*Nicolaus Copernicus University, Toruń, Poland
**USDA, Forestry Sciences Laboratory, Corvallis, USA

EFFECT OF ENDOPHYTIC BACTERIUM *PSEUDOMONAS FULVA* ON GROWTH OF PINE SEEDLINGS (*PINUS SYLVESTRIS*), FORMATION OF MYCORRHIZAE AND PROTECTION AGAINST PATHOGENS[1]

*A. Pokojska-Burdziej, *E. Strzelczyk, *H. Dahm and **C.Y. Li[2]

Abstract

The effect of endophytic bacterium *Pseudomonas fulva* isolated from interior tissues of non-mycorrhizal, suberized roots of Scots pine on growth of seedlings, formation of mycorrhizae, and protection against fungal root pathogens *Fusarium oxysporum* and *Rhizoctonia solani* was studied.

Inoculation of *P. fulva* significantly stimulated the growth of pine seedlings, increased the number of mycorrhizal roots in seedlings grown in non-pasteurized and pasteurized soil (depend on experimental combination). The inoculated bacterium also protected the seedlings from infection by *Rhizoctonia solani*. The potential for *P. fulva* to increasing tree growth and reducing root diseases could play an important role in maintaining sustainability of pine forests.

Key words: endophytic bacteria, *Pseudomonas fulva*, pathogenic fungi, biocontrol, mycorrhizae

Introduction

Detection of numerous endophytic microbial populations in tissues of healthy plants indicates that plants represent an important ecological niche for microorganisms (Sturz et al. 2000). It is a relatively unexplored habitat that has been received relatively little attention. Until recently the term endophyte had usually

[1]This work was financed by the State Committee for Scientific Research, grant No. 6 P04C 061 21.
[2]Dr. C.Y. Li is thankful to the Fullbright Fundation for the grant allowing him to stay and work in Poland.

Phytopathol. Pol. 32: 33–47

ISSN 1230-0462

been applied to fungi (Chanway 1996). Bacterial endophytes in agricultural crops such as potato, maize, rice, sugar cane, sugar beets have been documented more frequency than those in forest tree species (Kobayashi and Palumbo 2000, Sturz et al. 2000). Shishido et al. (1995) had found internal colonization of lodgepole pine seedlings roots (*Pinus contorta* var. *latifolia* (Dougl.) Engelm.) by *Bacillus polymyxa*. He also isolated *Pseudomonas fluorescens* from within stem tissue of young hybrid spruce (*Picea glauca* × *P. engelmanii*) (Shishido et al. 1999). Strzelczyk and Li (2000) described the occurrence of bacteria *P. fulva* in interior tissues of suberized, non-mycorrhizal roots of Scots pine (*Pinus sylvestris* L.) growing in sand dunes at the Baltic Sea of Poland.

Endophytic bacteria exert marked influence on plant growth and development. They are usually categorized by the effect on plant as plant growth promoting, plant growth inhibiting or plant growth neutral bacteria (Sturz et al. 2000). The mechanisms of such actions have not been elucidated. Plant growth stimulation can be attributed, in some extent, to ability of nitrogen fixation by variety of endophytic bacteria (Baldani J.I. et al. 1997, Reinhold-Hurek and Hurek 1998), production of growth hormones (Arshad and Frankenberger 1991) or siderophores (De Weger et al. 1987, Buyer and Sikora 1990). The biocontrol of phytopathogens in the root zone indirectly can lead to plant growth stimulation. Interactions between bacterial endophytes and plants can be viewed as relatively similar to those observed in rhizospheres (Glick 1995, Strzelczyk 2001).

The aim of this work was to study the effect of endophytic bacterium *P. fulva* isolated from the interior tissues of pine roots on growth of pine seedlings, formation of mycorrhizae with mycorrhizal fungi: *Suillus luteus* (Fr.) S.F. Gray, *Boletus edulis* Vitt. and *Xerocomus badius* (Fr.) Quél) and protection against infection by *Rhizoctonia solani* Kühn and *Fusarium oxysporum* Schlecht., the two important, serious damping-off root pathogens on coniferous seedlings in Poland.

Materials and methods

Microorganisms

The endophytic bacterium was isolated from interior tissues of non-mycorrhizal, suberized roots (7–8 mm in diameter) of Scots pine growing in sand dunes at the Baltic Sea of Poland (Strzelczyk and Li 2000). The bacterium was identified as *P. fulva* by MIDI Labs Inc. (Newark, Delaware, USA) on the basis of 16S rRNA gene sequence similarity.

Sporocarps of mycorrhizal fungi: *S. luteus*, *B. edulis* and *X. badius* were collected from the forest near Toruń.

Root pathogenic fungi: *R. solani* and *F. oxysporum* were derived from roots of Scots pine seedlings with damping-off symptoms. The isolates were from the culture collections of the Department of Plant Pathology, Agricultural University, Poznań and the Department of Forest Phytopathology, Agricultural University, Cracow, respectively.

Culturing of pine seedlings

The sandy soil for this study was collected from under the pine trees. One portion of the soil was pasteurized three times, each time for 3 h in every five days; the other portion of the soil was left unsterile. Pine seeds of uniform size were surface sterilized with 30% H_2O_2 (20 min) followed by several washings with sterile distilled water. The seeds dried on sterile filter paper were planted in plastic leach tubes of 200 cm^3 soil. The emerging seedlings were thinned to one per tube. The seedlings were grown in a plant growth chamber with a 24–20°C (day–night) and under a 16–8-h-photoperiod under the light from sodium vapor lamps at about 100 $\mu mol/s/m^2$ PAR.

Inoculation of seedlings

Pseudomonas fulva was cultured on R2A (Difco) agar medium. After 48 h of incubation at 26°C the bacterial cells were washed off with 10 mM phosphate buffer, pH 6.7. The optical dentisity of the suspension was adjusted using of turbidimeter (model 21101, BIOLOG, Hayward, California, USA) at 590 nm (it was assumed to be over 10^8 cells per 1 ml). In this suspension the seeds were dipped for 30 min before sowing.

