# Biological effect of *Pseudomonas oryzihabitans* and *Gliocladium virens* on *Rhizoctonia solani*, the causal agent of damping-off

*A.V. Kapsalis[1], S.R. Gowen[1], I.K.Vagelas[2] and F.T. Gravanis[2]*
*[1]The University of Reading, Berkshire UNITED KINGDOM*
*[2]Technological Education Institution of Larissa, Larissa GREECE*
*Correspondence author Kapsalis_tegos@yahoo.com; a.kapsalis@rdg.ac.uk*

## Abstract

*Cotton (*Gossypium hirsutum*) seedlings are vulnerable to attack by a number of soilborne pathogens, including* Rhizoctonia solani. *Biocontrol with antagonist microorganisms seems to be a promising approach for managing cotton seedling damping-off. Therefore, studies were conducted both* in vitro *and* in vivo *to test the efficacy of* Pseudomonas oryzihabitans, *a symbiont of the entomopathogenic nematode* Steinernema abbasi *and an isolate of* Gliocladium virens *isolated from greenhouse soil in Larissa region (Greece), against* R. solani. *Different concentrations of both biocontrol agents that tested were evaluated* in vitro *for their efficacy on* R. solani. P. oryzihabitans *was also tested* in vivo *as soil drench for its efficacy to control cotton seedling damping-off.* R. solani *was not parasitized in vitro by* P. oryzihabitans *but was strongly inhibited probably by antibiosis.* G. virens *parasitized the hyphae of* R. solani *very quickly. Growth of the fungi was monitored by measuring hyphal length and colony density where* R. solani *was either in contact or not with the biological agent that was employed. In all treatments with* P. oryzihabitans *there was healthy cotton seedling establishment up to 70% compared to the untreated seedlings (30%). Cotton seedling treatments with the* P. oryzihabitans *significantly ($P<0.05$) increased seedling stand, plant height and fresh weight, and decreased root rot severity compared to plants derived from untreated controls.*

## Introduction

The use of antagonists to control diseases incited by soil-borne pathogenic fungi is being intensively studied and may ultimately augment or replace current chemical methods of control.

*Rhizoctonia solani* is a major cause of seed rot, pre-emergence damping-off, and especially post-emergence damping-off of cotton throughout the world. The objective of this study was to determine the potential use of *Pseudomonas oryzihabitans* and *Gliocladium virens* for controlling *R. solani,* causing of cotton seedling damping-off.

## Experimental procedure

### Microbial cultures

The bacterial strain used in this study, *Pseudomonas oryzihabitans,* was grown at 27 °C on plate cultures containing 37g/l Nutrient Agar (Oxoid) (NA). Both *G. virens* and *R. solani* were grown on PDA (Difco) agar plates, at 25 °C in the dark. *R.solani* and *P. oryzihabitans* were obtained from the University of Reading culture collection, whereas *G. virens,* which was isolated from a greenhouse soil in Larissa region (Greece), was obtained from Crop Protection Laboratory, TEI of Larissa, Greece, culture collection.

### *In vitro biological effect of* Pseudomonas oryzihabitans *and* Gliocladium virens *against* Rhizoctonia solani

Three concentrations [$10^6$, $10^5$ and $10^4$ colony-forming units (cfu)/ml] of the bacterium *P. oryzihabitans*, suspension were streaked on a line onto NA plates at the one edge of the Petri dish. The density of the bacterial suspensions was measured spectrophometrically at 580 nm and adjusted accordingly. On a distance 4.5 cm apart (at the other edge of the Petri dish), a 3 mm agar plug, taken from a fresh *R. solani* culture, was placed. Replicates were 10-fold. *R. solani* 3 mm agar plugs were placed in the middle of 10 NA plates, were used as controls. A drop of spore suspension of *G. virens* in three concentrations ($10^6$, $10^5$ and $10^4$ spores/ml) adjusted by a haemocytometer, was placed on PDA plates in dual culture with *R. solani*. Three mm agar plugs of *R. solani* were placed 4.5 cm apart of *G. virens* inoculum and replicateted 10 times. *R. solani* plugs placed in the middle of 10 PDA plates, were used as controls. All cultures were randomly placed and incubated at 27 °C. The zone of inhibition between the bacteria and the leading edge of the fungal colony was measured with every 24 h and the incubation extended up to 96 h. The experiment was performed twice.

