J. AMER. SOC. HORT. SCI. 117(3):407-410. 1992.

Evaluation of *Penicillium janthinellum* as a Biological Control of Phytophthora Root Rot of Azalea

B.H. Ownley[1] and D.M. Benson[2]

Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616

Additional index words. ornamental, *Phytophthora cinnamomi,* pine bark, *Rhododendron obtusum,* root disease

Abstract. **Wheat bran inoculum of *Penicillium janthinellum* (Biourge) [1% w/w added to pine bark (PB) container medium] suppressed "'root rot of azalea *(Rhododendron obtusum* Planch.) caused by *Phytophthora cinnamomi* Rands in greenhouse experiments. Shoot fresh weight was increased by 31% to 91% and mortality reduced by 30% to 50% for azaleas planted in natural (nonsterile) PB amended with *P. janthinellum* compared with the infested control. The population densities of *P. janthinellum* exceeded 10^5 to 10^6 cfu/g dry PB within 7 days and remained stable over time. *Penicillium janthinellum,* a natural colonizer of PB container media, shows potential as a biological control of phytophthora root rot of azalea.**

Phytophthora root rot is the single most important disease in the commercial production of azaleas in the southeastern United States. The most frequently isolated pathogen is *Phytophthora cinnamomi* Rands (Lambe et al., 1982). Azaleas are produced commercially in containers and in the field. Container production is the most common method in the South. Container media amended with composted hardwood bark or PB suppress various root-infecting pathogens, including *P. cinnamomi* (Gerrettson-Cornell et al., 1978; Hoitink et al., 1977; Spencer and Benson, 1981, 1982). Reproduction, survival, and pathogenicity of *P. cinnamomi* are adversely affected by tree bark media. Significant inhibition of linear growth, formation of chlamydospores and sporangia, and lysis of zoospores and mycelium have been demonstrated in composted hardwood bark (Hoitink et al., 1977; Spencer and Benson, 1982). Suppression of sporangium production has been reported in PB (Gerrettson-Cornell and Humphreys, 1978; Spencer and Benson, 1982). Significant suppression of phytophthora root rot of various species (Hoitink et al., 1977; Spencer and Benson, 1981, 1982; Gerrettson-Cornell et al., 1976) has been reported in composted hardwood bark (CHB) (Hoitink et al., 1977; Spencer and Benson, 1981, 1982) and PB (Gerrettson-Cornell et al., 1976; Spencer and Benson, 1981, 1982).

Our study was initiated to evaluate *Penicillium janthinellum,* a natural inhabitant of PB, as a biological control of phytophthora root rot of azalea. In addition, the population dynamics of introduced and naturally occurring *P. janthinellum* and other fungi were quantified to better understand the relation between disease suppression and *P. janthinellum* activity.

Materials and Methods

Oat inoculum of *P. cinnamomi* (ATCC 46292) was prepared as described by Ownley and Benson (1990). Oat cultures were maintained at 25C for 2 weeks before infestation of PB container medium. *Penicillium janthinellum* (NRRL A-27551) was isolated in late summer from a 3 pine bark : 1 sand (v/v) (PBS) container medium to which a vermiculite-V-8 juice medium colonized by *P. cinnamomi* had been added as inoculum in June. *Rhododendron* L. 'Nova Zembla' grown in the infested PBS did not exhibit symptoms of root rot, and *P. cinnamomi* could not be recovered from either the vermiculite–V-8 inoculum or from the rhododendron roots when plated onto two media semi-selective for *Phytophthora* (Eckert and Tsao, 1962; Shew and Benson, 1982). Using the classification scheme of Pitt (1979), the predominant fungal colonist of the vermiculite-V-8 inoculum was identified as *P. janthinellum.*

Conidial suspensions of *P. janthinellum* for introduction into wheat bran medium (Lewis and Papavizas, 1985) were prepared from 9-day-old colonies growing on V-8 juice agar (200 ml V-8 juice, 800 ml water, 3 g $CaCO_3$, 1 g glucose, 20 g agar, and 6.0 ml 1.0 N NaOH). Wheat bran (100 g) was mixed with water (100 ml) and autoclave for 1 h. Plates of V-8 agar with colonies of *P. janthinellum* were flooded with sterile, demineralized water and 10-ml samples of the suspension were added to sterile, moistened wheat bran to provide 10^7 cfu/100 g bran. The bran cultures were incubated for 4 days at 25C.

