A RAPID TECHNIQUE FOR SCREENING FUNGICIDES FOR CONTROL OF LEAF CURL (*Taphrina deformans* (Berk.) Tul.) IN STONEFRUIT

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Leaf curl, caused by the fungus *Taphrina deformans*, is a disease that is potentially devastating both to crop yields and tree longevity of stonefruit. In the last three seasons, the disease has become of major concern to growers in Hawkes Bay as it is not being effectively controlled by current fungicides (copper and captafol) in spite of good timing and complete coverage by the sprays. Resistance to the fungicides was suspected to be the main cause of the problem.

T. deformans grows as a yeast in pure culture so standard methods for filamentous fungi cannot be used to obtain quantitative data. This research describes a rapid and simple technique to measure quantitatively the growth of the fungus in pure culture. The technique was used to test for possible resistance to current fungicides and to screen new systemic fungicides for improved disease control.

T. deformans was isolated by attaching small pieces of curled leaves bearing mature asci (indicated by a white 'bloom') to the lid of an inverted petri plate containing potato dextrose agar (PDA) + streptomycin (50 ppm). The ascospores were ejected and stuck to the agar surface. Single clusters of spores were then located with a microscope, removed by cutting out a small portion of agar with a micro-scalpel, transferred to PDA and incubated at $20 \pm 2^\circ\text{C}$. This resulted in pale pink cultures, each derived from a single ascus.

Spore suspensions ($10^7/\text{ml}$) of *T. deformans* were prepared from a 14 day old culture. One millilitre of this suspension was seeded onto the agar surface of various culture media in petri plates (Table 1). The suspension was evenly spread with a glass rod to ensure complete coverage. Excess suspension was then removed with a capillary pipette, leaving a thin film of spores covering the agar surface. Four petri plate replications were used for each treatment. Tests showed that the spores were evenly spread on the agar surface.

TABLE 1: Growth of *T. deformans* on different culture media.

Culture media (pH 4.5)	Growth ¹ (spore $\times 10^2$ /10 mm diam. plug)
PDMA	4.32
PDA	6.58
PDMA + peptone*	8.28
PDA + peptone*	8.37
Gibco PDA + peptone*	8.21
Nutrient media	5.86
LSD (P = 5%)	0.69

*Difco bacto-peptone 10 g was added to 1 litre of agar medium

¹Incremental number, mean of 4 replications

PDMA = Potato 200 g, dextrose 20 g, agar 20 g, Marmite 1 g, water 1 litre.

PDA = Potato 200 g, dextrose 20 g, agar 20 g, water 1 litre.

Gibco PDA = Gibco PDA 39 g, water 1 litre.

Nutrient media = KH_2PO_4 2.7 g, MgSO_4 1.2 g, dextrose 10.0 g, KNO_3 2.00, Asparagine 2.00 g, agar 20 g, water 1 litre.

Proc. 39th N.Z. Weed and Pest Control Conf.

Initial spore concentrations per unit area was estimated by taking a 10 mm diam. agar plug from the centre of each plate, vigorously shaking it in a test tube containing 10 ml of water and counting the number of spores present with a haemocytometer. Final spore concentration was assessed by the same procedure 4 days after incubation at $20 \pm 2^\circ\text{C}$. Growth of fungus was calculated as the difference between initial and final spore numbers.

PDMA + peptone, PDA + peptone and Gibco PDA + peptone showed significantly better growth than PDMA, PDA and nutrient media (Table 1).

To test for resistance to fungicides, captafol and copper-amended agar plates (Pennycook and Corbin 1970) at 4 concentrations (500, 1000, 2000 and 3000 mg ai/litre) were prepared using PDA + peptone media (Table 2). Four isolates from different localities were tested and growth assessed as above. Growth of all isolates was completely inhibited at 500 mg ai/litre for captafol and 1000 mg ai/litre for cupric hydroxide (Table 2).

TABLE 2: Effect of captafol and cupric hydroxide on growth of *T. deformans* on agar medium.

Fungicide	Concentration (mg ai/litre)	Mean growth ¹ (spores $\times 10^2$ /10 mm diam. plug)			
		TdI*	TdG	TdH	TdC
captafol	0	7.52 (0.18)#	6.93 (0.31)	7.06 (0.24)	7.13 (0.33)
	500	0**	0	0	0
	1000	0	0	0	0
	2000	0	0	0	0
	3000	0	0	0	0
cupric hydroxide	0	7.99 (0.90)	6.86 (0.26)	9.45 (1.09)	9.04 (0.52)
	500	0.71 (0.03)	1.92 (0.15)	0.14 (0.02)	5.05 (0.57)
	1000	0	0	0	0
	2000	0	0	0	0
	3000	0	0	0	0

*Isolates from four Hastings orchards where problems in controlling leaf curl had been experienced.

**Zero results were not included in the statistical analysis.

¹Incremental number, mean of 4 replications.

Std. deviation

Fungicide screening tests at 4 concentrations (1, 5, 50 and 100 ppm of active ingredients) were similarly carried out. Bitertanol, DPX-H6573, penconazole, prochloraz, propiconazole and S3308 gave complete inhibition of the yeast growth at 1 ppm. The other fungicides were less effective (Table 3).

The results do not indicate any resistance to copper or captafol in *T. deformans* since complete growth inhibition occurred at concentrations well below the recommended field rates of 800 and 1250 mg ai/litre for captafol and copper respectively. Of the 12 systemic fungicides tested, six were very effective in inhibiting growth. This suggests that the disease may be controlled in the field by the systemic action of the fungicides.

The technique proved to be a simple and rapid method to measure quantitatively the growth of *T. deformans* in pure culture. Its advantages are that growth can be assessed both visually and quantitatively within 3-4 days as compared to 21 days for potato dextrose broth culture (Mix 1953). This is useful for fungicide screening as there is a good contrast in growth rate between untreated and fungicide-amended plates. Furthermore, since the culture grows rapidly on agar plates there is less problem with contamination, and the contaminant if present can be easily removed. In broth culture, however, contamination cannot be removed and ruins the culture.

TABLE 3: Inhibition of growth of *T. deformans* by fungicides in laboratory screening tests.

Fungicide		Conc. for complete inhibition (ppm)	
benomyl	Benlate	50 WP	(50-100)*
bitertanol	Baycor	50WP	<1
bupirimate	Nimrod	25EC	>100
DPX.H6573	Nbl	20WP	<1
fenarimol	Rubigan	12EC	(5-50)
fenpropimorph	Mistral	75EC	(1-5)
penconazole	Topas	10EC	<1
prochloraz	Sportak	50WP	<1
propiconazole	Tilt	25EC	<1
S3308L	Nb2	12.5WP	<1
triadimefon	Bayleton	5WP	(1-5)
triforine	Saprol	19EC	>100

*Value written (50-100 etc) indicate that the value lies between these limits; the experimental results do not allow any more precise estimate.

Nbl: bis (4-fluorophenyl) methyl(1H-1, 2, 4-triazol-1-ylmethyl)silane)

Nb2: 1-(2, 4-dichlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-penten-3-01).

ACKNOWLEDGEMENTS

We acknowledge the partial funding of this project by the Hawkes Bay Fruitgrowers Association.

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