Fruit body hymenium of *S. luteus*, *B. edulis* and *X. badius* were removed with a sterile scalpel. Then they were homogenized in sterile distilled water. The suspension was filtered through 50 μm nylon screens to obtained spore suspensions. The number of spore was determined in a Thom's chamber. 1 ml of suspension, which contained over 10^6 spores, was used to inoculate each three-week-old seedling.

Fusarium oxysporum and *R. solani* were cultured on slants prepared from potato dextrose agar (Difco). Mycelium (and the spores in case of *Fusarium*) were washed off with sterile distilled water and agitated with glass beads in Erlenmayer flasks. 1 ml of mycelial fragment suspension thus formed was used as inoculum for each seedling.

Experimental combinations

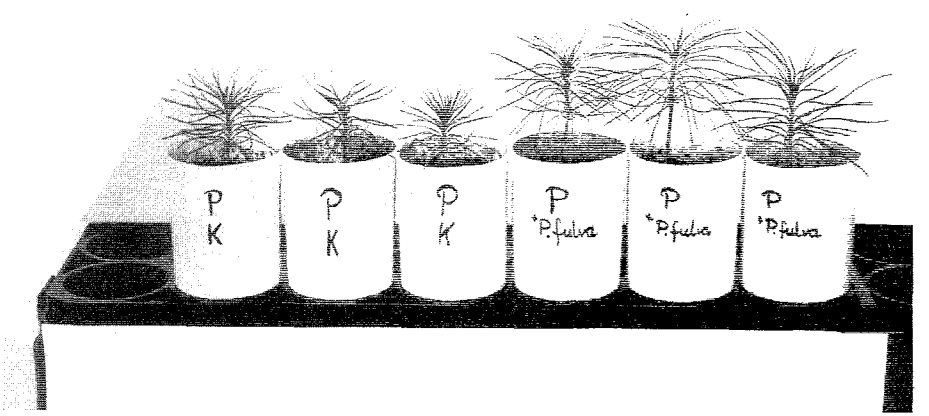
The seedlings were inoculated with mycorrhizal or pathogenic fungi only, or inoculated additionally with *P. fulva*. Non-inoculated seedlings were served as controls. The growth of seedlings inoculated with *P. fulva* only was also observed. The same treatment combinations were applied to the pasteurized and the non-pasteurized soil. Seven replicates were conducted for each treatment combination. Every two–three days the seedlings were watered with distilled water. After four months seedlings were removed from the soil. The length of main root, root collar diameter and shoot height were measured. Determination of root and shoot dry weight was made and the number of mycorrhizal root tips was counted.

Statistical analyses

The growth of seedlings from each experimental combination was compared with the growth of non-inoculated seedlings (control) by use of Student's t-test for independent samples. One-factor ANOVA comparing the growth of seedlings in all experimental combinations was made too. Three-factor ANOVA was used for comparing the effects of: 1) soil treatment (pasteurized – non-pasteurized), 2) kind of inoculation (non-inoculated seedlings and inoculated with individual mycorrhizal or pathogenic fungus) and 3) presence or absence of *P. fulva* on the seedlings growth parameters. Means were compared using the Newman-Keuls multiple range test. STATISTICA/win 5.1 (1966) programme was applied (Stat Soft, Tulsa, Oklahoma, USA).

Results

The seedlings inoculated with *P. fulva* and grown in the pasteurized soil grew significantly better than the non-inoculated ones grown in the same soil (Phot. 1). Root and shoot dry weights significantly increased about 50% and 40% over the controls, respectively (Table 1). Inoculation of seedlings with mycorrhizal and pathogenic fungi resulted in reduction of main root length. Simultaneously the increase of root collar diameter in seedlings inoculated with mycorrhizal fungi as compared to control ones was observed. Seedlings inoculated with mycorrhizal fungi produced more lateral roots, thus making the root dry weight bigger (especially in presence of *B. edulis* and *X. badius*). In the pasteurized soil mycorrhizae were detected on the roots of seedlings inoculated with only mycorrhizal fungi. The greatest number of mycorrhizal roots was noted with *S. luteus* as the inoculum.



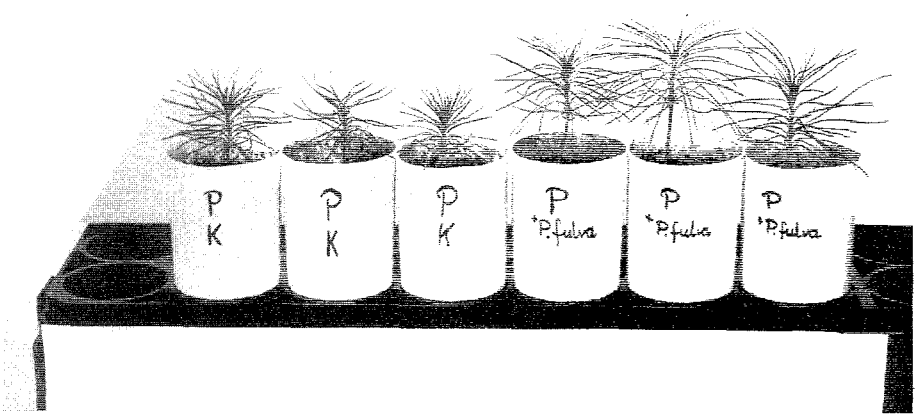
Phot. 1. Pine seedlings inoculated with *Pseudomonas fulva* in comparison to non-inoculated ones (P – pasteurized soil) (photo by D. Dworznikowska)

Statistical analyses

The growth of seedlings from each experimental combination was compared with the growth of non-inoculated seedlings (control) by use of Student's t-test for independent samples. One-factor ANOVA comparing the growth of seedlings in all experimental combinations was made too. Three-factor ANOVA was used for comparing the effects of: 1) soil treatment (pasteurized – non-pasteurized), 2) kind of inoculation (non-inoculated seedlings and inoculated with individual mycorrhizal or pathogenic fungus) and 3) presence or absence of *P. fulva* on the seedlings growth parameters. Means were compared using the Newman-Keuls multiple range test. STATISTICA/win 5.1 (1966) programme was applied (Stat Soft, Tulsa, Oklahoma, USA).

