BIOLOGICAL CONTROL OF FUSARIUM STORAGE ROT OF SQUASH WITH YEASTS

L.H. CHEAH and A.P. MARSHALL

*N.Z. Institute for Crop & Food Research,
Private Bag 4005, Levin, New Zealand*

Keywords: biological control, fusarium storage rot, *Fusarium sambucium*, squash, yeasts, population dynamics

Storage rot of squash (*Cucurbita maxima*) is a major problem to the squash industry (Hawthorne pers. comm.). Several fungi may be involved but *Fusarium* spp. are the most common pathogens associated with the rot (Hawthorne 1988; Hawthorne *et al.* 1992). Dipping fruit in fungicide is the primary means of control, but it has recently come under scrutiny as posing potential oncogenic risks (Anon. 1987). Further postharvest pathogens often develop resistance to fungicides (Spotts and Cervantes 1986). For these reasons there is a need for new and effective means of postharvest disease control that poses less risk to human health and the environment.

Over the past 6 years, we have isolated and identified yeasts, fungi and bacteria that have potential as biocontrol agents effective against the fungi which cause postharvest diseases including *Botrytis cinerea* and *Penicillium* spp. We found three isolates of *Trichoderma* spp that were highly effective against *B. cinerea* in kiwifruit (Cheah *et al.* 1992) but these fungal isolates produce an active toxic compound, harziano-pyridone (Cutler and Jacyno 1991). We therefore decided to screen yeast as safe and effective biocontrol agents that do not produce mycotoxins or antibiotics (Droby *et al.* 1991). Last year, we found that several industrial yeast isolates and two yeasts isolated from kiwifruit (Cheah *et al.* 1995) were highly effective against *B. cinerea* in kiwifruit (Cheah and Hunt 1994) and *Penicillium* spp. in lemon (Cheah and Tran 1995). This paper reports the evaluation of these yeasts for the control of fusarium storage rot in squash.

Two types of yeasts were used: (i) industrial yeasts (S20, K52 and K58) obtained from the culture collection at Levin Research Centre and the Processing and Environmental Technology Department of Massey University, Palmerston North; (ii) kiwifruit yeasts (NZ10 and NZ11) isolated from fruit of unsprayed kiwifruit vines growing at the Levin Research Centre (Cheah *et al.* 1995).

Fusarium sambucium cultures were established on potato dextrose agar (PDA), each from a single spore isolated from a diseased squash 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 fruit for testing the effectiveness of the yeast isolates and for a population dynamic study.

Unsprayed squash fruit picked from Levin Research Centre were used for the tests. Yeast isolates were screened by a modification of the method used by Roberts (1990) and Cheah and Tran (1995). Cultures of individual isolates to be tested were grown on nutrient yeast dextrose agar plates (NYDA: 20 g of agar, 8 g of nutrient broth, 4 g of yeast extract, 1.5 g of dextrose and 1 litre of distilled water) for 2 days. Yeast cell suspension of the isolates were made by taking a loop of the culture diluted in sterile distilled water in a test tube and the cell concentrations were adjusted to about 10^8 colony-forming units per ml determined by dilution plating. Squash fruit which had been washed in 70% alcohol and dried, were wounded on the side of the fruit with a sterilised nail. The wound size was about 2 mm (diam.) x 3 mm (deep). The wounds were filled with yeast cell suspensions (10 µl per wound) using a micropipette. After the wound site had dried, each wound was challenged with 10 µl of *F. sambucium* spore suspensions. Control wounds were treated with water. Treated fruit were placed

Proc. 48th N.Z. Plant Protection Conf. 1995: 337-339

in a covered plastic container with moistened paper towels to maintain high humidity. Three wounds were used for each treatment and six treatments (Table 1) were randomised on a fruit. The experiment was repeated six times for each treatment. Disease assessment was made 5 days after incubation at 20°C by recording the number of rot and lesion size caused by *F. sambucium*. Data were analysed using GLM procedures of the Genstat package.

All five yeast isolates tested significantly reduced fusarium rot incidence compared to the untreated control wounds (Table 1). Isolates, NZ10 and NZ11 were the most effective in controlling the rot and also provided the least lesion size on the wounds.

TABLE 1: Fusarium rot in squash after treatment with five yeast isolates.

Isolate	Yeast	Percent rot	Confidence interval ¹	Lesion size (mm)	Sem (48 df)
NZ10	<i>Pichia</i> sp	14.2	(2.1;56.4)	1.6	1.8
NZ11	unidentified	5.7	(0.8;32.2)	4.2	2.5
S20	<i>Saccharomyces</i> sp	49.0	(10.9;88.3)	9.5	1.1
K52	<i>Kluyveromyces</i> sp	85.8	(43.6;97.9)	9.0	0.9
K58	<i>Kluyveromyces</i> sp	73.1	(25.7;95.5)	9.9	1.0
Untreated	water	100	-	14.9	0.8

¹The confidence intervals (95%) are calculated for the percentages on the transformed (logit) scale, and then back-transformed.

