.

weight of shoot was found in the treatment where strain Pa-3 was used in soybean and uridbean, strain Pa-7 used in sunflower and wheat bran used alone in cotton. Similarly in 60 day old plants, strain Pa-3 in cotton and uridbean, strain Pa-7 on sunflower and wheat bran used alone in soybean showed maximum fresh weight of shoot (Table 2).

Discussion

Seed dressing or soil drench is a common method of introducing biocontrol agents in the soil and root environment (Kommedahl & Windels, 1981). For field application of these microorganisms, various formulations have been made which include alginate

Table 1. Effect of *Pseudomonas aeruginosa* multiplied on wheat bran on growth of cotton, soybean, sunflower and uridbean under field conditions.

Treatment	Plant height (cm)		Shoot weight (gm)	
	Harvest time (Days)			
	30	60	30	60
Cotton				
Control	11.3	18.5	2.7	6.8
Wheat bran	12.3	15.6	7.5	6.0
Pa3-multiplied on wheat bran	15.9	35.5	5.6	18.5
Pa-5 multiplied on wheat bran	11.8	25.6	3.6	17.6
Pa-7 multiplied on wheat bran	16.2	22.6	4.5	11.2
LSD <0.05	Treatment=7.3,	Time=4.6	Treatment=7.3	Time=4.6
Soybean				
Control	27.0	41.2	6.2	16.1
Wheat bran	19.3	34.8	5.1	24.3
Pa3-multiplied on wheat bran	23.5	37.8	6.3	20.0
Pa-5 multiplied on wheat bran	19.9	28.2	5.4	15.3
Pa-7 multiplied on wheat bran	20.6	37.9	5.6	20.6
LSD <0.05	Treatment=13.4,	Time=8.5	Treatment=13.3	Time=8.4
Sunflower				
Control	34.6	82.0	11.9	67.5
Wheat bran	42.8	122.1	24.3	162.5
Pa3-multiplied on wheat bran	53.0	119.5	28.0	187.8
Pa5-multiplied on wheat bran	36.4	84.1	21.6	81.8
Pa7-multiplied on wheat bran	47.8	149.0	29.2	290.4
LSD <0.05	Treatment=39.6,	Time=25.0	Treatment=114.4	Time=72.3
Uridbean				
Control	17.3	42.2	5.2	33.5
Wheat bran	15.3	39.6	4.6	48.0
Pa3-multiplied on wheat bran	13.6	48.1	5.4	62.5
Pa5 multiplied on wheat bran	16.2	30.2	3.6	24.7
Pa7 multiplied on wheat bran	17.5	39.2	4.7	18.1
LSD <0.05	Treatment=11.2,	Time=7.0	Treatment=25.4	Time=16.1

Table 2. Effect of *Pseudomonas aeruginosa* multiplied on wheat bran in the control of root infecting fungi on cotton, soybean, sunflower and uridbean under field conditions.

Treatmnet	Infection %					
	M. phaseolina		*F. solani*		*R. solani*	
	Harvest time (Days)					
	30	60	30	60	30	60
Cotton						
Control	26.6	64.9	93.3	65.0	51.6	16.6
Wheat bran	0.0	25.0	47.9	75.0	31.2	31.2
Pa3-multiplied on wheat bran	8.3	44.4	80.5	58.3	8.3	27.7
Pa-5 multiplied on wheat bran	8.3	60.4	79.1	79.1	14.5	0.0
Pa-7 multiplied on wheat bran	0.0	37.5	54.1	66.6	0.0	8.3
Soybean						
Control	41.6	60.0	53.2	65.0	54.9	25.0
Wheat bran	12.5	37.5	54.1	62.5	16.6	0.0
Pa3-multiplied on wheat bran	0.0	58.3	63.8	66.6	8.3	25.0
Pa-5 multiplied on wheat bran	25.0	47.9	50.0	54.1	0.0	0.0
Pa-7 multiplied on wheat bran	37.5	62.5	62.5	66.6	16.6	14.5
Sunflower						
Control	81.6	46.6	55.0	80.0	10.0	16.6
Wheat bran	62.5	43.7	25.0	75.0	12.5	12.5
Pa3-multiplied on wheat bran	0.0	33.3	75.0	75.0	11.1	16.6
Pa-5 multiplied on wheat bran	20.8	56.2	87.5	43.7	8.3	12.5
Pa-7 multiplied on wheat bran	43.7	37.5	68.7	68.7	0.0	0.0
Uridbean						
Control	41.6	55.0	61.6	45.0	26.6	45.0
Wheat bran	43.7	62.5	56.2	68.7	12.5	12.5
Pa3-multiplied on wheat bran	22.2	16.6	61.1	75.0	8.3	8.3
Pa-5 multiplied on wheat bran	25.0	25.0	50.0	25.0	18.7	31.2
Pa-7 multiplied on wheat bran	52.0	31.2	52.0	50.0	8.2	18.7
LSD $p<0.05$ Treatment =	18.6		17.7		36.5	
Time =	11.7		10.8		23.0	
Host =	16.6		15.2		32.6	