Results

The seedlings inoculated with *P. fulva* and grown in the pasteurized soil grew significantly better than the non-inoculated ones grown in the same soil (Phot. 1). Root and shoot dry weights significantly increased about 50% and 40% over the controls, respectively (Table 1). Inoculation of seedlings with mycorrhizal and pathogenic fungi resulted in reduction of main root length. Simultaneously the increase of root collar diameter in seedlings inoculated with mycorrhizal fungi as compared to control ones was observed. Seedlings inoculated with mycorrhizal fungi produced more lateral roots, thus making the root dry weight bigger (especially in presence of *B. edulis* and *X. badius*). In the pasteurized soil mycorrhizae were detected on the roots of seedlings inoculated with only mycorrhizal fungi. The greatest number of mycorrhizal roots was noted with *S. luteus* as the inoculum.



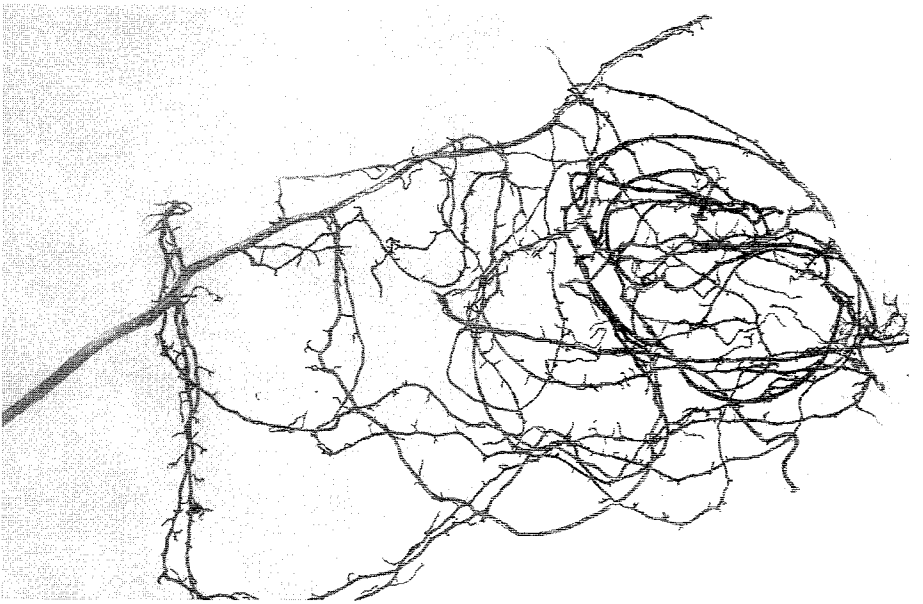
Phot. 1. Pine seedlings inoculated with *Pseudomonas fulva* in comparison to non-inoculated ones (P – pasteurized soil) (photo by D. Dworznikowska)

Table 1

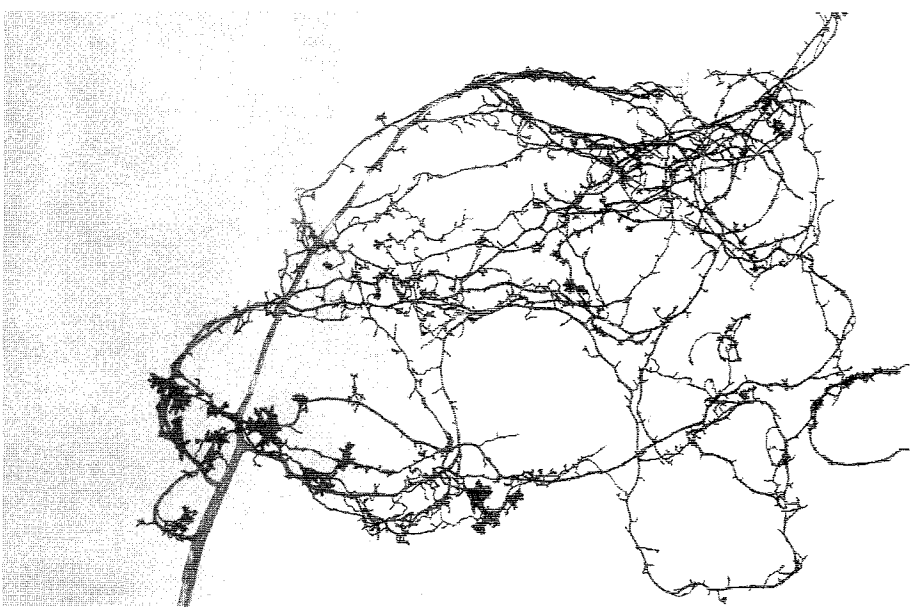
Effect of *Pseudomonas fulva* on growth of pine seedlings inoculated with mycorrhizal and pathogenic fungi in pasteurized soil

