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Responses of non-primed or primed seeds of 'Marketmore 76' cucumber (*Cucumis sativus* L.) slurry coated with *Trichoderma* species to planting in growth media infested with *Pythium aphanidermatum*

W.G. Pill *, C.M. Collins, B. Goldberger, N. Gregory

Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19716-2170, USA

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A B S T R A C T

Aqueous slurries of commercial preparations of *Trichoderma harzianum* Rifai strain KRL-AG2 G41 (*Th*), *T. virens* Strain G-41 (*Tv*), or their combination (*ThTv*, at half rates each of the single application rate) were applied to 'Marktetmore 76' cucumber seeds (*Cucumis sativus* L.) that were non-primed or primed for 3 days at 25 °C either osmotically (−2.5 MPa from 0.337 molal $Ca(NO_3)_2$) or osmomatrically (−1.0 MPa from 0.135 molal $Ca(NO_3)_2$ plus −1.5 MPa from 50% water in exfoliated grade 5 vermiculite). Slurries were applied to seeds (1 mg per seed) either before or after priming. Seeds were sown in soilless, peat-based media with or without inoculation with *Pythium aphanidermatum* (*Pa*). Protection against damping-off caused by high pressures of *Pa* (16% emergence in non-coated, non-primed seeds) was increased by slurry coating *Th* on non-primed (76.4% emergence) or on osmotically primed seeds, with coating either before or after priming having no effect on efficacy (average 62.6% emergence). Slurry coating *Th* on osmomatrically primed seeds failed to increase final emergence percentage (FEP). Colony forming units per three seeds (CFU, all 10^3) was 2.8 for non-primed seeds, and 3.2 and 2.6, respectively, when osmotically and osmomatrically primed seeds were coated after priming. In a second study with lower disease pressure (58.1 FEP from non-coated, non-primed seeds), slurry coating of non-primed or osmotically primed seeds with *Th*, *Tv* or *ThTv* reduced percentage damping-off and increased FEP. The combination coating eliminated damping-off only in non-primed seeds, and tended to reduce percentage damping-off in osmotically or osmomatrically primed seeds compared to coating with *Th* or *Tv* alone. In a third study using only non-primed seeds, slurry coatings with mefenoxam fungicide, *Th*, *Tv*, or *ThTv* decreased total damping-off to 2.6%, 7.4%, 2.0%, and 0%, respectively, from the 30.1% occurring in non-coated seeds. *Th*, *Tv* or *ThTv* applied to growth media at the same rate as the seed coating (1 mg per seed) were generally as effective as the seed coatings, and only the *ThTv* growth medium application eliminated damping-off. A fourth experiment revealed that *Th*, *Tv* or *ThTv* remained viable on non-primed seeds for up to 4 weeks (the longest storage duration) at 21 or 4 °C, but 21 °C storage resulted in faster seed germination by week 3 and higher CFU per three seeds by week 4. In summary, coating of non-primed seeds with *Th*, *Tv* or *ThTv* was more effective than coating primed seeds in reducing percentage damping-off. While priming treatments generally led to faster seedling emergence and greater seedling shoot fresh weight than was achieved with non-primed seeds, only for non-primed seeds was damping-off eliminated by the *ThTv* seed coating or growth medium application.

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1. Introduction

Several soil-borne pathogenic fungi including various species of *Pythium*, *Phytophthora*, *Fusarium*, *Aphanomyces*, and *Rhizoctonia* can cause pre- or post-emergence damping-off (Agrios, 1997). Damping-off is a common problem in many field and greenhouse crops, with little natural plant resistance to infection. Biological control (biocontrol) provides a non-chemical means to control damping-off organisms that can reduce or eliminate the use of synthetic fungicides, and their use frequently is permitted within organic farming certification.

Actinomycetes, bacteria and fungi have been used as both seed treatments and seed bed drenches to control damping-off (Fravel et al., 1998). While the Actinomycete *Streptomyces griseoviridis* and numerous strains of bacterial species including *Bacillus subtilis*, *Enterobacter cloacae* and *Pseudomonas* are available as commercial preparations, *Trichoderma*, one of several biocontrol fungal species, probably has received the most attention as a seed treatment

* Corresponding author.
E-mail address: wgpill@udel.edu (W.G. Pill).

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(Taylor et al., 1994; McQuilken et al., 1998). Seed treatment is more economical than drenching due to the smaller volume of inoculum needed (Bennett et al., 1992).

Trichoderma, a mycoparasite that both attacks other fungi and stimulates growth of the host plant, is the most common saprophytic fungus in the rhizosphere. Modes of action of this fungus include mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance, and inactivation of the pathogen's enzymes (Scala et al., 2007). Yedida et al. (1999) found that *Trichoderma harzianum* (*Th*) entered primarily the epidermis and outer cortex of cucumber roots where strengthening of cell walls (increased callose and cellulose) occurred. They also noted increased activities of peroxidase and chitinase in leaves and roots providing evidence that *Th* may induce systemic resistance mechanisms. Many fungal biocontrol agents, including *Trichoderma*, are applied to seed as conidia or resting spores which must become active before interaction with the pathogen. The active fungal agent must persist in the spermoplane or spermosphere in sufficient quantity to protect the germinating seed (see reviews by Harman, 2006; Neumann and Laing, 2006).

