RESEARCH REPORT 10-10

THE USE OF PROLINE® FOR THE CONTROL OF *PYTHIUM* AND *BOTRYTIS* SPP.

by
Paul Jackson and Scott Enebak

INTRODUCTION

Long-term cold storage (> 1 week) from October to mid-December has been shown to decrease bareroot seedling survival compared to when seedlings are lifted and stored in January (Kahler and Gilmore 1961; Dierauf 1976; Hebb 1982; Venator 1984). During lifting, bareroot seedling roots are wounded (May 1984), which could serve as infection sites for soil-borne fungi.

Pythium is a common pathogen found in nursery soils and is associated with "damping-off disease" before and after germination and "fine feeder root disease" several weeks after germination (Kelley and Oak 1989). *Pythium* spores can move throughout the soil profile and nursery bed after rainfall or irrigation (Kelley and Oak 1989). Factors that can contribute to increased *Pythium* activity include altered soil drainage caused by the use of machinery (Dumroese and James 2005), high soil pH, and the use of contaminated equipment (Kelley and Oak 1989).

In an effort to control soil-borne *Pythium*, nursery soils are routinely treated every 2 to 3 years with fumigants such as methyl bromide, chloropicrin, metam sodium, or dazomet (Fraedrich and Dwinell 2003; Cram et al. 2007). Occasionally, these fumigants do not completely sterilize nursery beds, and patches of soil containing *Pythium* remain (Duniway 2002). In addition to soil fumigation, fungicides are often applied to control *Pythium*. Boyer and South (1984) surveyed 63 nursery managers and found that Fermate®, Captan®, and Benlate® were the most popular fungicides for controlling damping-off. Today, some of the most common fungicides used include Captan®, Subdue®, and Aliette®. These fungicides are usually applied to seedlings within weeks of germination but not at all when lifted. If *Pythium* is being transferred from nursery soils and into cold storage on infected seedling roots, once seedlings are placed in storage, the moist, cool (1-5°C) conditions may provide an environment conducive for *Pythium* growth that results in seedling mortality after outplanting.

In addition to *Pythium* spp, another nursery pathogen, *Botrytis cinerea*, the causal agent of gray mold, can develop in the nursery and continue disease progression in cold storage (Srago and McCain 1989). Unlike *Pythium*, *B. cinerea* is a foliage pathogen that infects seedlings in the nursery via airborne spores (Srago and Mccain 1989), yet both pathogens require moist conditions to grow. Perhaps a fungicide treatment prior to lifting would reduce outplanting mortality caused by these two pathogens.

Although not yet registered for use in forest seedling nurseries, the fungicide Proline® (41% prothioconazole-Bayer CropScience) is currently labeled for the control of ascomycetes, basidiomycetes, and deuteromycetes diseases on food crops such as peanuts, barley, wheat, sugar beets, beans, soybeans, and rapeseed. In field, laboratory, and greenhouse studies it has shown promise for controlling fusiform rust (*Cronartium quercuum f.sp. fusiforme)*, pitch canker (*Fusarium circinatum*), and *Rhizoctonia* foliar blight (*Rhizoctonia solani*) (Starkey and Enebak, in press). Since Proline® has been effective in controlling three major nursery diseases, it might be useful to prevent seedling diseases in cold storage caused by *Pythium* and *Botrytis* spp. To determine the effect that Proline® had on *Pythium* growth, the null hypothesis that Proline®-amended agar has no effect on the survival of *Pythium dimorphum*, *Pythium irregulare*, and *Botrytis cinerea* was tested.

METHODOLOGY

Pythium dimorphum and *P. irregulare* were obtained from American Type Culture Collection (ATCC®, Manassas, VA) and aseptically transferred to oatmeal agar (Kim et al. 2005). From the advancing margin of the mycelium, three 0.5 cm disks of *P. dimorphum* and *P. irregulare* were transferred to oatmeal agar for use later in the study. *Botrytis cinerea* was obtained from loblolly pine (*Pinus taeda*) seedlings infected in cold storage. Infected needles were surface sterilized in a 10% bleach/water solution and rinsed two times in sterile, distilled water. After sterilization, five 2.5-cm needle segments were randomly chosen and placed onto PDA. From the advancing margin of the mycelium, three 0.5 cm disks of *B. cinerea* were transferred to potato dextrose agar (PDA) (Difco®) for use later in the study.