### *In vivo biological effect of* P. oryzihabitans *against* R. solani *cotton seedlings damping-off*

Non-treated cotton seed (cv. Cocker) were planted four seeds per pot 3.8-cm deep in sterilized soil-sand-vermiculite (3:1:1 v/v) in 25-cm diameter plastic pots placed randomly on a greenhouse bench. The seedlings in each pot were thinned to one plant 10 days after planting. Replicates were 16-fold. *R. solani* was grown in PDA broth (Difco, 100-ml in 250-ml flasks) for 10-12 days in a shake culture at room temperature (25-27 °C). The resulting mycelial mass was filtered through sterilized cheesecloth, rinsed in sterile distilled water, and fragmented in sterile distilled water in a Waring Blender for approximately 10 seconds. Concentration of the mycelial suspension was adjusted to give 50% light transmission (580 nm) on a Bausch and Lomb Spectronic-20 spectrophotometer. Inoculum was pipetted (50 ml/pot) onto the soil surface around 10-day old cotton seedlings, and the pots were watered thoroughly to spread the inoculum evenly around the hypocotyls. As regards to *P. oryzihabitans*, the same three concentrations and preparation method that used in *in vitro* tests were also employed in greenhouse ex-

periment. One hundred ml of each bacterial cfu/ml concentration was applied per pot just after *R. solani* application. After 60 days of incubation, plants were uprooted and assessed.

### Statistical analysis

The SPSS 10.0 for Windows statistical package was used. The data were then subjected to analysis of variance (ANOVA) to identify significant differences between treatments. Multiple range tests were used to identify statistical differences between means.

## Results and Discussion

The results of the *in vitro* tests were presented in Figure 1. Both biological agents were effective against *R. solani* after 48 h of incubation. The same pattern was appeared also after 72 h and 96 h of incubation. *R. solani* growth was inhibited by *P. oryzihabitans,* much more compared to inhibition effected by *G. virens*. After 24 h of incubation there was statistically different inhibition ($p=0.01$) of *R. solani* growth, caused by *P. oryzihabitans* in all concentrations used. *G. virens* was proved effective in suspension of $10^5$ spores/ml and $10^6$ spores/ml, but not in $10^4$ spores/ml.

Apart of *R. solani* inhibition by *P. oryzihabitans*, mycelia disintegration and vacuolization were observed microscopically, probably due to metabolites production. This is in agreement with Vagelas (2002) observations.

Vacuolation and coagulation were observed in hyphae of *R. solani* after 48 h period in all *G. virens* treatments. The efficacy of *G. virens* against *R. solani* was also reported by Vagelas *et al*. (2002c). In the present work lytic activity of the parasite on *R. solani* hyphae was observed microscopically. Analogous behaviour has been reported by Elad *et al*. (1983) in the case of *Trichoderma* spp. parasitism on *R. solani,* where after attachment and coiling of the antagonist around the phytopathogenic fungus, the parasitic hyphae were removed, revealing lysed sites and penetration holes in hyphae of the host fungus. There was no evidence in the present study of antibiotic activity of *G. virens*. However, Loper (1992) reported a correlation of the antibiotic gliovirin, produced by *G. virens* with its biocontrol activity on *Pythium* cotton damping-off. Also, Howell (1981) and Howel and Stipanovic (1995) stated that in agar medium the mycelium of *R. solani* was inhibited by antibiosis.

All bacteria treatments ($10^6$ cfu/ml, $10^5$ cfu/ml and $10^4$ cfu/ml) were effective in disease suppression in glasshouse tests and exhibited statistically significant differences in disease index, compared to the untreated control. The $10^4$ cfu/ml did not affect root fresh weight and nor plant fresh weight (Table 1). *P. oryzihabitans* applied to *R. solani*-infected soil at rates $10^6$, $10^5$ and $10^4$ cells/ml, delayed the appearance of symptoms and decreased the percentage of diseased plants from 68 to 24% (Table 1).

*P. oryzihabitans* has been reported as biological agent against many soilborne pathogens (Vagelas *et al.*, 2000a; Vagelas *et al.*, 2000b; Vagelas *et al.*, 2001a; Vagelas *et al.*, 2001b; Vagelas *et al.*, 2001c; Vagelas, 2002; Vagelas *et al.*, 2002a; Vagelas *et al.*, 2002b; Kapsalis *et al.*, 2002). The same bacterium also has been reported acting as biological agent against plant parasitic nematodes (Andreoglou *et al.,* 2000; Samailev *et al.,* 2000; Andreoglou, 2001a; Andreoglou *et al*., 2001b; Andreoglou, 2001; Leontopoulos *et al.,* 2001; Vagelas, 2002; Andreoglou *et al.,* 2003). In particular, the biological effect of *P. oryzihabitans* towards *R. solani* was also reported by Kapsalis *et al.* (2002).