In the first experiment, rooted azalea ('Hinodegiri') cuttings (≈16weeks old) were potted into natural (nonsterile) PB amended (1% w/w) with autoclave wheat bran or *P. janthinellum* (10^6 cfu/g bran), or into medium without amendment. Lime and fertilizer were added to PB container medium as described by Ownley and Benson (1990). *Phytophthora cinnamomi* (nine colonized oat kernels per container) was added 4 days after potting. The inoculum was divided among three sites around the periphery of the root ball at a depth of 2.5 to 5 cm. Noninfested controls received autoclave wheat bran or *P. janthinellum.* The plants were watered by hand twice daily and containers were placed in saucers 2 days-week^{-1} to maintain moisture conditions near saturation. The design was a randomized complete block with eight replicates per treatment.

In the second and third experiments, l-year-old azaleas were used. Treatments were as described previously. To eliminate

Received for publication 23 May 1991. Accepted for publication 2 Dec. 1991. This research was supported by the North Carolina Agricultural Research Service (NCARS), North Carolina State Univ., Raleigh, 27695-7643. Use of trade names in this publication does not imply endorsement by the NCARS of the products mentioned nor criticism of similar ones not mentioned. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Former Graduate Research Assistant. Current address: USDA–ARS, Root Disease and Biological Control Research Unit, 367 Johnson Hall, Washington State Univ., Pullman, WA 99164-6430.

[2]Professor.

Abbreviations: PB, pine bark.

Table 1. Effect of *Penicillium janthinellum* on phytophthora root rot of azalea grown in PB container medium.

Treatment	Expt. 1[z] Root[x] rot severity (rating)	Expt. 1[z] Shoot[x] fresh wt (g)	Expt. 2[z,y] Root rot severity (rating)	Expt. 2[z,y] Shoot fresh wt (g)	Expt. 2[z,y] Dead plants[x] (%)	Expt. 3[z,y] Root rot severity (rating)	Expt. 3[z,y] Shoot fresh wt (g)	Expt. 3[z,y] Dead plants (%)
Infested control[w]	2.88 a[v]	11.3 c	4.00 a	18.1 c	54.5	3.82 a	27.9 b	27.3
Infested bran control	2.00 b	13.9 bc	3.82 a	31.1 bc	36.4	3.18 ab	43.6 b	27.3
Infested + *P. janthinellum* in natural PB	1.50 c	15.4 ab	3.45 a	34.5 b	27.3	2.73 b	42.6 b	18.2
Noninfested control + bran	1.00 d	18.3 a	1.18 b	61.4 a	0	1.36 c	69.6 a	9.1
LSD	0.45	3.5	1.07	16.2		1.06	20.4	

[z]Expt. 1 = 134 days, Expt. 2 = 67 days, Expt. 3 = 91 days.
[y]Plants received a 24-h post-inoculation flooding treatment on day 40 of experiment.
[x]Root rot severity, shoot fresh weight, and percent dead plants were determined on the last day of each experiment (days 134, 67, and 91 for Expts. 1, 2, and 3, respectively). No plants died in Expt. 1.
[w]Expt. 1 = natural PB, Expts. 2 and 3 = autoclaved PB.
[v]Means separation (in columns) by F-protected LSD test, P = 0.05.

Table 2. Effect of type of amendment on the population density of *total fungi*, *Pencillium janthinellum*, and percent P. *janthinellum* as a proportion of the total fungal population density in a PB medium at the conclusion of Expt. 1.

Treatment	Fungal population: Total[z] (count)	*P. janthinellum*[z] (count)	*P. janthinellum*[y,x] (%)
Infested control	5.34 b[w]	4.55 b	18.2 c
Infested bran control	5.78 a	5.30 a	33.8 b
Infested + *P. janthinellum* in natural PB	5.39 b	5.07 a	49.3 a
Noninfested control + bran	5.70 a	5.19 a	33.8 b
LSD	0.18	0.25	13.4

[z]log(cfu + 1) per gram of dry PB.
[y]Percent of *P. janthinellum* as a proportion of total fungi.
[x]Data were transformed to (arcsin) $\sqrt{\text{percentage}}$ before analysis (Steel and Torrie, 1980).
[w]Means separation (in columns) by F-protected LSD test, $P = 0.05$.