Proline® fungicide was added to PDA after autoclaving but before pouring into plates. Three rates of the fungicide were used: 1.25 fl oz/ac (325 ppm; 0.25x label rate), 2.5 fl oz/ac (650 ppm; 0.5x), 5 fl oz/ac (1,300 ppm; 1x), and a control (no fungicide). There were 13 plates (replications) of each treatment per fungus species. Using a #4 cork borer, an 8 mm plug of either *P. dimorphum*, *P. irregulare*, or *B. cinerea* were placed at the center of an agar plate. The plates were wrapped in parafilm, incubated on a laboratory bench at 21°C, and the fungal growth measured over a 10-day period. An agar plate was considered covered when mycelia reached 80 mm.

To determine if the treatments were fungicidal (killed the fungus) or fungistatic (stopped fungal growth), on day 11, the fungal plugs from the amended and control plates were transferred to non-amended agar plates. Radial growth was measured again for 9 days. Statistical analyses were conducted using a General Linear Model (GLM) in SAS statistical software (9th ed., SAS Institute, Cary, NC). Means of radial fungal growth were analyzed using Analysis of Variance (ANOVA) in a factorial design.

RESULTS

The effect of the Proline® on control of the fungi was dependent upon the species. By day 8, *P. dimorphum* had completely covered (80 mm) the non-amended PDA plates (Figure 1). However, after 10 days, *Pythium dimorphum* mycelia (growth) were not observed on any of the amended PDA plates (Figure 1). In contrast, *P. irregulare* was not affected by Proline® and was similar to the control plates growing 80 mm by day 4 (Figure 2). Although, *B. cinerea* growth was minimal (< 6 mm) on amended agar (Figure 3), the Proline® resulted in *B. cinerea* to grow aerially instead of along the media surface (Figure 4).

On day 11, the agar plugs with mycelia were removed from the amended agar plates and placed onto non-amended PDA. After 9 days, there was no mycelia growth of *P. dimorphum*, indicating 100% control with Proline® (Figure 5). In contrast, mycelia growth of *B. cinerea* was > 45 mm on non-amended media (Figure 6). While growth was less than the control, it appears that Proline® is fungistatic to *B. cinerea*. *Pythium irregulare* was not affected by any of the fungicide treatments and were not included in the re-isolation trial.

DISCUSSION

Proline® was variable in its ability to control *Pythium* and *Botrytis* spp. when amended into agar media in the laboratory. *Pythium irregulare* was not affected by any rate of Proline®, while *P. dimorphum* was controlled at all rates. Trials using Proline® on seedlings in storage are needed to determine if *P. dimorphum* could be controlled under normal operating conditions. Likewise, due to the fungistatic nature with *B. cinerea,* Proline® applications would only slow fungal growth and could allow the possibility of carry-over into the field.

Other studies conducted by the Nursery Cooperative have reported that Proline® controlled *Cronartium quercuum f.sp. fusiforme*, *Fusarium circinatum* and *Rhizoctonia solani* and reduced seedling disease in the field (Starkey and Enebak, in press). It appears the fungicidal nature of Proline® may be species specific within the oomycetes (*Pythium* spp.) as seen with control differences between *P. dimorphum* and *P. irregulare*. According to the label, Proline® does not list activity against any fungi in the fungal class, oomycetes, so these test results with *P. dimorphum* were unexpected. Proline® has not been tested on pine seedlings for control of *B. cinerea*. Captan® controlled the fungus on lodgepole pine (*Pinus contorta*), while benomyl did not (James and Woo 1984). Captan® is still used in nurseries and may be useful in preventing *B. cinerea* prior to lifting. The use of fungicides to control *Pythium* damping-off after seedling germination is common, but the efficacy of fungicides remains unknown for controlling the dynamic levels of *Pythium* in the soil during the lifting season.

MANAGEMENT IMPLICATIONS

The fungicide Proline® was effective in controlling *Pythium dimorphum* but not *Pythium irregulare* or *Botrytis cinerea* when grown on Proline® -amended agar media. *Pythium dimorphum* (Sun 1996) and *B. cinerea* (Srago and McCain 1989) can kill seedlings in storage, and recently, *P. irregulare* was also identified as a pine seedling storage pathogen (Jackson 2010). Due to inconsistent control between the three fungal species tested, applying Proline®

over-the-top of seedlings prior to lifting to prevent fungal infection in cold storage would not be recommended.

