POTENTIAL FOR BIOLOGICAL CONTROL OF BOTRYTIS STEM-END ROT OF KIWIFRUIT WITH *TRICHODERMA* SPP.

L.H. CHEAH¹, R. HILL² and A.W. HUNT¹

¹Levin Horticultural Research Centre, Private Bag 4005, Levin
²Ruakura Agricultural Centre, Private Bag, Ruakura

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Storage stem-end rot of kiwifruit, caused by *Botrytis cinerea* Pers. is a major problem for the New Zealand kiwifruit industry (Hopkirk and Clark 1990) and caused up to \$10M of losses in 1991 (Peter Bull, NZKMB, pers. comm.).

Botrytis stem-end rot first appears as soft and water-soaked rot at the stem-end of fruit. As the disease develops, the rot progresses down the fruit and sporulation may occur at the picking wound. The rot arises from infection of the wound tissue of the picking scars by the spores at, or soon after, harvest (Pennycook 1984). These spores may originate from the infected calyces attached to the fruit or from fruit surfaces.

Field application of dicarboximide fungicides reduces the level of *B. cinerea* inoculum present at harvest. Since strains of *B. cinerea* that are resistant to the dicarboximide fungicides have become established on many New Zealand orchards (Pennycook 1985), application of these fungicides is restricted to the preharvest period only, and spraying during the flowering period is not recommended. However, the preharvest spray alone does not protect the picking scar from infection and consequently often fails to keep the storage rot below an acceptable level (Pennycook 1985). An alternative method of control is to apply biocontrol agents to reduce the level of *B. cinerea* inoculum during the growing season and hence reduce the chance of infection (NZKMB Research Committee Meeting 1990).

Many species of *Trichoderma* have been shown to be effective for control of a wide range of plant pathogens (Papavizas 1985). An isolate of *T. harzianum* Rifai was found to be effective for botrytis rot control on apple (Tronsmo and Ystass 1980; Tronsmo 1991), on carrot (Tronsmo 1989) and on strawberries (Tronsmo 1986). One of our three effective isolates was also found to be effective for control of armillaria root rot in kiwifruit (unpublished data). An active compound, harziano-pyridone, has been isolated from *T. harzianum* (Cutler and Jacyno 1991) and shown to be active against a wide range of plant pathogens.

This paper describes the effect of six isolates (three species) of *Trichoderma* on control of *B. cinerea* and their ability to inhibit *B. cinerea* spore production on fruit. The survival of the three effective *Trichoderma* isolates in the field is also described.

Botrytis cinerea cultures were established on potato dextrose agar (PDA), each from a single spore isolated from a diseased fruit. Spore suspensions were made by washing spores from a 10 to 14 day-old PDA culture with water. This suspension was used to inoculate the fruit.

Trichoderma isolates were obtained from the culture collection at Ruakura Agricultural Centre, Hamilton, N.Z. The fungi were originally isolated from orchard soil near Hamilton. *Trichoderma* cultures were established on PDA. Spore suspensions used for laboratory tests were made by washing spores from 14-day-old PDA cultures with water. Spore suspensions used for field trials were made by blending the cultures with agar and then diluting with water to 10⁶ spores/ml.

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson cv. 'Hayward') were harvested (Brix=8.5) from vines growing at Levin Horticultural Research Centre and stored at 0°C (about 95% RH) before being used. In the laboratory, fruit were

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labelled and surface sterilised with 1% sodium hypochlorite for 2 minutes. They were air dried and placed in commercial kiwifruit trays. Single wounds (3 mm deep x 3 mm wide) were made on the side of each fruit. Immediately after wounding, each wound was inoculated with *B. cinerea* spores (2000 spores per wound site) in water suspensions. After the spore droplets were air dried, each wound was challenge-inoculated with conidia of one of six isolates of *Trichoderma* (Table 1) by pipetting 30 µl of conidia suspensions (10^8 /ml) into each wound site. Control fruit were inoculated with *B. cinerea* but were not treated with *Trichoderma*. Treatments were repeated four times with 20 fruit in each treatment. Fruit from each treatment were randomised and packed into commercial trays and stored at 15°C (about 75% RH). After 10 days of incubation, individual fruit were examined for *B. cinerea* at the wound site. About 1 cm² of fruit skin around each wound site was cut and placed in a small bottle containing 5 ml of water. The bottles were mechanically shaken for 2 minutes to release the spores. The number of *B. cinerea* spores was estimated with a haemocytometer under a microscope.

