**DECREASED SENSITIVITY OF *TAPHRINA DEFORMANS*
(PEACH LEAF CURL)
TO COPPER FUNGICIDES IN HAWKES BAY**

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Leaf curl of peaches, caused by *Taphrina deformans* (Berk.) Tul. may significantly reduce both crop yields and tree longevity (Atkinson 1971). This disease has been effectively controlled with copper fungicides applied during leaf bud movement. However, in the past up to seven applications of copper have been made from leaf fall to bud movement for additional control of bacterial diseases. In 1985, orchardists in Hawkes Bay reported difficulty in controlling leaf curl. Isolates of *T. deformans* from orchards with control failure were grown on potato dextrose agar amended with a range of cupric hydroxide [Cu(OH)₂] concentrations to test their sensitivity to this compound. Growth of these isolates was completely inhibited at 1000 ppm. Since the field rate application of Cu(OH)₂ was 1250 ppm, it was concluded that development of fungicide insensitivity was probably not a factor in the poor control achieved (Cheah and Tate 1986). However, in spring 1988 a strain of *T. deformans* was isolated that grew well on media amended with 1500 ppm Cu(OH)₂, suggesting that strains resistant to Cu(OH)₂ at the field rate could be present and might be responsible for failure to control leaf curl on certain orchards. A survey of Hawkes Bay orchards was carried out to determine occurrence and incidence of copper insensitivity in *T. deformans*.

Field survey

In October 1990 17 nectarine and peach orchards were sampled and diseased shoots taken at widely-spaced intervals in each block. In the laboratory *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 water agar. Single ascospores ejected onto the agar surface, were separated from the typical clumps by streaking with a loop, and removed by cutting out a small portion of agar with a micro-scalpel. These were then transferred to potato dextrose agar plus peptone (Difco, 1%) and incubated at 20°C. This resulted in pale pink cultures derived from single ascospores. Because *T. deformans* in culture has a yeast-like growth stage, growth of the culture was assessed by counting the increase in the number of individual cells.

Sensitivity of *T. deformans*

To determine a baseline sensitivity of *T. deformans*, eight single spore isolates from trees that had never been exposed to copper fungicides were used (Table 1). The baseline sensitivity of these isolates was estimated by exposing the spores to a range of Cu(OH)₂ concentrations (0, 500, 750, 1000, 1250, 1500 and 2000 ppm) using methods described by Cheah and Tate (1986). All eight isolates grew at 1250 ppm but were inhibited at 1500 ppm (baseline sensitivity).

For mass screening of isolates of *T. deformans* obtained from the field survey, spores were exposed to 1500 ppm of Cu(OH)₂ only using the same method as used for determining the baseline sensitivity.

The EC₅₀ was estimated also using similar methods to those described by Cheah and Tate (1986) except that spores were exposed to a series of Cu(OH)₂ concentrations. Data were fitted into a model to estimate the EC₅₀ of each isolate using the SAS statistical package.

A total of 127 *T. deformans* isolates were collected from 17 orchards. To date, 74 isolates have been screened and 33 were found to grow on 1500 ppm of Cu(OH)₂.

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When these isolates were further tested to estimate their EC_{50} values, only nine were found to have EC_{50} values higher than the mean of the sensitive isolate (Table 1). The remaining 24 isolates seemed to lose their insensitivity through repeated subculturing. The mean EC_{50} of the less sensitive isolates (953 ppm) was about double that of the sensitive ones (439 ppm). Recently Menkissoglu and Lindow (1991) have shown that a high proportion of Cu^{++} (cupric) ions are locked up in complexes when incorporated into media and that the concentration of free Cu^{++} ions increases logarithmically with the rate of incorporation in the media. On this basis the EC_{50} s of our isolates in pure aqueous $Cu(OH)_2$ solutions would be lower than those we have determined, but our less sensitive isolates would have EC_{50} s considerably more than twice that of the sensitive isolates. All nine of the less sensitive isolates were collected from orchards that had a history of disease control failure despite being sprayed with recommended rates of copper fungicides.

TABLE 1: EC_{50} s of *Taphrina deformans* isolates to $Cu(OH)_2$ tested on potato dextrose agar.

Sensitive isolate	Source	EC_{50} (ppm)	Insensitive isolate	Source (Hawkes Bay)	EC_{50} (ppm)
BE	Levin	291	2.4	Haumoana	599
DL	Palmerston N	296	2.6	Haumoana	704
U6.2	Hawkes Bay	360	2.7	Haumoana	783
U6.1	Hawkes Bay	480	2.5	Haumoana	863
19	Hawkes Bay	518	3.6	Clive	868
NB	Levin	523	4.0	Lawn Rd	968
LC	Levin	604	3.4	Clive	1074
			C14	Roys Hill	1300
			C12	Roys Hill	1421
Mean		439			953
Std. error		46			90

The results of this survey strongly suggest that *T. deformans* strains with reduced sensitivity to $Cu(OH)_2$ are present in Hawkes Bay. The evidence includes the long history and high frequency of use of copper fungicides (including $Cu(OH)_2$) and failure to control the disease with recommended rates. In 1985, the baseline sensitivity was about 1000 ppm of $Cu(OH)_2$ (Cheah and Tate 1986). In the current survey (1990), the baseline had shifted to 1500 ppm and almost half of the isolates tested grew at this rate. Also, in recent replicated field trials (Tate, unpublished data) copper fungicides have been ineffective in preventing severe leaf curl, while non-copper fungicides (e.g. ziram) were effective. These trials also confirm recent grower experience with copper fungicides compared with alternatives such as ziram.

Resistance to copper sulphate has been found in the yeast *Saccharomyces cerevisiae* Meyen ex Hansen in cultures (Brenes-Pomales *et al* 1955; Antoine 1965). Resistance to copper oxychloride was also recorded in *Colletotrichum capsici* (Syd.) Bult. & Bisby (Thind and Jhooty 1980) and *Helminthosporium maydis* Nisikado (Bains and Mohan 1982) in cultures. Although that resistance was found only in cultures, this indicates that the yeast phase of *T. deformans* may become less sensitive to copper when constantly exposed to high selection pressure. Resistance to other heavy metal fungicides (e.g. nickel) has also been found in *Neurospora crassa* Shear & Dodge (Mohan and Sastry 1983).

The present results also indicate that the reduced sensitivity may be 'low level', as the mean EC_{50} value of the less sensitive isolates was only double that of sensitive isolates. Furthermore, we have observed that some less sensitive isolates became sensitive to copper after several subculturing on agar plates. This may indicate that

there was a shift-back to copper sensitivity similar to that reported in *Penicillium digitatum* (Pers. ex St. -Am.) Sacc. to imazalil (van Gestel 1988). Although the fitness of the less sensitive isolates was not investigated, this shiftback behaviour may also indicate that less sensitive isolates are not as ecologically fit. Similar results were noted in *Botrytis cinerea* Pers. exposed to dicarboximides (Beever and Bryde 1982; Beever and Brien 1983).

To ensure control of *T. deformans*, it is recommended that an effective alternative to copper fungicides, such as chlorothalonil (Cheah *et al.* 1988) is applied twice during the bud movement period.

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