This study suggested that adding *P. oryzihabitans* into soil prevents growth of *R. solani* and reduce the incidence of cotton damping-off. The study also confirmed that *G. virens* is a promising biocontrol agent towards *R. solani*.

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## References

- Andreoglou, F.I. Samaliev, H.Y. and Gowen, S.R. (2000). Temperature response of the bacterium *Pseudomonas oryzihabitans* from the entomopathogenic nematodes *Steinernema abbasi,* on the potato cyst nematode *Globodera rostochiensis*. *Aspects of Applied Biology,* **59**: 67-73.
- Andreoglou, F., Samaliev, H., Elawad, S., Hague, N., and Gowen, S.R. (2001a). The susceptibility of the root-knot nematode *Meloidogyne javanica* to the bacterium *Pseudomonas oryzihabitans* isolated from the entomopathogenic nematode *Steinernema abbasi* (Steinermatidae: Nematoda). *Tri-trophic interactions in the rhizosphere, IOBC/wprs Bulletin,* **24**: 5-8.
- Andreoglou, F.I., Vagelas, I. K., Samaliev, H.Y., and Gowen, S.R. (2001b). Control of potato cyst nematode *Globodera rostochiencies* with the entomopathogenic bacterium *Pseudomonas oryzihabitans*. *IOBC WPRS BULLETIN* (proof in press).
- Andreoglou, F.I. (2001). *Control of potato cyst nematode* Globodera rostochiensis *with Pseudomonas oryzihabirans*, The University of Reading, UK, Ph.D. 229 pp.
- Andreoglou, F.I., Vagelas, I.K., Wood, M., Samaliev, H.Y., and Gowen, S.R. (2003). Influence of temperature on the motility of *Pseudomonas oryzihabitans* and control of *Globodera*

*rostochiensis. Soil and Biotechnology* (proof in press).
- Elad, Y., Chet, I., Boule, P., and Henis, Y. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* – SEM studies and fluorescence microscopy. *Phytopathology,* **73**: 85-88.
- Howell, C.R. (1981). Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani*, and Damping-Off of cotton seedling. *Phytopathology,* **72**: 496-498.
- Howell, C.R. and Stipanovic, R.D. (1995). Mechanisms in the biocontrol of *Rhizoctonia solani*-induced cotton seedling disease by *Gliocladium virens*: antibiosis. *Phytopathology,* **85**: 469-472.
- Kapsalis, A.V., Gowen, S.R. and Gravanis, F.T. (2002). Nematode entomopathogenic symbiotic bacteria acting as biological agents against tomato seedlings fungal pathogens. *Proceedings of the BCPC Conference - Pests & Diseases 2002,* **2**: 749-752. Brighton, U.K.
- Leontopoulos, S.V., Vagelas, I.K., Gravanis, F.T. and Gowen, S.R. (2001). The effect of certain bacteria and fungi on the biology of the root-knot nematode *Meloidogyne* sp. *International Organization for Biological Control (IOBC) Bulletin* (proof in press).
- Loper, J.E (1992). *Biological control of plant diseases*. APS Press slide collections. ISBN: 0-89054-150-7.
- Samaliev, H.Y., Andreoglou, F.I., Elawad, S.A., Hague, N.G.M. and Gowen, S.R. (2000). The nematicidal effects of the bacteria *Pseudomonas oryzihabitns* and *Xenorhabdus nematophilus* on the root-knot nematode *Meloidogyne javanica*. *Journal of Nematology,* **2**: 507-514.
- Vagelas, I.K., Andreoglou, F.I., Peters, J.C., Gravanis, F.T. and Gowen, S.R. (2000a). Tests of the biological effect of the nematode symbiotic bacterium *Flavimonas oryzihabitans* (=*Pseudomonas* oryzihabitans) Holmes *et al.* in *Fusarium oxysporum* f.sp. *lycopersici. In* 6th European Fusarium Seminar & Third COST 835 Workshop (Agriculturally Important Toxigenic Fungi. p. 106. Edited by H.I. Nirenberg. Biologische Bundesanstalt fur Land- und Forstwirtschaft Institut fur Pflanzenvirologie, Mikrobiologie und Biologische Sicherheit. ISBN: 3-8263-3352-7.
- Vagelas, I.K., Andreoglou, F.I., Peters, J.C., Gravanis, F.T. and Gowen, S.R. (2000b). Testing the efficacy of microorganisms to *Fusarium oxysporum*. *Proceedings of the BCPC Conference - Pests & Diseases 2000,* **3**: 1049-1054. Brighton, U.K.
- Vagelas I.K., Gravanis F.T. & Gowen S.R. (2001a). Antifungal activity of entomopathogenic bacterium *P. oryzihabitans* towards *Fusarium oxysporum*. *International Organization for Biological Control (IOBC) Bulletin (proof in press).*
- Vagelas, I.K., Gravanis, F.T. and Gowen, S.R. (2001b). Soilborne fungi and entomopathogenic bacteria acting as biological agents against Fusarium wilt of tomato. *International Organization for Biological Control (IOBC) Bulletin* (proof in press).
- Vagelas, I.K., Kapsalis, A., Gravanis, F.T. and Gowen, S.R. (2001c). Biological control of *Rhizoctonia* solani Damping-off with a bacterium symbiotically associated with *Steinernema abbasi*. *International Organization for Biological Control (IOBC) Bulletin* (proof in press).
- Vagelas, I.K. (2002). *Efficacy of* Pseudomonas oryzihabitans *as a biocontrol agent of root pathogens.* The University of Reading, UK, PhD. Thesis, 216 pp.
- Vagelas, I.K., Gravanis, F.T. and Gowen, S.R. (2002a). Biological activity of *Gliocladium virens* towards soilborne plant pathogens. (Abstr.) Proceedings of 11th Panhellenic Phytopathological Conference, p. 44.
- Vagelas, I.K., Gravanis, F.T., Wood, M., Davies, K.G. and Gowen, S.R. (2002b). Antifungal activity of *Pseudomonas oryzihabitans* a bacterium symbiotically associated with *Steinernema abbasi* towards *Fusarium oxysporum* and *Rhizoctonia solani*. *Proceedings of the BCPC Conference - Pests & Diseases 2002,* **1**: 85-90.
- Vagelas, I.K., Gravanis, F.T. and Gowen, S.R. (2002c). Biological activity of *Gliocladium virens* and *Trichoderma* spp. towards soilborne plant pathogens. (Abstr.) Proceedings of 11th Panhellenic Phytopathological Conference, p. 44.