To assess conidia production of *Trichoderma* the trial was repeated and after 15 days incubation at 15°C (75% RH) the number of *Trichoderma* conidia on each fruit was estimated by the method described above.

Data were subjected to analysis of variance using the SAS statistical package.

In a field trial, survival of *Trichoderma* was monitored on fruit of four kiwifruit vines grown at Levin Horticultural Research Centre. These vines were part of a biological control trial and had been inoculated twice with *B. cinerea* spore suspensions (10^7 /ml) during flowering. They had also received a total of three *Trichoderma* treatments (mixture of the three most effective isolates; TV, M1037 and TBHPP7 at 10^8 conidia/ml) at 50% flower (Dec), 100% flower (Jan) and mid-season (Feb) periods by spraying the spore suspensions with a knapsack sprayer. Survival was measured by sampling twenty flowers or fruit/vine at monthly intervals from December 1991 to April 1992, washing these in water, and plating the solutions onto *Botrytis*-selective media (Keressies 1990). *Trichoderma* spp. will also grow on this medium but other fungi will not. The plates were incubated at 22°C for 8 days and the growth of *Trichoderma* spp. and *B. cinerea* was recorded.

No botrytis rot developed on any of the *Trichoderma* treated fruit, but *B. cinerea* sporulation occurred at the wound site of some treated fruit. Three isolates of *Trichoderma*, TV (*T. viridae*), M1037 (*T. harmatum*) and TBHPP7 (*T. harmatum*), gave complete inhibition of *B. cinerea* spore production (Table 1). Isolates MTM and D also gave good levels of inhibition, but isolate KEK was not as effective. Two of the most effective isolates themselves produced large number of conidia at wound sites on fruit (Table 1). The *Trichoderma* also survived well in the field from December 1991 to April 1992 on fruit (Table 2) and no *B. cinerea* spores were found throughout this period.

TABLE 1: Sporulation of *B. cinerea* and *Trichoderma* spp. on postharvest kiwifruit after inoculation with *Botrytis cinerea* and treatment with six isolates of *Trichoderma* spp.

Isolate	<i>Trichoderma</i> spp.	<i>Botrytis cinerea</i> spores/fruit ($\times 10^4$ /ml)	<i>Trichoderma</i> spores/fruit ($\times 10^5$ /ml)
TV	<i>T. viridae</i>	0 b*	30.2 a*
M1037	<i>T. harmatum</i>	0 b	7.9 b
TBHPP7	<i>T. harmatum</i>	0 b	3.8 c
MTM	<i>T. harmatum</i>	0.5 b	3.6 c
D	<i>T. harzianum</i>	1.8 b	0.1 c
KEK	<i>T. harmatum</i>	13.9 a	0 c
untreated		12.8 a	0 c

* Any two means in a column followed by the same letter are not significant at 0.05 probability level.

TABLE 2: Number of *Trichoderma* and *Botrytis* spores on kiwifruit flowers or fruit after treatment of vines with *Trichoderma*.

Sampling Date	No. of surviving spores/fruit	
	<i>Trichoderma</i>	<i>Botrytis</i>
Dec '91	++ ^a	–
Jan '92	++	–
Feb '92	+++	–
Mar '92	+++	–
Apr '92	+++	–
untreated ^b	–	+

^a – = No spores; + = 1-1000 spores; ++ = 1000-2000 spores; +++ = 2000-3000 spores

^b for each sampling date

The results of laboratory tests indicate that three isolates of *Trichoderma* were highly effective, giving good control of *B. cinerea*, inhibiting *B. cinerea* sporulation and producing large numbers of conidia. *Botrytis cinerea* spores are important in the spreading and initiating of new infections in kiwifruit (Pennycook 1985). Thus inhibition of spore production will result in a reduction of *B. cinerea* inoculum in the field and hence reduced fruit infection. At least one of the isolates could survive for long periods in the field and effectively control *B. cinerea* on fruit. Preliminary tests by H.G. Cutler (Richard Russell Centre, Georgia, U.S.A., unpublished data) indicate that these three isolates produce harziano-pyridone which may explain their efficacy against *B. cinerea*. These isolates are potentially good biological control agents for botrytis stem-end rot control in kiwifruit. In view of their activity more field trials are being conducted in which conidia suspensions of these fungi will be applied for control of *B. cinerea*. Further work is also being planned to study their mode of action and to evaluate their efficacy for the control of other diseases.

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