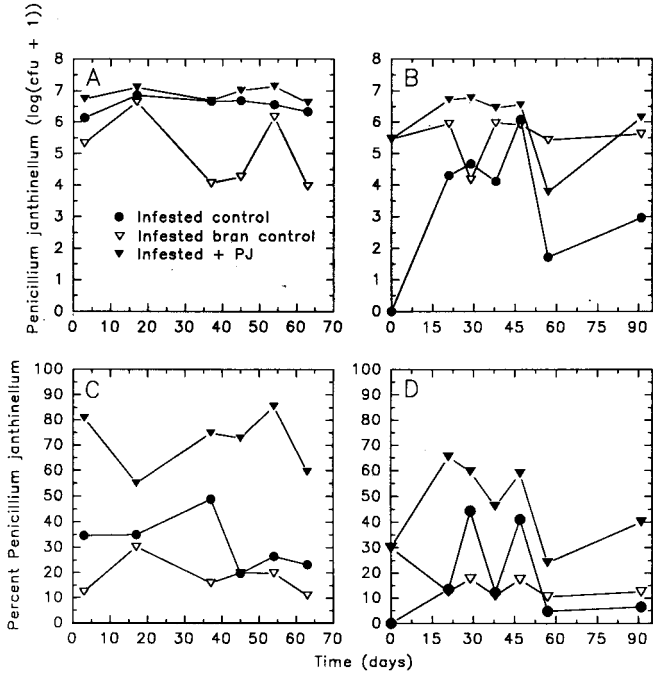

Fig. 1. Effect of amendments on the population density of *Penicillium janthinellum* **(A, B)** and on the percent *P. janthinellum* as a proportion of the total fungal population density **(C, D).** Azaleas were potted in sterile PB infested with *Phytophthora cinnamomi* and received no amendment (Infested control), or plants were potted in natural (nonsterile) PB infested in *P. cinnamomi* and amended with 1% w/w sterile bran (Infested bran control), or 1% w/w bran colonized by *P. janthinellum* (Infested + *PJ).* Expt. 2 **(A, C).** Expt. 3 **(B, D).**

natural populations of *P. janthinellum,* sterile PB (autoclaved 1 h, on two successive days) was used in the infested control. At day 40 of the experiment, plants were placed, by treatment, in plastic-lined wooden boxes(18.5 × 46 × 102 cm); water was added until 2 to 4 cm was ponded on the surface of the container medium for 24 h. The design was a randomized complete block with 11 replicates per treatment.

After all plants with symptoms of severe root rot had died, shoot fresh weight and root rot severity were determined. Root samples were collected from each plant, as described by Ownley and Benson (1990); the severity of root rot was evaluated on a visual assessment scale, with 1 = healthy, 2 = necrosis and discoloration of feeder roots, 3 = necrosis of larger roots, 4 = stem necrosis, and 5 = dead plant, and infection of roots by *P. cinnamomi* was confirmed. Data were analyzed for significance by the general linear models procedure and the F-protected least significant difference test *(P* = 0.05) (SAS Institute, 1985).

The population densities of all fungi present and of *P. janthinellum* were determined at the end of the first experiment, and at least six times throughout the course of the second and third experiments. A 1.5-cm-diameter soil-sampling tube was used to take PB container medium samples to a depth of 11 cm (total volume = 50 to 60 cm^3 of medium). A 10-g subsample was removed and stirred for 2 min in 99 ml sterile, demineralized water. Serial dilutions were prepared with l-ml samples transferred to duplicate plates of peptone–dextrose-rose bengal agar (Johnson and Curl, 1972). Plates were incubated at 25C for 4 to 5 days, and developing colonies were counted. Add tional subsamples (5 to 10 g) were oven-dried (105C, 24 h) to determine percent moisture, to express population density as

colony-forming units per gram of dry PB. Three to seven replicates per treatment were used to determine fungal population densities.