Experimental combination	Main root length (mm)	Root collar diameter (mm)	Root dry weight (mg)	Shoot height (mm)	Shoot dry weight (mg)	Mycorrhizal roots (number)
Non-inoculated seedlings (control)	18.90 b ± 1.91 100%	0.75 ab ± 0.41 100%	74.84 a ± 21.40 100%	3.63 a ± 0.43 100%	75.51 a ± 14.17 100%	0
P. fulva	18.75 b ± 0.86 99.5%	0.78 b ± 0.06 104%	117.59 ab ± 30.63 157.2%*	3.84 a ± 0.42 105.8%	105.01 ab ± 28.59 139.1%*	0
F. oxysporum	14.50 ab ± 3.64 76.7%*	0.63 a ± 0.15 84%	75.07 a ± 44.74 100.4%	3.74 a ± 0.41 103%	88.36 ab ± 25.51 117.1%	0
F. oxysporum + *P. fulva*	19.00 b ± 2.16 100.5%	0.81 b ± 0.03 108%**	150.99 b ± 52.32 201.9%**	3.81 a ± 0.31 105%	109.96 ab ± 21.36 145.7%**	0
R. solani	17.00 ab ± 7.03 89.9%	0.76 ab ± 0.17 101.3%	124.06 ab ± 61.45 166%	3.51 a ± 0.64 96.7%	107.40 ab ± 52.51 142.3%	0
R. solani + *P. fulva*	15.00 ab ± 5.88 79.4%	0.73 a ± 0.19 97.3%	89.09 ab ± 49.90 119.1%	3.59 a ± 0.59 98.9%	95.06 ab ± 51.06 126%	0
S. luteus	15.79 ab ± 3.77 83.5%	0.87 b ± 0.06 116%***	76.69 a ± 23.95 102.5%	3.86 a ± 0.48 106.3%	120.30 ab ± 32.40 159.3%**	70.00 ± 21.81 100%
S. luteus + *P. fulva*	11.57 a ± 2.79 61.4%***	0.90 b ± 0.04 120%***	81.31 a ± 17.83 108.7%	4.01 a ± 0.49 110.5%	170.73 c ± 26.39 226.1%***	190.71 ± 82.94 272.4%***
B. edulis	17.71 ab ± 3.70 93.7%	0.89 b ± 0.06 118.7%***	147.64 b ± 41.03 197.3%**	3.89 a ± 0.23 107.2%	143.14 bc ± 24.87 189.5%***	6.57 ± 6.19 100%
B. edulis + *P. fulva*	17.93 ab ± 2.35 94.7%	0.87 b ± 0.04 116%***	132.53 ab ± 40.44 177.1%**	3.64 a ± 0.49 100.3%	127.71 b ± 26.53 169.1%***	25.00 ± 24.03 380.5%
X. badius	12.86 ab ± 1.52 68.3%***	0.91 b ± 0.05 121.3%***	127.64 ab ± 20.16 170.6%***	3.61 a ± 0.37 99.4%	126.41 ab ± 37.03 167.4%***	6.86 ± 13.75 100%
X. badius + *P. fulva*	15.50 ab ± 3.10 82%*	0.91 b ± 0.04 121.3%***	119.77 ab ± 22.26 160.2%**	3.74 a ± 0.36 103%	120.34 ab ± 17.33 159.3%***	4.00 ± 2.24 58.3%

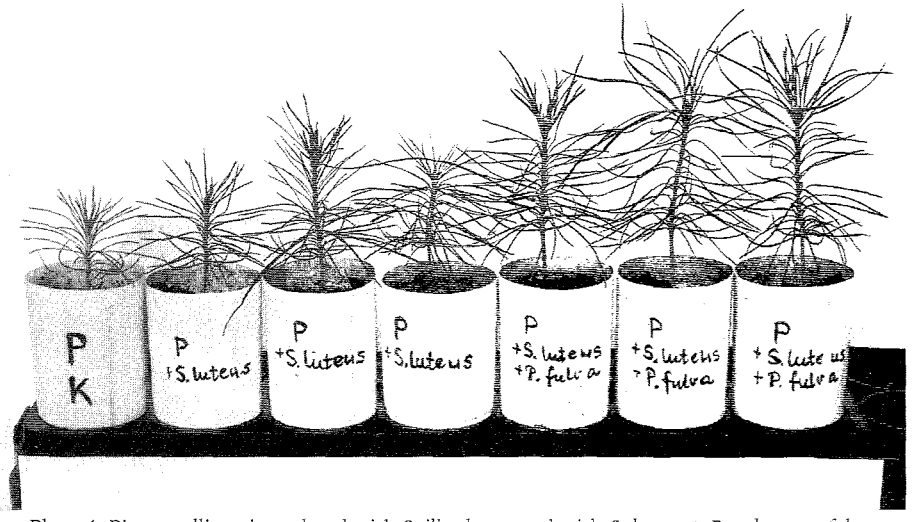
Data are means of seven replicates ± standard deviations.
Significance of differences as compared with control (t-test): *$0.01 < p < 0.05$, **$0.001 < p < 0.01$, ***$p < 0.001$.
Values within a given column followed by different letters are significantly different ($p < 0.05$).



Phot. 2. Formation of ectomycorrhizae on pine seedlings roots by *Suillus luteus* (pasteurized soil) (photo by D. Dworznikowska)



Phot. 3. Formation of ectomycorrhizae on pine seedlings roots by *Suillus luteus* in presence of *Pseudomonas fulva* (pasteurized soil) (photo by D. Dworznikowska)



Phot. 4. Pine seedlings inoculated with *Suillus luteus* and with *S. luteus* + *Pseudomonas fulva* (the seedling on the left is control, P – pasteurized soil) (photo by D. Dworznikowska)

Additionally inoculation of the seedlings with *P. fulva* caused significant increase of mycorrhizal roots (Table 1, Phots 2–3).

Differences in shoot height in various treatment combinations were not statistically significant. However, shoot dry weight was significantly higher for the seedlings inoculated with mycorrhizal fungi due to the greater number and longer needles produced. *Pseudomonas fulva* enhanced this effect in seedlings inoculated with *S. luteus* (Phot. 4). Similar stimulating effect of *P. fulva* on shoot dry weight of seedlings inoculated with *F. oxysporum* was observed (Table 1).

The response of pine seedlings to different inocula was less pronounced in non-pasteurized soil. After inoculation of seedlings with *P. fulva* in that soil stimulation of root and shoot dry weight reached 12% and 22%, respectively, over non-inoculated seedlings but this effect was not statistically significant (Table 2). As in the pasteurized soil, the reduction of the main root length occurred upon inoculation of seedlings. Some treatment combinations with simultaneously inoculation with *P. fulva* resulted in increase of root dry weight. Mycorrhizal inoculation affected the increase of shoot dry weight. In non-pasteurized soil, mycorrhizal roots were observed in seedlings of each treatment combination but presence of *P. fulva* significantly increased the number of mycorrhizae.