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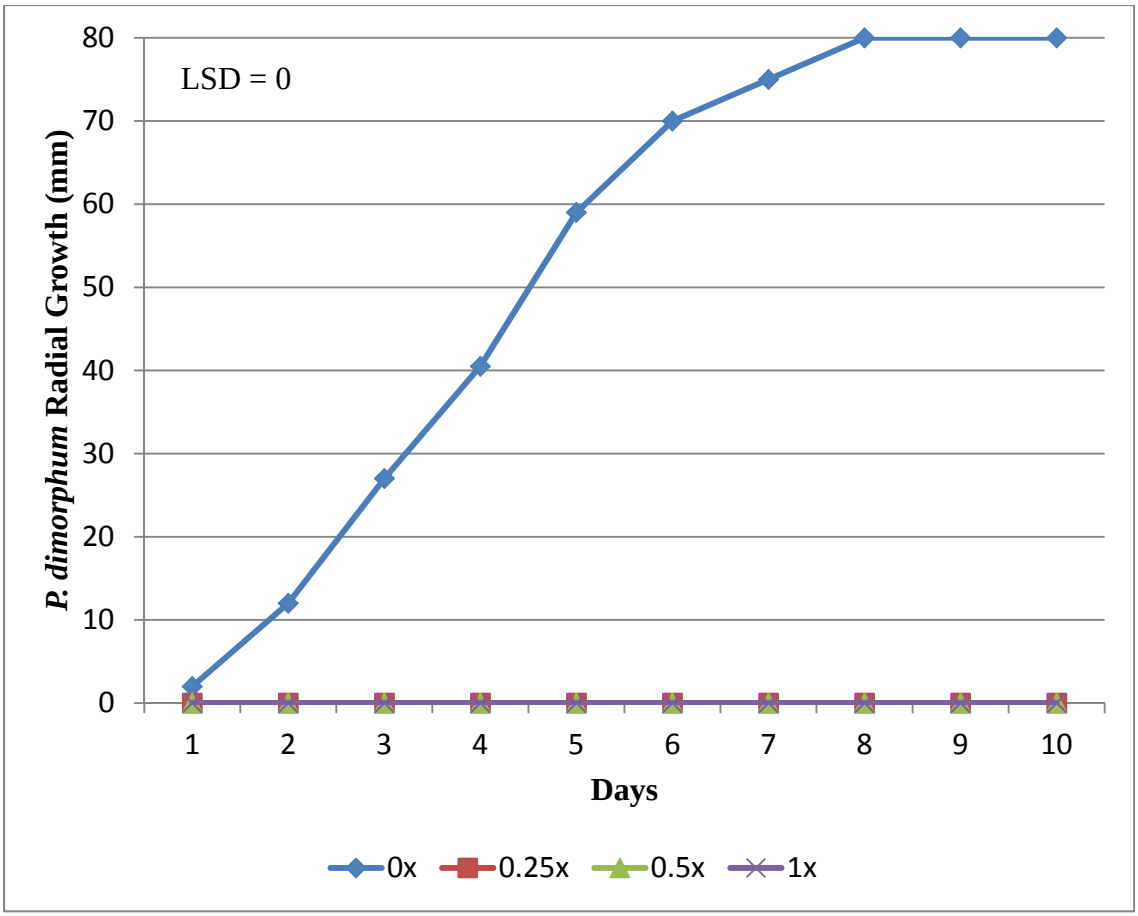

Figure 1. Radial growth of *Pythium dimorphum* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