***Table 1.*** *Efficacy of* P. oryzihabitans *as a biological control agent of* R. solani *cotton damping-off.*

| Treatment | Diseased plants (%) | Total root fresh weight (mg) | Height of fresh cotton plant (cm) | Disease index[a] |
|---|---|---|---|---|
| *R. solani* (ontrol) | 68 | 23.693a* | 32.44a | 3.6b |
| *R. solani*/*P. oryzihabitans* $10^6$ cfu/ml | 24 | 27.515b | 36.94b | 1.6a |
| *R. solani*/*P. oryzihabitans* $10^5$ cfu/ml | 29 | 25.717ab | 36.06ab | 1.8a |
| *R. solani*/*P. oryzihabitans* $10^4$ cfu/ml | 34 | 25.679ab | 33.44a | 1.9a |

[a] Disease index was evaluated using a 1-5 scale: 0 (healthy plants) to 5 (completely girdled).

* Values within a column followed by the same letter do not differ significantly ($P$=0.05) according to Duncan's multiple range test.

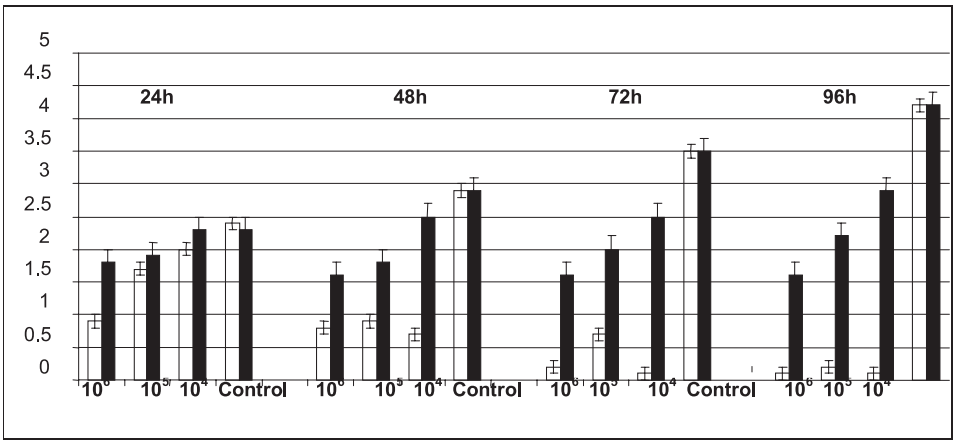


***Figure 1.*** *Effect of* P. oryzihabitans *and* G. virens *on* R. solani *growth. *Bars representing* R. solani *challenged by* P. oryzihabitans *(□) and* R. solani *challenged by* G. virens *(□) mycelia growth (cm) in four different incubation times. Bars show means; error bars show means (±1.0) standard error.*