Pseudomonas fulva, in great extent, protected seedlings against infection by *R. solani* (Phot. 5). *Rhizoctonia solani* significantly reduced growth of seedlings in non-pasteurized soil. Application of *R. solani* together with *P. fulva* rendered the seedlings the same as the seedlings without infection by *R. solani.*

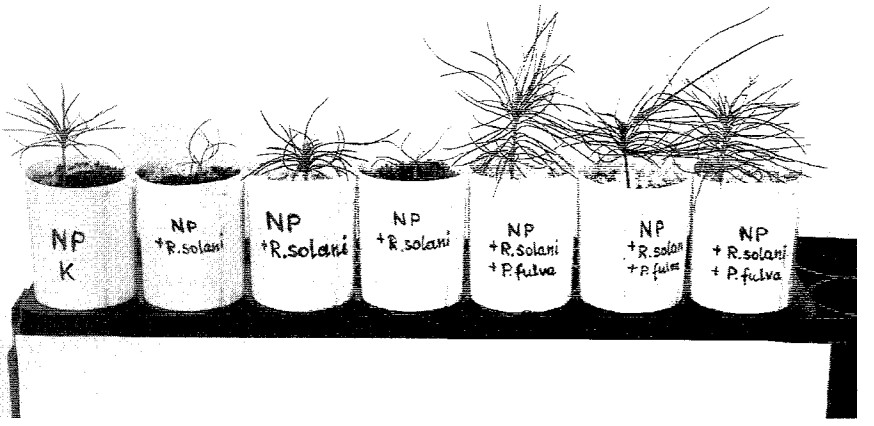
The seedlings with or without *F. oxysporum* inoculation didn't differ significantly either in pasteurized or non-pasteurized soil. *Fusarium oxysporum* appeared

Table 2

Effect of *Pseudomonas fulva* on growth of pine seedlings inoculated with mycorrhizal and pathogenic fungi in non-pasteurized soil

Experimental combination	Main root length (mm)	Root collar diameter (mm)	Root dry weight (mg)	Shoot height (mm)	Shoot dry weight (mg)	Mycorrhizal roots (number)
Non inoculated seedlings (control)	18.71 b ± 4.64 100%	0.83 b ± 0.09 100%	74.84 a ± 21.40 100%	3.60 b ± 0.61 100%	65.31 b ± 24.69 100%	13.86 ab ± 8.43 100%
P. fulva	16.90 b ± 3.93 90.4%	0.85 b ± 0.05 102.4%	117.59 ab ± 30.63 157.2%*	3.64 b ± 0.24 101.1%	80.10 b ± 11.61 122.7%	10.43 ab ± 5.53 74.8%
F. oxysporum	16.57 b ± 3.79 88.8%	0.86 b ± 0.05 103.6%	75.07 a ± 44.74 100.4%	3.50 b ± 0.44 97.2%	73.91 b ± 15.30 113.2%	10.86 ab ± 7.52 78.4%
F. oxysporum + *P. fulva*	14.29 ab ± 4.72 76.5%	0.91 b ± 0.04 109.6%	150.99 b ± 52.32 201.9%**	3.67 b ± 0.51 101.9%	91.60 b ± 25.10 140.3%	31.43 b ± 17.81 225.9%*
R. solani	7.07 a ± 5.46 38%**	0.70 a ± 0.19 84.3%	124.06 ab ± 61.45 166%	2.54 a ± 1.15 70.6%	38.11 a ± 31.82 58.3%	5.86 a ± 6.20 42.4%
R. solani + *P. fulva*	11.23 ab ± 4.01 59.9%**	0.86 b ± 0.07 103.6%	89.09 ab ± 49.90 119.1%	3.84 b ± 0.64 106.7%	84.30 b ± 33.57 129.1%	15.43 ab ± 11.57 110.8%
S. luteus	13.21 ab ± 4.00 0.6%*	0.83 b ± 0.03 100%	76.69 a ± 23.95 102.5%	4.00 b ± 0.50 111.1%	80.54 b ± 25.90 123.3%	23.86 ab ± 17.29 171.9%
S. luteus + *P. fulva*	13.07 ab ± 4.76 70.1%*	0.81 b ± 0.07 97.6%	81.31 a ± 17.83 108.7%	3.64 b ± 0.29 101.1%	87.31 b ± 11.54 133.7%	46.00 b ± 18.54 330.9%**
B. edulis	10.21 ab ± 3.78 54.5%**	0.81 b ± 0.04 97.6%	147.64 b ± 41.03 197.3%**	3.81 b ± 0.55 105.8%	91.70 b ± 11.64 140.4%*	23.57 ab ± 8.28 169.8%
B. edulis + *P. fulva*	14.40 ab ± 4.44 77%	0.85 b ± 0.02 102.4%	132.53 ab ± 40.44 177.1%**	3.67 b ± 0.51 101.9%	99.33 b ± 32.59 152.1%*	29.43 b ± 11.15 211.5%*
X. badius	11.00 ab ± 4.14 58.8%**	0.83 b ± 0.05 100%	127.64 ab ± 20.16 170.6%***	3.60 b ± 0.20 100%	84.83 b ± 14.64 129.9%	17.14 ab ± 6.84 123%
X. badius + *P. fulva*	13.71 ab ± 3.58 73.3%*	0.88 b ± 0.04 106%	119.77 ab ± 22.26 160.2%**	3.71 b ± 0.39 103%	102.44 b ± 41.10 156.8%	38.00 b ± 20.66 273.4%*

Explanations: see Table 1.



Phot. 5. Pine seedlings inoculated with *Rhizoctonia solani* and with *R. solani* – *Pseudomonas fulva* (the seedling on the left is control, NP – non-pasteurized soil) (photo by D. Dworznikowska)

to have lost its pathogenicity. However, seedlings inoculated with *F. oxysporum* and *P. fulva* increased shoot and root dry weights.

Table 3 presents the data from three-factor ANOVA comparing the effects of: soil treatment (pasteurized – non-pasteurized), kind of inoculation and presence or absence of *P. fulva* on the seedlings growth. Three experimental factors strongly affected root and shoot dry weight ($p < 0.05$). Their effect on the shoot height was the weakest one. Statistical interactions between main experimental factors were significant in the following cases: a) soil treatment × kind of inoculation: slightly for root and shoot dry weights ($p \sim 0.04$) and root length ($p \sim 0.01$), highly for the root collar diameter ($p = 2 \times 10^{-6}$), b) soil treatment × kind of inoculation × inoc-

Table 3

Significance level (p) from three-factor ANOVA comparing the effects of: soil treatment, kind of inoculation and inoculation with *Pseudomonas fulva* on the growth of pine seedlings

Effect of:	df	Main root length	Root collar diameter	Root dry weight	Shoot height	Shoot dry weight
Soil treatment [1]	1	0.000008	0.1833	0.000001	0.0811	0.0000
Kind of inoculation [2]	5	0.000001	0.00003	0.000021	0.0110	0.0000
Inoculation with *P. fulva* [3]	1	0.2917	0.0034	0.0075	0.1043	0.0010
[1] × [2]	5	0.0110	0.000002	0.0484	0.8232	0.0388
[1] × [3]	1	0.4323	0.6059	0.5393	0.4372	0.4211
[2] × [3]	5	0.1792	0.1398	0.0218	0.0299	0.3174
[1] × [2] × [3]	5	0.0335	0.0172	0.0044	0.0414	0.0238
Error	144	–	–	–	–	–

df – degrees of freedom (the same for each growth parameter measured).