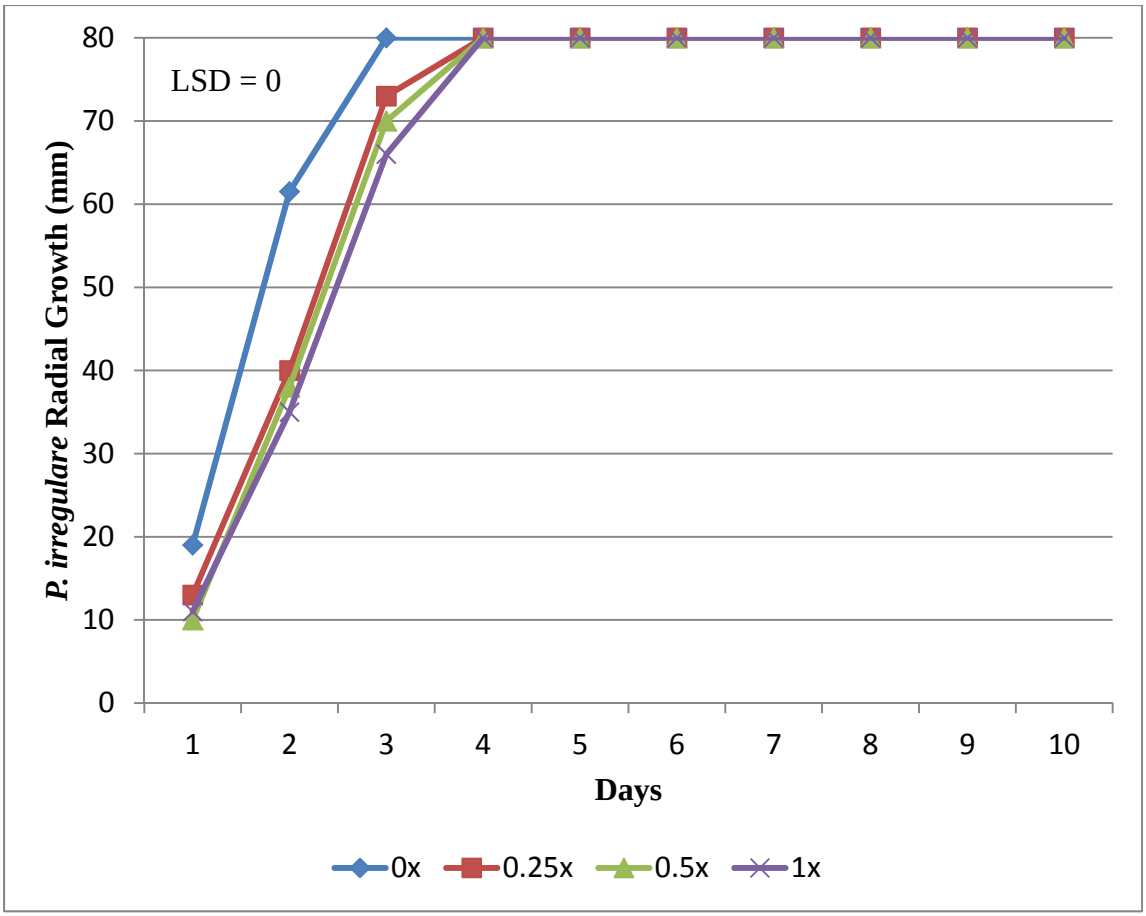

Figure 2. Radial growth of *Pythium irregulare* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

