Biological Control Agents for Damping-off Disease in Bedding Plants

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INTRODUCTION

Damping-off diseases are common wherever seedlings are grown. Nurseries experience disease outbreaks despite the use of good hygiene and fungicides, and would welcome an economical, environmentally safe biological control product (Harris, 1995). Some bacteria and binucleate *Rhizoctonia* isolates can control the fungi that most commonly cause damping-off diseases in nursery seedlings: *Pythium* spp. (Broadbent et al., 1971; Harris and Adkins, 1993; Harris et al., 1993b, 1994c), and *Rhizoctonia solani* Kühn (Cardoso and Echandi, 1987; Harris et al., 1994a,b; Howell and Stipanovic, 1979; Kommedahl and Windels, 1978). Plant growth promotion in the absence of known pathogens also has been reported for soil bacteria (e.g. Broadbent et al., 1977; Lifshitz et al., 1987) and binucleate *Rhizoctonia* (Harris et al., 1993b, 1994b).

This paper summarises experiments designed to screen several isolates of soil bacteria and binucleate *Rhizoctonia* for biological control of damping-off diseases and growth promotion of *Capsicum* seedlings in pasteurised potting medium.

MATERIALS AND METHODS

The following methods were common to all experiments. Bacteria and fungi were isolated from samples of potting media collected from plant nurseries around Adelaide, South Australia (Harris et al., 1993b, 1994a; Schisler et al., 1993). Fungal isolates were cultured on sterilised rice hulls or wheat bran, and added to pasteurised potting medium in plastic punnets (seedling trays) (Harris et al., 1993a). *Rhizoctonia solani* anastomosis group 4 (AG 4), isolate D1B1, on organic substrate was mixed with potting medium, and 7 cm^3 of this mix was added to the bottom of each punnet cell. Punnets then were filled with potting medium with or without a binucleate *Rhizoctonia* isolate. *Pythium ultimum* var. *sporangiiferum* Drechsler isolate 2 was mixed throughout the potting medium in punnets.

Seeds were sown near the top of the potting mix in each punnet cell, and covered with 5 to 10 mm (approx. 7 cm^3) of sterilised, washed coarse sand. Bacterial isolates were grown on 1/2- or 1/5-strength tryptic soy agar (Difco) at 25C, suspended in sterile deionised water at different concentrations, then 2.25 ml of suspension was pipetted onto the sand in each punnet cell. Doses for each experiment are indicated below.

Biological control by our microbial antagonists was compared with *Bacillus subtilis* Cohn emend. Prazm. isolate A13, and the fungicides quintozene and propamocarb. *Bacillus subtilis* A13 controls *R. solani* (Broadbent et al., 1971) and is used commercially for biological control of several soil fungi on field crops. Unformulated *B. subtilis* A13 was obtained from P. Barkley (nee Broadbent), NSW Agriculture, Rydalmere, Australia. Quintozene (PCNB) (Terraclor, Uniroyal Aus-

tralia) was suspended in deionised water at 5 g litre^{-1} and propamocarb (Previcur, Schering AG) at 1.78 ml litre^{-1}. An aliquot (2.25 ml) of each suspension was pipetted onto the soil in each punnet cell to give doses of 8.4 mg a.i. quintozene, or 2.4 mg a.i. propamocarb, per punnet cell, which approximate the recommended commercial rates.

Punnets were arranged in randomised complete block designs in either a glasshouse or growth chamber at 25C (±7C) (Harris et al., 1993b). When most seedlings had four true leaves and damping-off had apparently ceased (3 to 4 weeks), the seedlings that had survived or collapsed were counted separately. Seedlings that were still standing were excised at soil level, and the tops were dried at 60C and weighed. Data for each variable were subjected to analysis of variance, and treatment means were compared to the controls by Fisher's protected least significant difference (PLSD).

Experiment I. Effects of Biological Control Organisms on *Capsicum* With or Without *R. solani*. We tested the efficacy of selected micro-organisms at different doses to suppress damping-off, caused by *R. solani* AG 4, in seedlings of *Capsicum annuum* L. 'Green Giant' (capsicum, syn. bell pepper). We also assessed their ability to stimulate shoot growth in the absence of added *R. solani*. For treatments with *R. solani*, the fungus on wheat bran substrate was mixed with potting medium at 0.28% (v/v), then 7 cm^3 of this mix was added to the bottom of each punnet cell. Binucleate *Rhizoctonia* isolates BNR1 or BNR2 on wheat bran substrate were mixed with potting medium at doses of 0.018, 0.035, 0.07, 0.14, or 0.35% (v/v), and punnets were filled with these mixes. Bacterial isolates *B. subtilis* A13, BAC1, BAC2, and BAC3 were suspended at concentrations of $10^{2.5}$, 10^4, $10^{5.5}$, 10^7, and $10^{8.5}$ bacterial cells ml^{-1}. To determine the direct effects of microbial isolates on plant growth, each isolate was also added at the same five doses to the remainder of the punnets without *R. solani*. There were three replicate punnets for each dose of each biological control organism. Quintozene and control treatments each had nine replicate punnets. Means for the five doses of each microbial isolate were subjected to regression analysis. As no dose responses were observed, the means of the 15 punnets for each treatment (across all doses) were calculated and subjected to analysis of variance.