Table 4

Results of Newman-Keuls multiple range test for the main experimental factors

Main root length (mm)		
Soil treatment:	non-pasteurized 13.37 a	pasteurized 16.21 b
Kind of inoculation:	control 18.32 c, *F. oxysporum* 16.09 b, *R. solani* 12.58 a, *S. luteus* 13.41 a, *B. edulis* 15.06 ab, *X. badius* 13.27 a	
Inoculation with *P. fulva*:	non-inoculated 14.46 a	inoculated 15.11 a
Root collar diameter (mm)		
Soil treatment:	non-pasteurized 0.83 a	pasteurized 0.82 a
Kind of inoculation:	control 0.80 a, *F. oxysporum* 0.80 a, *R. solani* 0.76 a, *S. luteus* 0.85 b, *B. edulis* 0.86 b, *X. badius* 0.88 b	
Inoculation with *P. fulva*:	non-inoculated 0.81 a	inoculated 0.84 b
Root dry weight (mg)		
Soil treatment:	non-pasteurized 83.04 a	pasteurized 109.18 b
Kind of inoculation:	control 82.98 a, *F. oxysporum* 102.51 b, *R. solani* 83.29 a, *S. luteus* 81.15 a, *B. edulis* 117.41 b, *X. badius* 109.32 b	
Inoculation with *P. fulva*:	non-inoculated 89.26 a	inoculated 102.96 b
Shoot height (mm)		
Soil treatment:	non-pasteurized 3.60 a	pasteurized 3.74 a
Kind of inoculation:	control 3.68 ab, *F. oxysporum* 3.68 ab, *R. solani* 3.37 a, *S. luteus* 3.88 b, *B. edulis* 3.75 b, *X. badius* 3.67 b	
Inoculation with *P. fulva*:	non-inoculated 3.61 a	inoculated 3.74 a
Shoot dry weight (mg)		
Soil treatment:	non-pasteurized 81.63 a	pasteurized 115.83 b
Kind of inoculation:	control 81.49 a, *F. oxysporum* 90.96 a, *R. solani* 81.22 a, *S. luteus* 114.73 b, *B. edulis* 115.47 b, *X. badius* 108.51 b	
Inoculation with *P. fulva*:	non-inoculated 91.30 a	inoculated 106.16 b

Values followed by the same letter do not differ significantly ($p < 0.05$).

ulation with *P. fulva* (triple interaction) – slightly or moderately significant for all the growth parameter studied (p from ~ 0.004 to ~ 0.03). The results of Newman-Keuls multiple range test comparing the means are presented in Table 4. The most important observation for this study is that the seedlings inoculated with *P. fulva* regardless of pasteurized or non-pasteurized soil and the treatment combinations grew significantly better than the seedlings without inoculation with this endophytic bacterium.

Discussion

Certain plant growth promoting rhizobacterial (PGPR) strains, particularly those belonging to the genera *Bacillus* and *Pseudomonas* proliferate not only on and around plant roots, but also inside root tissues of plants (Shishido et al. 1999). The

discovery of bacterial populations in the endodermis and root cortex of plants has supported the idea that many bacteria in the rhizosphere are able to penetrate and colonize plant roots (Darbyshire and Greaves 1973, Petersen et al. 1981). In contrast, van Peer et al. (1990) using an analysis of lypopolysaccharide patterns and cell envelope protein patterns, reported that endophytic and rhizobacterial strains belonging to the same genera formed discrete subpopulations each specifically suited to colonizing their respective niches.

Endophytic as well as exo-root bacteria can stimulate, inhibit or be neutral for plant growth (Chanway and Holl 1994). Plant growth stimulating effects that have encompassed endophytes, include growth stimulation indirectly through the biocontrol of phytopathogens, through the induction of phytohormone synthesis by the plant or through the enhanced availability of minerals (Sturz et al. 2000).

In our studies the pine seedlings inoculated with *P. fulva* grew significantly better than non-inoculated ones. Root and shoot dry weights in the pasteurized soil increased significantly about 50% and 40%, respectively, over the control. Similar results were obtained with lodgepole pine inoculated with other endophytic bacteria (Chanway et al. 1994). In greenhouse trials with forest soils, inoculation of spruce (*Picea glauca*) with root-endophytic bacteria significantly increased both shoot and root relative growth rates by 10–234% (Shishido and Chanway 2000).

Some research efforts have focussed on the development of endophytic diazotrophs that are able to supply biologically fixed nitrogen directly to their host (Quispel 1991, Barraquio et al. 1997, Reinhold-Hurek and Hurek 1998). However our previous studies have shown that *P. fulva* was not able to fix N_2 (Strzelczyk and Li 2000) but was an active producer of indole-3-acetic acid and 3-indoleacetonitrile (unpublished data). Microbial production of phytohormons has been regarded as an important source of plant growth promotion (Lebuhn et al. 1997).

The mycorrhizosphere supports large bacterial populations (Linderman and Paulitz 1990). The potential for many of these strains to affect infectivity and growth of mycorrhizal fungi *in vitro* and on plant roots has been demonstrated (Garbaye 1994). Our results show that *P. fulva* acts relatively similar to mycorrhizosphere bacteria enhancing mycorrhizae formation on the roots of pine seedlings. Garbaye and Duponnois (1992) demonstrated that the stimulatory effect of bacterial isolates (helper bacteria) was not plant specific, but in a striking degree of bacteria-fungus specifity. The endophyte *P. fulva* in this study was not fungus specific; it promoted mycorrhizae formation with different ectomycorrhizal fungi as *S. luteus* or *B. edulis* – fungi of different stage of plant development ("early or later-stage" fungus, respectively).