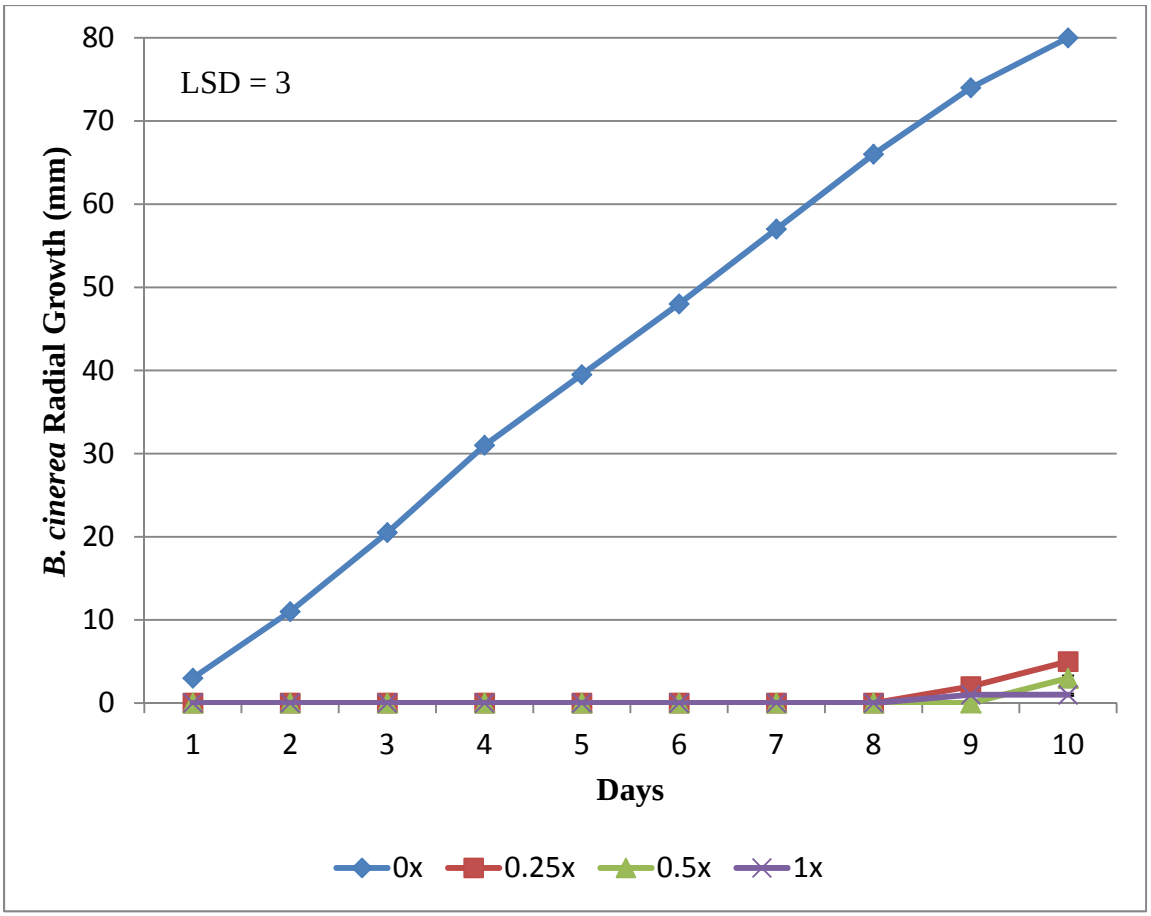

Figure 3. Radial growth of *Botrytis cinerea* on Proline®-amended agar. The least significance difference (LSD) value corresponds with radial growth on day 10.