pellets with wheat bran as food base (Lewis & Papavizas, 1987), wheat bran and saw dust inocula (Elad *et al.*, 1980) and peat-bran inocula (Sivan *et al.*, 1984). Plant growth-promoting rhizobacteria (PGPR) have been reported to survive in certain dry formulations (Suslow, 1980). Kloepper & Schroth (1981), developed a dried formulation of PGPR strains for potatoes by mixing bacteria with xanthan gum and then adding talc. Chickpea seeds treated with talc based inoculum of *Pseudomonas fluorescens* effectively controlled chickpea wilt disease and increased the yield (Vidhyasekaran & Muthamilan, 1995). In the present study also, multiplication of some strains of *P. aeruginosa*, a plant growth promoting-rhizobacterium (Siddiqui *et al.*, 1999) on wheat bran showed better growth of cotton plants as compared to seed or soil treatment with cell suspension. Presumably wheat bran provides additional food base for the multiplication of bacteria in the rhizosphere which increases its population. A better control of root infecting fungi viz., *M. phaseolina, R. solani* and *Fusarium* spp., on okra has been reported by rhizobial inoculum multiplied on organic substrates than seed or soil treatment with cell suspension (Ehteshamul-Haque, 1994). Wheat bran culture of *Trichoderma* and *Gliocladium* also effectively reduced the population of *R. solani* and incidence of damping-off of radish, cotton and sugar beet (Lewis & Papavizas, 1985). It is interesting to note that bacterial antagonists showed better biocontrol and growth promoting effects in pot experiments compared with the field trial. Presumably, field soil might contain variety of microorganisms and application of wheat bran inoculum also supported the growth of other soil microorganisms that successfully competed with *P. aeruginosa* and limited its multiplication in soil. The efficacy of biological agents is likely to be affected by the density of pathogen, host plant, and other biotic and abiotic factors. The significance of these factors needs to be elucidated so that application rates and methods can be developed to deliver sufficient inoculum to give effective pathogen control in a range of conditions.

Acknowledgement

The present work was carried out under the research grant of Pakistan Atomic Energy Commission which is sincerely acknowledged.

References

Ehteshamul-Haque, S. 1994. *Use of rhizobia in the control of soilborne plant diseases caused by root infecting fungi*. Ph.D Thesis, Department of Botany, University of Karachi, Karachi-75270, Pakistan. pp. 239.

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

Gremida, J.J. 1988. Growth of indigenous *Rhizobium leguminosarum* and *Rhizobium meliloti* in soils amended with organic nutrients. *Appl. Environ. Microbiol.*, 54: 257-263.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical procedures for agricultural research*. 2nd. ed. Wiley, New York. pp. 680.

Keen, B.A. and H. Raczkowski. 1921. The relation between clay content and certain physical properties of soil. *J. Agric. Sci.*, 11: 441-449.

Kommedahl, T. and C. Windels. 1981. Introduction of microbial antagonists to specific court of infection: seed, seedlings and wounds. pp. 227-248. In: *Biological control in crop production*.

Lewis, J.A. and G.C. Papavizas. 1987. Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of *Rhizoctonia* damping off. *Plant Pathology*, 36: 438-446.

Lewis, J.A. and G.C. Papavizas. 1985. Effect of mycelial preparation of *Trichoderma* and *Gliocladium* on population of *Rhizoctonia solani* and the incidence of damping off. *Phytopathology*, 75: 812-817.

Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soil. *Phytopathology*, 52: 567-572.

Parker, C.A., M.J. Trinick and D.L. Chatel. 1977. Rhizobia as soil and rhizosphere inhabitants. pp. 312-352. In: *A Treatise on Dinitrogen Fixation*. section IV. (Eds.) R.W.F. Hardy and A.H. Gibson. John Wiley & Sons, New York.

Pena-Cabriales, J.J. and M. Alexander. 1983. Growth of *Rhizobium* in soil amended with organic matter. *Soil Sci. Soc. Am. J.*, 241-245.

Sharma, A.H. and J. Nowak. 1998. Enhancement of *Verticillium* wilt resistance in tomato transplant by *in vitro* co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). *Can. J. Microbiol.*, 44: 528-536.

Sheikh, A.H. and A. Ghaffar. 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot.*, 7: 13-17.

Sivan, A., Y. Elad and I. Chet. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathology*, 74: 498-501.

Suslow, T.V. 1980. Growth and yield enhancement of sugarbeets by pelleting seed with specific *Pseudomonas* spp. *Phytopathology*, 79: 640-646.

Vidhyasekaran, P. and M. Muthamilan. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.*, 79: 782-786.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.*, 26: 379-407.

Wilhelm, S. 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology*, 45: 180-181.

(Received for publication 11 October 1999)