Mechanisms by which endophytes can act as biocontrol agents include the production of antifungal or antibacterial agents, siderophore production, nutrient competition, niche exclusion and indirectly through the induction of systemic acquired host resistance or immunity (Tuzun and Kloepper 1994, Chen et al. 1995). Reddy et al. (1994) reported significant reduction in disease occurrence of conifer seedlings infested with *F. oxysporum* after seed inoculation with *Pseudomonas*. Selected strains of pseudomonads also reduced disease appearance caused by *F. oxysporum* or *Pythium*

ultimum Trow at a conifer nursery. The mechanisms by *P. fulva* to protect the Scots pine from infection by *R. solani* are unknown and need to be studied.

In previous studies we have detected siderophore production by *P. fulva* (Dahm et al. 2003). *Pseudomonas fluorescens, P. putida, P. aeruginosa* isolated from within older (90-year-old) pine tree roots were unable to synthesize these compounds.

Interesting mechanism of biological control efficacy of plant associated *Enterobacter cloacae* on various plant species against *P. ultimum* was demonstrated by Kageyama and Nelson (2003). Their data have shown that *E. cloacae* was effective in suppressing *P. ultimum* by reducing the stimulatory activity of plant seed exudates to sporangia of the pathogen. It is not excluded that *P. fulva* could also have this kind of biocontrol mechanism, as reported by van Dijk and Nelson (1998) that *P. fulva* KN4 was effective in biological control of seed rot and *P. ultimum* damping-off of cotton by reducing the stimulatory activity of cotton seed exudates.

Most experimentation with "helper" bacteria and plants has been performed under controlled environmental conditions. In addition we have only circumstantial evidence regarding mechanisms of growth promotion and almost no data regarding bacterial persistance in the field. According to Sturz et al. (2000) the utilization of endophytic bacteria in agricultural and forestry production will depend on investigators ability to maintain and modify beneficial populations under field conditions.

Summary

The effect of endophytic bacterium *Pseudomonas fulva* isolated from interior tissues of non-mycorrhizal suberized roots of Scots pine on the growth of pine seedlings, formation of mycorrhizae and protection against fungal pathogens was studied. The growth of seedlings inoculated with suspension of *Suillus luteus, Boletus edulis, Xerocomus badius* spores and with pathogenic fungi *Rhizoctonia solani* and *Fusarium oxysporum* was compared with the growth of seedlings inoculated with these fungi and additionally with *P. fulva* . Comparison of the growth of non-inoculated (control) seedlings with the growth of seedlings inoculated with *P. fulva* only, was also made. The seedlings were grown in pasteurized and non-pasteurized sandy soil. *Pseudomonas fulva* stimulated significantly the growth of pine seedlings in the pasteurized soil and a weaker stimulating effect was noted in the non-pasteurized one. *Pseudomonas fulva* increased the number of mycorrhizal roots in seedlings grown in the non-pasteurized soil (in different experimental combinations) and significantly stimulated the shoot growth and number of mycorrhizal roots in seedlings inoculated with *S. luteus* in the pasteurized soil. The bacterium protected the seedlings grown in non-pasteurized soil against *R. solani*. This study indicates that Scots pine seedlings inoculated with *P. fulva* grew better than the seedlings without inoculation with this bacterium, and the effect was more pronounced with pasteurized soil than with non-pasteurized one.

Streszczenie

WPŁYW ENDOFITYCZNEJ BAKTERII *PSEUDOMONAS FULVA* NA WZROST SIEWEK SOSNY (*PINUS SYLVESTRIS*), POWSTAWANIE MIKORYZY ORAZ OCHRONĘ PRZED PATOGENAMI

Zbadano wpływ endofitycznej bakterii *Pseudomonas fulva* wyizolowanej z wnętrza zdrewniałych, niemikoryzowych korzeni sosny na wzrost siewek sosny, powstawanie mikoryzy i ochronę przed patogenami. Wzrost siewek sosny zaszczepionych zawiesiną zarodników *Suillus luteus, Boletus edulis, Xerocomus badius* oraz patogenami: *Rhizoctonia solani* i *Fusarium oxysporum* porównano ze wzrostem siewek zaszczepionych tymi grzybami i dodatkowo *P. fulva*. Porównano też wzrost siewek kontrolnych (niezaszczepionych) i zaszczepionych jedynie *P. fulva*. Siewki hodowano w glebie piaszczystej sterylizowanej i niesterylnej.

Stwierdzono, że endofityczna bakteria *P. fulva* istotnie stymulowała wzrost siewek sosny w podłożu sterylnym; słabszy efekt stymulacyjny obserwowano w podłożu niesterylnym. Bakteria *Pseudomonas fulva* wpłynęła korzystnie na stopień mikoryzacji siewek sosny hodowanych w glebie niesterylnej (w różnych kombinacjach doświadczalnych), a także istotnie stymulowała wzrost pędu i rozwój mikoryz u siewek zaszczepionych *S. luteus* w glebie sterylnej. Bakteria ta chroniła siewki hodowane w glebie niesterylnej przed *R. solani*.

Wyniki trójczynnikowej analizy wariancji porównującej wpływ rodzaju podłoża, kombinacji doświadczalnych oraz szczepienia *P. fulva* na wzrost siewek pozwalają na ogólne stwierdzenie, że siewki zaszczepione *P. fulva* rosły lepiej niż niezaszczepione tą bakterią oraz że siewki hodowane w podłożu sterylnym rosły lepiej niż w podłożu niesterylnym.

Literature

Arshad M., Frankenberger W.T., 1991: Microbial production of plant hormones. Plant Soil 133: 1–8.

Baldani J.I., Caruso L., Baldani V.L.D., Goi S.R., Döbereiner J., 1997: Recent advances in BNF with non-legume plants. Soil Biol. Biochem. 29: 911–922.

Barraquio W.L., Revilla L., Ladha J.K., 1997: Isolation of endophytic diazotrophic bacteria from wetland rice. Plant Soil 194: 15–24.

Buyer J.S., Sikora L.J., 1990: Rhizosphere interactions and siderophores. Plant Soil 129: 101–107.

Chanway C.P., 1996: Endophytes: they're not just fungi! Can. J. Bot. 74: 321–322.

Chanway C.P., Holl F.B., 1994: Growth of outplanted lodgepole pine seedlings one year after inoculation with growth promoting rhizobacteria. For. Sci. 40: 238–246.