Inhibition of colony growth of *P. cinnamomi* by *P. janthinellum* was determined with paired cultures on cornmeal agar (Difco), Czapek's yeast extract agar (Pitt, 1979), and malt extract agar (Pitt, 1979), adjusted to pH 4.0, 5.0, or 6.0 with 1.0 N NaOH or concentrated HCl. Plates were seeded with 4-day-old, 5.0-mm-diameter mycelial plugs, grown on the corresponding medium, of *P. janthinellum* and *P. cinnamomi* or *P. cinnamomi* alone. After incubation for 4 days at 25C, the colony diameters of *P. cinnamomi* were measured. The experiment was a 3 × 3 factorial in a completely randomized design with 10 replicates per treatment.

Results and Discussion

Wheat bran inoculum of *P. janthinellum*, added to natural PB, suppressed phytophthora root rot of azalea in greenhouse studies. Disease suppression was demonstrated by lower ratings for root rot severity, higher shoot fresh weights, and reduced plant mortality compared with the infested control (Table 1). Disease suppression also was observed when plants were subjected to 24 h of post-inoculation flooding, conditions highly suitable for development of phytophthora root rot (Kenerley et al., 1984). In Expt. 1 (Table 1), plants in natural PB infested with *P. cinnamomi* and amended with *P. janthinellum* had less *(P* = 0.05) root rot than either the infested control or the infested bran control. The shoot fresh weight of plants in infested PB treated with *P. janthinellum* was higher *(P* = 0.05) than the infested control. In Expt. 2 (Table 1), root rot severity was similar for plants in natural PB infested with *P. cinnamomi* and amended with *P. janthinellum* as for both infested controls, but the shoot fresh weight of plants in infested, natural PB amended with *P. janthinellum* was higher *(P = 0.05)* and plant mortality was reduced by 50% compared with the infested control. In Expt. 3 (Table 1), there were no differences in shoot fresh weight for plants in natural PB amended with *P. janthinellum* compared to both infested controls, but root rot severity was less *(P* = 0.05) for plants in infested, natural PB amended with *P. janthinellum* and plant mortality was 30% less than in the infested control. In the absence of *P. cinnamomi,* there was no evidence of either phytotoxicity or growth promotion of azalea in PB amended with *P. janthinellum.* There were no differences in shoot fresh weight or root rot severity for plants in noninfected PB amended with *P. janthinellum* compared with plants in noninfested PB amended with bran (data not shown).

Bran also reduced root rot severity, but this amendment was less effective than *P. janthinellum.* At the conclusion of Expt. 1, the total population density of fungi was higher *(P* = 0.05) in infested and noninfested PB amended with bran than in the infested control or in PB + infestation and *P. janthinellum* (Table 2). An increase in microbial activity increases fungistasis, resulting in a degree of general suppression (Cook and Baker, 1983).

In all experiments, *P. janthinellum* was found in the infested control (Table 2, Fig. 1). In Expt. 1, natural populations were the source of *P. janthinellum.* In Expts. 2 and 3, because autoclave PB was used in the infested control treatment, air-borne conidia from the surface of PB container medium that had been amended with *P. janthinellum* probably were the source of the fungus.

There were no differences in the population densities of *P. janthinellum* in either infested or noninfested PB amended with bran, compared with the population in infested PB amended with *P. janthinellum* (Table 2, Fig. 1 A and B). The density of *P. janthinellum* as a proportion of the total fungal population was, however, higher in PB amended with *P. janthinellum* than in either noninfested or infested PB amended with bran (Table 2, Fig. 1 C and D). In all experiments, *P. janthinellum* made up a higher proportion of the fungal population in PB amended with *P. janthinellum* than in the infested control. The observed relationship between higher percentages of *P. janthinellum* in the total population of fungi and reduced root rot severity suggests that *P. janthinellum* may need to predominate the PB mycoflora for suppression of phytophthora root rot to occur. Addition of bran alone was apparently insufficient to increase the relative population density of naturally occurring *P. janthinellum* to levels that effected specific suppression.