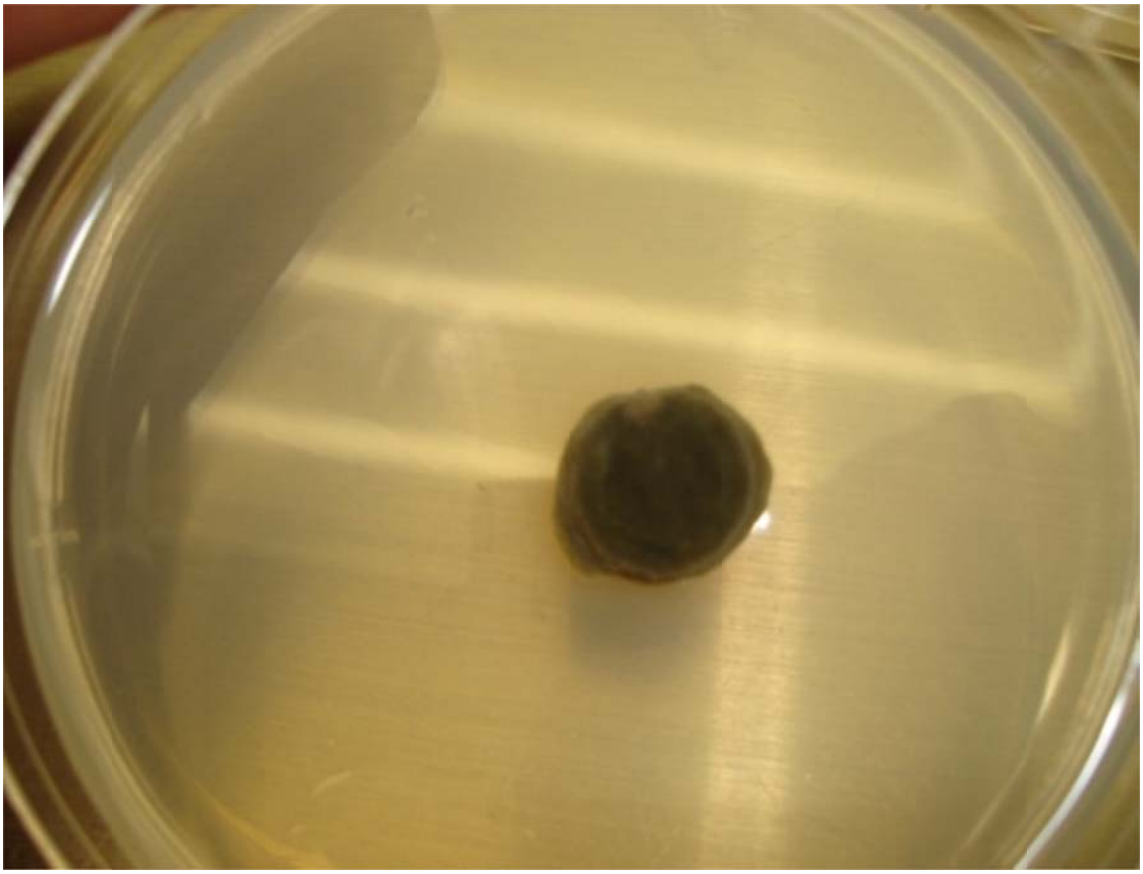

Figure 4. Aerial growth of *Botrytis cinerea*.

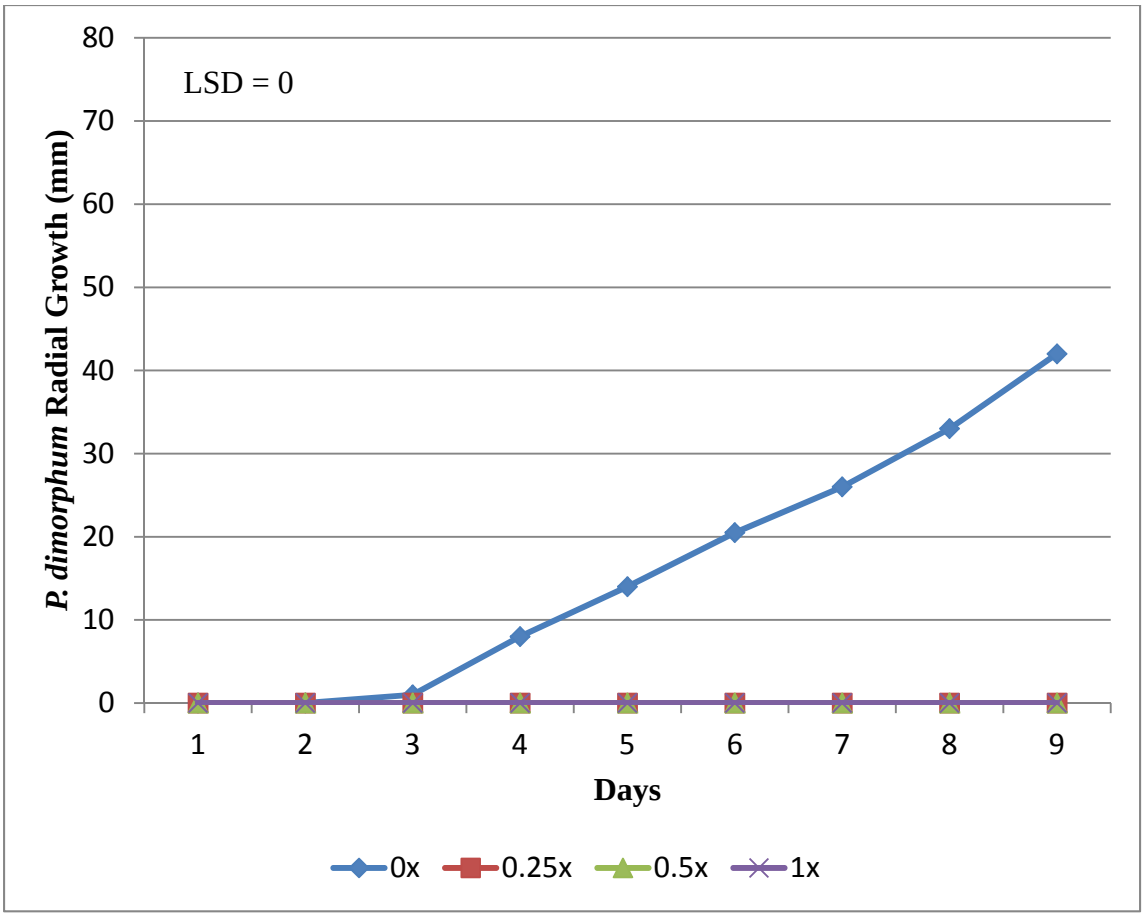

Figure 5. Radial growth of *Pythium dimorphum* that was re-isolated on non-amended potato dextrose agar (PDA). The least significance difference (LSD) value corresponds with radial growth on day 9.