The ability of cells of NZ10 to survive and multiply in wounds was studied to determine if NZ10 is an effective colonizer of squash fruit wounds. Experimental methods and design were similar to those described above, except for the sampling procedures. Three wounds per treatment were sampled 0 and 4 h after inoculation, then every 24 h until 96 h had elapsed. Samples were taken by excising the entire wound from the squash fruit with sterilised cork borer (6 mm diam.), trimming the resultant tissue cylinder to about 6 mm in length, and placing the sample in 10 ml of sterile distilled water. The tissue plug then was macerated with a glass rod, vortexed, dilution-plated in triplicate onto NYDA, and incubated at 25°C for 2 days when colonies were counted.

Population densities of NZ10 in wounds without *Fusarium* increased with incubation time from 4 to 48 h then levelled off (Fig. 1). In the presence of *Fusarium* inoculum the population density increased significantly at each sampling for up to 72 h after inoculation. The mechanism involved in the increase of NZ10 yeast population

FIGURE 1: Growth of NZ10 yeast isolate in squash wounds

of in the presence of *Fusarium* inoculum is not clear, but there is evidence that the multiplication of bacteria or yeasts is partially stimulated by nutrients leaked from conidia (Blakeman 1980). Further work is needed to study the mode of action of disease control and the interaction of the two organisms in the wounds.

In this study we found all five yeast isolates tested gave significant control of fusarium storage rot. This is the first report of biocontrol of storage rot in squash with yeasts. As none of the yeast isolates tested produced antibiotics (Cheah and Hunt 1994; Cheah *et al.* 1995), it could be assumed that antibiosis is not an important aspect of their mode of action (Chalutz *et al.* 1988). Droby *et al.* (1989) found that an effective yeast isolate, *Debaryomyces hansenii* did not produce antibiotic substances in culture but showed that competition for nutrients may play a role in the biocontrol of *Penicillium digitatum* on grapefruit. *Cryptococcus laurentii* gave effective control of botrytis rot in apples (Roberts 1990) by effectively colonizing space in the wounds. Our results also indicate that NZ10 is a fast growing isolate, and may be an effective colonizer for space.

ACKNOWLEDGEMENTS

We thank Ruth Butler for statistical analysis and Anne-Marie Jackson of Processing and Environment Technology Dept., Massey University for providing some yeast cultures.

REFERENCES

- Anon, 1987. Regulating pesticides in food - the Delaney paradox. Board of Agriculture. National Research Council Washington D.C. National Academy Press. 272 pp
- Blakeman, J.P., 1980. Behaviour of conidia on aerial plant surfaces. Pp 115-151 In: The Biology of *Botrytis*. Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R (Eds), Academic Press, London.
- Chalutz, E., Droby, S. and Wilson, C.L., 1988. Mechanism of action of postharvest biocontrol agents. *Proc. 5th International Congress of Plant Pathology*: 422.
- Cheah, L.H., Hill, R. and Hunt, A.W., 1992. Potential for biological control of botrytis stem-end rot of kiwifruit with *Trichoderma* spp. *Proc. 45th N.Z. Plant Protection Conf.*: 193-196.
- Cheah, L.H. and Hunt, A.W., 1994. Screening of industrial yeasts for biocontrol of botrytis storage rot in kiwifruit. *Proc. 47th N.Z. Plant Protection Conf.*: 362-363.
- Cheah, L.H. and Tran, T.B., 1995. Postharvest biocontrol of *Penicillium* rot of lemons with industrial yeasts. *Proc. 48th N.Z. Plant Protection Conf.*: this volume.
- Cheah, L.H., Wilson, C.L. and Marshall, A.P., 1995. Rapid screening for antagonists against botrytis storage rots using leaf and fruit tissues. *Postharvest Biology and Technology*: in press.
- Cutler, H.G. and Jacyno, J.M., 1991. Biological activity of (-)-Harziano-pyridone isolated from *Trichoderma harzianum*. *Agri. Biol. Chem.* 55: 2629-2631
- Droby, S., Chalutz, E. and Wilson, C.L., 1991. Antagonistic microorganisms as biological control agents of postharvest diseases of fruits and vegetables. *Postharvest News and Information* 2(3): 196-173.
- Droby, S., Chalutz, E., Wilson, C.L. and Wisniewski, M., 1989. Characterisation of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grapefruit. *Can. J. Microbiol.* 35: 794-800.
- Hawthorne, B.T., 1988. Fungi causing storage rots on fruits of Cucurbita spp. *N.Z. J. Exp. Agric.* 16: 151-157.
- Hawthorne, B.T., Rees-George, J. and Broadhurst, P.G., 1992. Mating behaviour and pathogenicity of New Zealand isolates of *Nectria haematococca* (*Fusarium solani*). *N.Z. J. Crop and Hort. Sci.* 20: 51-57.
- Roberts, R.G., 1990. Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. *Phytopathology* 80: 526-530.
- Spotts, R.A. and Cervantes, L.A., 1986. Population, pathogenicity and benomyl resistance of *Botrytis* spp. *Penicillium* spp. and *Mucor piriformis* in packing house. *Plant disease* 70: 106-108.