Experiment II. Suppression of *P. ultimum* var. *sporangiiferum* on *Capsicum*. Microbial isolates were screened for ability to suppress damping-off caused by *P. ultimum* var. *sporangiiferum* on *Capsicum*. *Pythium ultimum* var. *sporangiiferum* on rice hulls substrate was mixed with potting medium at 0.7% (v/v). For the treatments BNR1, BNR2, and BNR3, an isolate of binucleate *Rhizoctonia* on rice hull substrate was also mixed with potting medium at 0.7% (v/v). Each of 15 bacterial isolates, including *B. subtilis* A13, BAC2, BAC3 and BAC4, but not BAC1, was suspended at approx. 3×10^8 bacterial cells ml^{-1}, and pipetted onto the potting medium to give 7×10^8 bacteria per punnet cell. There were four replicate punnets for each antagonist and propamocarb treatment, and 16 for each of the two controls with or without *Pythium*. Plants were grown for 20 days at 25C in a controlled-environment growth chamber with a 12-h photoperiod and daytime illuminance of approximately 250 μEm^{-2}s^{-1}.

Experiment III. Suppression of *P. irregulare* on *Capsicum*. We also tested whether selected microbial isolates were effective against a different species of *Pythium*. *Pythium irregulare* on rice hull substrate was mixed with potting medium at 7.4% (v/v). There were 20 replicate punnets for each antagonist treatment, and eight for propamocarb and control treatments with and without *P. ultimum* var. *sporangiiferum*. Plants were grown in a glasshouse for 35days.

RESULTS

In experiment I, in which *R. solani* was added to potting medium at the same time as the putative biological control organisms, two binucleate *Rhizoctonia* isolates increased survival and growth of *Capsicum* seedlings more than any of the four bacterial isolates. *Bacillus subtilis* A13 did not control damping-off ($P < 0.05$) in Experiment I. In Experiment I the two binucleate *Rhizoctonia* treatments had similar seedling survival to the control without *R. solani*, or quintozene.

In Experiment II, in which *P. ultimum* var. *sporangiiferum* was added to potting medium at the same time as the putative biological control organisms, isolates BAC2, BAC3, BNR1, and BNR2 reduced damping-off in seedlings of *Capsicum*. The biological disease suppression was greater than that achieved with propamocarb. *Bacillus subtilis* A13 gave no significant control ($P < 0.05$). BAC4 increased ($P < 0.01$) survival of *Capsicum* seedlings. *Pythium irregulare* was totally controlled by both binucleate *Rhizoctonia* isolates on *Capsicum* ($P < 0.01$). In pasteurised potting medium without added pathogens, the four bacterial isolates increased ($P < 0.05$) shoot dry weights of *Capsicum* seedlings in Experiment I. The two binucleate *Rhizoctonia* isolates increased shoot dry weights of *Capsicum* seedlings.

DISCUSSION

The two isolates of binucleate *Rhizoctonia*, and quintozene, consistently suppressed damping-off caused by *R. solani*. Damping-off caused by *P. ultimum* var. *sporangiiferum* was suppressed consistently by BNR1, BNR2, BAC2, and BAC3 on *Capsicum*. Both binucleate *Rhizoctonia* isolates were effective against *P. irregulare*. Our five microbial treatments suppressed damping-off more than the commercial biological control bacterium, *B. subtilis* A13, and at least as well as standard fungicide drenches. The beneficial microbial isolates have not been identified conclusively yet. To date, beneficial effects have been demonstrated on five plant species that represent the dicotyledonous families, Amaranthaceae, Cruciferae, Solanaceae,and Violaceae (Harris et al., 1994a, b). The microbial isolates are being tested further for efficacy in various potting media and environmental conditions, and for suppression of other soil-borne pathogens on a wider range of plants (Harris and Adkins, 1993). It is possible that different microbial isolates, or combinations of isolates, may be needed for different nursery situations.

All six selected microbial isolates increased shoot growth of seedlings in pasteurised potting medium without added pathogens. The two binucleate *Rhizoctonia* isolates generally gave the largest increases in dry weights, while *B. subtilis* A13 and BAC2 were the least effective. BNR2 increased mean shoot weights per punnet for *Capsicum* seedlings by 33%. Growth promotion would enable seedling growers to reduce production time for seedlings and thereby reduce costs. We are

now using pure cultures of one plant species with one isolate of micro-organism in sterile conditions, to determine whether the plant growth promotion is due to direct stimulation through supply of plant nutrients or hormones, or to an indirect mechanism, such as biological control of a mildly pathogenic contaminant.

BNR1 and BNR2 were the most consistent organisms for disease control and plant growth promotion over all of these experiments. These binucleate *Rhizoctonia* isolates are known to be dense colonisers of seedling roots (Harris et al., 1991), but the mechanisms they use to promote seedling growth and suppress damping-off are still being investigated. These fungal isolates are effective at low dose (< 0.35% v/v or concentrations < pathogen dose) against unrelated fungal pathogens, and therefore could potentially be developed for economical treatments of commercial potting media.

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