The slurry technique is a common method for applying bioprotectants to seeds (Taylor and Harman, 1990), and consists of mixing the bioprotectant with an aqueous binder and applying this mixture to seeds. Applying a *Th* suspension to cucumber seeds (without a binder) failed to provide protection against *Pythium ultimum* (Taylor et al., 1991). Various binders have been employed that have increased the ability of *Trichoderma* to protect against damping-off organisms. Included among these are gels such as hydroxyethyl cellulose (Hadar et al., 1984) and Pelgel (Nitragen; Inbar et al., 1996), an industrial film coating (vinyl acetate sticker; Cliquet and Scheffer, 1996), gelatin (Roberts et al., 2005), and ground oil palm mesocarp (Kanjanamaneesathian et al., 2003). These binder or coating materials may provide a food base for the *Trichoderma*. Nelson et al. (1988) found that polysaccharides and polyhydroxyl alcohols promoted *Th* growth.

Stasz and Harman (1980) reported that *Pythium* species can infect seeds in less than 4 h after sowing, while *Trichoderma* spores can require about 12 h to germinate (Taylor et al., 1991). For *Trichoderma* to be effective, it must be active before attack by the pathogen. Taylor et al. (1991) found that when cucumber seeds were double coated, first with a *Th* slurry then a solid particulate (muck soil or Agro-Lig) plus binder (Pelgel or Polyox N-10), the resultant thin (<0.1 mm), uninterrupted layer ("film-coated") over the seed surface was sufficient to slow infection by *Pythium* by about 6 h, which substantially improved biocontrol over that achieved with only the inner layer.

In addition to improving biocontrol with *Trichoderma* by delaying attack by the pathogen with a physical barrier (Taylor et al., 1991), seed priming is another technique to promote *Trichoderma* colonization of the seed coat before planting. During priming, seeds are exposed to an environment of low water potential created osmotically or matrically (see reviews by Parera and Cantliffe, 1994; Pill, 1994; Welbaum et al., 1997), or by addition of a limited predetermined amount of water during drum priming (Bennett and Warren, 1997; Bennett and Whipps, 2008a,b). Primed seeds are held at the plateau phase of water concentration (phase II) following a rapid increase in water uptake (phase I of imbibition). During this plateau phase, pregerminative biochemical, physiological and anatomical activities occur that benefit subsequent germination, particularly under stressful seedbed conditions. Ideally, biological control agents added to seeds before or during priming ("biopriming") would proliferate to provide greater protection against soil-borne pathogens than adding these agents after seed priming or to non-primed seed.

Harman and Taylor (1988) applied *Th* to cucumber seeds as an aqueous slurry then added the solid matrix and water and incubated this mixture for 4 days at 20 °C (solid matrix priming). The number of *Trichoderma* propagules increased 10-fold during priming to 10^3–10^4 colony forming units (CFUs) per seed, levels that were sustained after drying and a few days of storage. Following priming with a solid matrix of lignaceous shale (Agro-Lig), these seeds gave 96% emergence (compared to 64% with the aqueous slurry alone) in a *P. ultimum*-infested soil. Including *Th* during priming rather than Thiram fungicide gave greater seedling emergence. Solid matrices of bitumous coal or sphagnum peat resulted in fewer healthier plants, indicating the importance of choice of solid matrix by affecting pH or nutrient supply to the fungus. These workers found that priming using a liquid priming system with polyethylene glycol (all other conditions the same as in solid matrix priming) resulted in no *Th* recovery and poorer performance in *P. ultimum*-infested soil than was achieved with solid matrix priming. Wright et al. (2003a) noted during drum priming that *Th* and *Tv* survived but did not increase on the surface of carrot (*Daucus carota* L.) and parsnip (*Pastinaca sativa* L.) seed, and decreased on leek (*Allium porrum* L.) seed. They further noted that when applied as a post-priming treatment in a large priming system, recovery was greater than achieved when applied during priming. However, successful dual application of beneficial microorganisms on onion or carrot during drum priming was demonstrated recently (Bennett and Whipps, 2008a,b). We know of no reports of biopriming osmotically using inorganic salts.

The primary objective of this study was to compare the efficacy of two commercially available *Trichoderma* species applied to cucumber seeds as a slurry coating before or after osmotic or osmomatric priming on seedling emergence and growth in a *Pythium aphanidermatum*-infested growth medium. A further objective was to examine the effects of storage (up to 4 weeks) of *Trichoderma*-coated non-primed seeds at 4 or 21 °C on seedling germination and on *Trichoderma* viability.

2. Materials and methods

Seeds of 'Marketmore 76' cucumber (*Cucumis sativus* L.) were soaked in 0.625% (v/v) NaOCl for 2 min (50 g seeds per 300 ml with two drops of Tween 20 surfactant) then rinsed three times with sterile distilled water.

2.1. Experiment 1: seed priming and T. harzianum

Initial work showed that when seeds were imbibed in water at 25 °C, germination started by 12 h and ended with 100% germination by 20 h. Seed moisture at germination was 41.7–43.3%, oven dry weight (130 °C for 2 h). Further initial studies revealed that a minimum solute potential (ψ_s) of −2.5 MPa at 25 °C was required to prevent seed germination for at least 3 days during seed priming. Osmotic priming of seeds was achieved using 0.337 molal $Ca(NO_3)_2$ (−2.5 MPa at 25 °C according to the Van't Hoff equation, $\psi_s = imRT$, in which i = the dissociation constant, m = molality, R = universal gas constant, and T = °K). Fifty seeds for each of four replications were placed on two layers of germination paper (Germination Blotter No. 385; Seedburo, Chicago, IL) moistened with 15 ml of the $Ca(NO_3)_2$ solution contained within 125 mm × 80 mm × 20 mm transparent polystyrene boxes. The boxes were placed in darkness in 25 °C incubators for 3 days.

Matric priming using grade 5 exfoliated vermiculite (W.R. Grace, Columbia, MD) can provide a minimum of −1.5 MPa (50% water of the vermiculite dry weight) with any accuracy according to the moisture characteristic curve developed by Khan et al. (1992). To reach a water potential of −2.5 MPa, a 0.135 molal