Biological control of *Botrytis cinerea* and *Sclerotinia sclerotiorum* on kiwifruit.

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Abstract

Botrytis cinerea and *Sclerotinia sclerotiorum* are the two most serious pathogens on kiwifruit in New Zealand. Because of the pesticide regulations in some of the countries to which New Zealand exports fruit, total protection from *Botrytis* stem end rot with current dicarboximide fungicides is not possible. The aim of this thesis was to investigate biological control measures for *Botrytis* stem end rot and *Sclerotinia* diseases of kiwifruit.

More than 1000 microorganisms, isolated from the leaves and flowers of kiwifruit during spring and autumn, and selected from BCAs reported to be effective against *B. cinerea* and/or *S. sclerotiorum*, were tested *in vitro* for their antagonistic ability against *B. cinerea* and *S. sclerotiorum*. Successful antagonists were those that, in dual culture on agar plates, produced a zone of inhibition, an area of browning of the pathogens, or grew rapidly over the pathogens and inhibited their growth.

The fifty most promising isolates from the initial screen were tested on fruit for their ability to reduce *Botrytis* and *Sclerotinia* fruit rots. Mature kiwifruit were artifically wounded and dual inoculated with a spore suspension of one of the fifty test organisms and either a conidial suspension of *B. cinerea* or a mycelial suspension of *S. sclerotiorum*. Following 8-12 weeks incubation in a cool store, fruit were assessed for *Botrytis* or *Sclerotinia* induced rot. Isolates of *Bacillus* spp., *Epicoccum purpurascens*, *Pseudomonas* sp. and *Trichoderma* spp. reduced *Botrytis* fruit rot from 92% (inoculated control) to 0%. Isolates of *Alternaria* spp., *Pestalotia* sp. and a non-sporulating isolate also reduced the number of fruit rotting to some extent. Similarly, isolates of *Bacillus* spp., *E. purpurascens* and *Trichoderma* spp. reduced *Sclerotinia* fruit rot from 100% (inoculated control) to 0%. Isolates of *Alternaria* spp., *Myrothecium verrucaria* and *Pestalotia* sp. were also successful at reducing the level of *Sclerotinia* fruit rot.

It was considered undesirable if potential biological control agents (BCAs) were able to colonize kiwifruit that were to be marketed for human consumption. In order to determine if microorganisms, shown to be effective in preventing *Botrytis* or *Sclerotinia* fruit rot, were capable of themselves colonizing fruit, isolations were made from fruit dual inoculated with *B. cinerea*, *S. sclerotiorum* and/or one of several BCAs. Strains of the BCAs *Bacillus* spp., *Pseudomonas* sp. and *E. purpurascens* were not found to be saprophytic on fruit.

Isolates of *Alternaria* sp., *Bacillus* sp., *E. purpurascens*, *Pestalotia* sp., *Pseudomonas* sp. and *T. harzianum* significantly inhibited germination and germ tube elongation of *B. cinerea* conidia *in vitro* in a nutrient solution, over a 24 h period. For example,the presence of *Alternaria alternata* A6 spores in a nutrient solution reduced germination of *B. cinerea* conidia from 100% to 20%. The presence of *E. purpurascens* A77 spores inhibited *B. cinerea* conidial germ tube elongation from >840 µm (in control conidia) to 27 µm. The presence of any one of the BCAs tested prevented germination of *B. cinerea* conidia in a non-nutrient water solution, in comparision to germination of up to 86% in controls.

A spore or cell suspension of each of the isolates *Bacillus* sp.M60, *E. purpurascens* A77 and *T. harzianum* C65 were spray inoculated onto kiwifruit blossoms produced *in vivo* in the glasshouse, immediately prior to inoculation of the blossoms with a condial suspension of *B. cinerea*. Application of the BCAs were completely effective in preventing colonization of blossoms by *B. cinerea* conidia.

The effectiveness of each of the isolates *E. purpurascens* A77, *T. harzianum* C65 and either *Bacillus* sp.M60 or M53 to reduce the viability of sclerotia of *B. cinerea* and *S. sclerotiorum* was tested in soil punnets. A spore or cell suspension of each respective BCA was applied to the surface of replicated punnets that were seeded with either *B. cinerea* or *S. sclerotiorum*. Following 8 weeks incubation, punnets were harvested and

viability of sclerotia assessed. *T. harzianum* C65 and *Bacillus* sp. M60 significantly reduced the viability of *B. cinerea* sclerotia from 8 sclerotia/punnet (control) to 4 sclerotia/punnet. *T. harzianum* C65 and *E. purpurascens* A77 caused a significant reduction in apothecia production of *S. sclerotiorum*, from 2.7 apothecia/punnet (control) to 0.7 apothecia/punnet.

Bacillus sp.M8 and *E. purpurascens* A77 were tested for their ability to reduce *Botrytis* stem end rot and *Sclerotinia* field rot in a kiwifruit orchard. The isolates tested did not successfully reduce either disease. Possible explanations for this are discussed.

In order to monitor the survival of particular isolates of BCAs in the field, a technique was developed to distinguish between individual strains of a BCA species. The polymerase chain reaction (PCR) was utilized to identify DNA polymorphisms within the genome of *T. harzianum* C65, in comparison with other strains of *Trichoderma* spp.. A sequence of polymorphic DNA was cloned, sequenced and used as a hybridization probe in Southern blotting to enable *T. harzianum* C65 to be distinguished from other strains of *Trichoderma* spp..

From the results obtained in this study, it was considered that *Bacillus* M60, *E. purpurascens* A77 and *Pseudomonas* M30 were the best isolates for the biological control of *Botrytis* stem end rot on kiwifruit. Further work to enable application of these isolates as postharvest BCAs is discussed. Of the isolates tested in this study, *T. harzianum* C65 was considered the best isolate for use against *Sclerotinia* diseases on kiwifruit. Methods of selecting more effective BCAs against *S. sclerotiorum* are discussed.

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