Hyphal growth of *P. cinnamomi* was inhibited by *P. janthinellum* in vitro. Inhibition was more effective at the higher culture medium pH values and most pronounced on malt extract agar (data not shown). After several days in paired culture, colonies of *P. cinnamomi* failed to grow, but colonies of *P. janthinellum* advanced and completely colonized the mycelium of *P. cinnamomi.* Inhibition in vitro by *P. janthinellum* against various plant pathogens has been reported (Domsch et al., 1980; Sztejnberg and Tsao, 1986). *Penicillium janthinellum* produces the antibiotic griseofulvin (Grove, 1973) and the fungitoxic metabolize janthinellin (Poltorak and Silaev, 1964).

In summary, these results indicate that *P. janthinellum,* a natural colonist of PB container medium, shows potential as a biological control of phytophthora root rot. Investigations on the interactions of *P. janthinellum* with other microorganisms indigenous to PB and an understanding of the mechanism of disease suppression are needed for effective use of this organism.

Literature Cited

Cook, R.J. and K.F. Baker. 1983. The nature and practice of biological control of plant pathogens. Amer. Phytopathol. Soc., St. Paul, Minn.

Domsch, K. H., W. Gains, and T.-H. Anderson. 1980. Compendium of soil fungi. vol. I. Academic, New York.

Eckert, J.W. and P.H. Tsao. 1962. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. Phytopathology 52:771-777.

Gerretson-Comell, L. and F.R. Humphreys. 1978. Results of an experiment on the effects of *Pinus radiata* bark on the formation of sporangia in *Phytophthora cinnamomi* Rands. Phyton 36:15–17.

Gerrettson-Cornell, L., F.R. Humphreys, and S.R. Townsend. 1976. Results of a preliminary investigation on the use of *Pinus radiata* bark against *Phytophthora cinnamomi* Rands. Phyton 34:3-6.

Grove, J.F. 1973. Grisans, p. 363-365. In: A.I. Laskin and H.A. Lechevalier, (eds.). Handbook of microbiology. vol. III. Microbial products. CRC, Cleveland, Ohio.

Hoitink, H.A.J., D.M. Vandoren, Jr., and A.F. Schmitthenner. 1977. Suppression of *Phytophthora cinnamomi* in a composted hardwood bark potting medium. Phytopathology 67:561-565.

Johnson, L. and E.A. Curl. 1972. Methods for research on the ecology of soil-borne plant pathogens. Burgess, Minneapolis.

Kenerley, C. M., K. Papke, and R.I. Bruck. 1984. Effect of flooding on development of phytophthora root rot in Fraser fir seedlings. Phytopathology 74:401-404.

Lambe, R. C., R.K. Jones, W.H. Wills, and D.M. Benson. 1982. Azalea diseases, p. 30-37. In: R.K. Jones and R.C. Lambe (eds.). Diseases of woody ornamental plants. North Carolina Agr. Ext. Sew., Raleigh.

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

Ownley, B.H. and D.M. Benson. 1990. Physical properties of pine bark media and relation to severity of phytophthora root rot of rhododendron. J. Amer. Soc. Hort. Sci. 115:564-570.
Pitt, J.I. 1979. The genus penicillium. Academic, New York.
Poltorak, V.A. and A.B. Silaev. 1964. Chemical and physio-chemical properties of janthinellin. Antibiotiki 9:25-27.
SAS Institute, Inc. 1985. SAS user's guide: Statistics. 1985 ed. SAS Institute Inc., Cary, N.C.
Shew, H.D. and D.M. Benson. 1982. Qualitative and quantitative soil assays for *Phytophthora cinnamomi.* Phytopathology 72:1029-1032.
Spencer, S, and D.M. Benson. 1981. Root rot of *Acuba japonica* caused by *Phytophthora cinnamomi* and *P. citricola* and suppressed with bark media. Plant Dis. 65:918–921.
Spencer, S. and D.M. Benson. 1982. Pine bark, hardwood bark compost, and peat amendment effects on development of *Phytophthora* spp. and lupine root rot. Phytopathology 72:346-351.
Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics. McGraw-Hill, New York.
Sztejnberg, A. and P.H. Tsao. 1986. Evaluation of certain hyphomycetes as biocontrol agents for azalea root rot caused by *Phytophthora cinnamomi.* Phytopathology 76:847. (Abstr.)