Chanway C.P., Shishido M., Holl F.B., 1994: Root-endophytic and rhizosphere plant growth promoting rhizobacteria for conifer seedlings. In: Improving plant productivity with rhizosphere bacteria. Proceedings of the 3rd International Workshop PGPR. Eds M.H. Ryder, P.M. Stephens, G.D. Bowen. CSIRO Div. Soils, Adelaide, Australia: 72–74.

Chen C., Bauske E.M., Musson G., Rodriguez-Kabana R., Kloepper J.W., 1995: Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. Biol. Conserv. 6: 83–91.

Dahm H., Hrynkiewicz K., Strzelczyk E., Wrótniak W., 2003: Studies on the production of siderophores by forest trees endophytic bacteria and fungi. Folia For. Pol. Ser. A 17, 45: 5–13.

Darbyshire J.F., Greaves M.P., 1973: Bacteria and protozoa in the rhizosphere. Pestic. Sci. 4: 349–360.

De Weger L.A., Schippers B., Lugtenberg B., 1987: Plant growth stimulation by biological interference in iron metabolism in the rhizosphere. In: Iron transport in microbes, plants and animals. Eds G. Winkelmann, D. Van der Helm, J.B. Neilands. VCH Verlagsgeseilschaft, Weinheim, Germany: 387–400.

Dijk van K., Nelson E.B., 1998: Inactivation of seed exudate stimulants of *Pythium ultimum* sporangium germination by biocontrol strains of *Enterobacter cloacae* and other seed-associated bacteria. Soil Biol. Biochem. 30: 183–192.

Garbaye J., 1994: Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. 128: 197–210.

Garbaye J., Duponnois R., 1992: Specifity and function of mycorrhization helper bacteria (MHB) associated with the *Pseudotsuga menziesii-Laccaria laccata* symbiosis. Symbiosis 14: 335–344.

Glick B.R., 1995: The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41: 109–117.

Kageyama K., Nelson E.B., 2003: Differential inactivation of seed exudate stimulation of *Pythium ultimum* sporangium germination by *Enterobacter cloacae* influences biological control efficacy on different plant species. Appl. Environ. Microbiol. 69: 114–120.

Kobayashi D.Y., Palumbo J.D., 2000: Bacterial endophytes and their effects on plants and uses in agriculture. In: Microbial endophytes. Eds C.W. Bacon, J.F. White, Jr. Dekker, New York: 199–232.

Lebuhn M., Heulin T., Hartmann A., 1997: Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiol. Ecol. 22: 325–334.

Linderman R.G., Paulitz T.C., 1990: Mycorrhizal-rhizobacterial interactions. In: Biological control of soil-borne plant pathogens. Ed. D. Hornby. CAB International, Wallington: 262–283.

Peer van R., Punte H.L.M., De Weger L.A., Schippers B., 1990: Characterization of root surface and endorhizosphere pseudomonads in relation to their colonization of roots. Appl. Environ. Microbiol. 56: 2462–2470.

Petersen C.A., Emanuel M.E., Humphreys G.B., 1981: Pathway of movement of apoplastic fluorescent dye tracers through the endodermis at the site of secondary root formation in corn (*Zea mays*) and broad bean (*Vicia faba*). Can. J. Bot. 59: 618–625.

Quispel A., 1991: A critical evaluation of the prospects for nitrogen fixation with non-legumes. Plant Soil 137: 1–11.

Reddy M.S., Axelrood P.E., Radley R., Rennie R.J., 1994: Evaluation of bacterial strains for pathogen suppression and enhancement of survival and growth of conifer seedlings. In: Improving plant productivity with rhizosphere bacteria. Proceedings of the 3rd International Workshop PGPR. Eds M.H. Ryder, P.M. Stephens, G.D. Bowen. CSIRO Div. Soils, Adelaide, Australia: 75–76.

Reinhold-Hurek B., Hurek T., 1998: Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: identification, localization and perspectives to study their function. Crit. Rev. Plant Sci. 17: 29–54.

Shishido M., Breuil C., Chanway C.P., 1999: Endotrophic colonization of spruce by plant-promoting rhizobacteria. FEMS Microbiol. Ecol. 29: 191–196.

Shishido M., Chanway C.P., 2000: Colonization and growth promotion of outplanted spruce seedlings pre-inoculated with plant growth-promoting rhizobacteria in the greenhouse. Can. J. For. Res. 30: 845–854.

Shishido M., Loeb M.B., Chanway C.P., 1995: External and internal root colonization of lodgepole pine seedlings by two growth-promoting *Bacillus* strains originated from different root microsites. Can. J. Microbiol. 41: 707–713.

Strzelczyk E., 2001: Endofity. In: Drobnoustroje środowiska glebowego – aspekty fizjologiczne, biochemiczne, genetyczne. Eds H. Dahm, A. Pokojska-Burdziej. Marszałek, Toruń: 97–108.

Strzelczyk E., Li C.Y., 2000: Bacterial endobionts in the big non-mycorrhizal roots of Scots pine (*Pinus sylvestris* L.). Microbiol. Res. 155: 229–232.

Sturz A.V., Christie B.R., Nowak J., 2000: Bacterial endophytes: potential role in developing sustainable systems in crop production. Crit. Rev. Plant Sci. 19: 1–30.

Tuzun S., Kloepper J.W., 1994: Induced systemic resistance by plant growth promoting rhizobacteria. In: Improving plant productivity with rhizosphere bacteria. Proceedings of the 3rd International Workshop PGPR. Eds M.H. Ryder, P.M. Stephens, G.D. Bowen. CSIRO Div. Soils, Adelaide, Australia: 104–109.

Authors' addresses: Dr Aleksandra Pokojska-Burdziej,
Prof. dr hab. Edmund Strzelczyk,
Prof. dr hab. Hanna Dahm,
Nicolaus Copernicus University,
Institute of General and Molecular Biology,
ul. Gagarina 9,
87-100 Toruń,
Poland

Dr Ching Yan Li,
USDA, Forestry Sciences Laboratory,
Pacific Northwest Research Station,
3200 SW Jefferson Way,
Corvallis, OR 97331,
USA

Accepted for publication: 16.04.2004