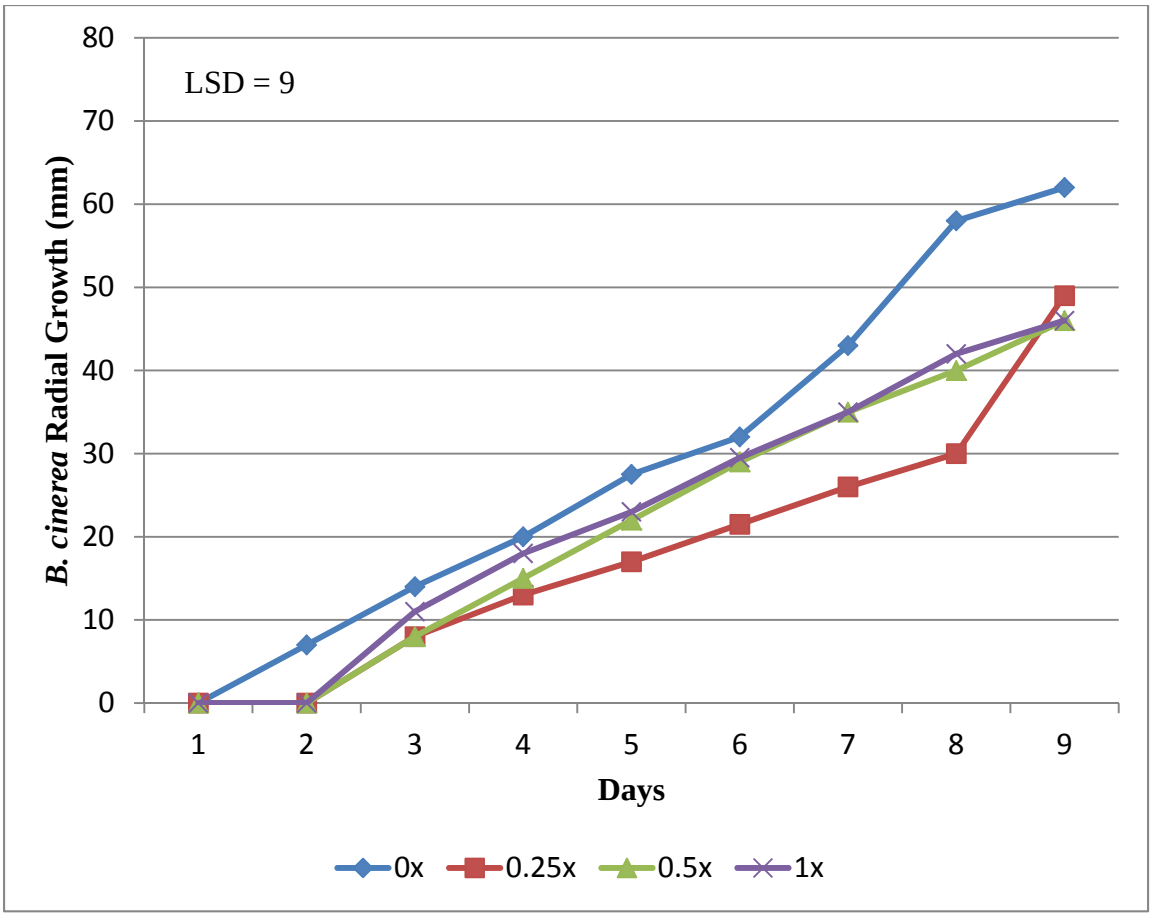

Figure 6. Radial growth of *Botrytis cinerea* that was re-isolated on non-amended potato dextrose agar (PDA). The least significance difference (LSD) value corresponds with radial growth on day 9.