**GREENHOUSE WHITEFLY (*TRIALEURODES VAPORARIORUM*
(WESTWOOD), HEMIPTERA: ALEYRODIDAE)
RESISTANCE TO BUPROFEZIN**

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ABSTRACT

First instar whitefly nymphs from five populations on bean leaf portions embedded on agar were sprayed under a Potter tower with several concentrations of buprofezin. Mortality was assessed when water-treated nymphs reached fourth instar. A Blenheim greenhouse tomato population was very resistant to buprofezin. The LC_{50} and LC_{99} values were 992 (95% confidence intervals, 546-1801) and 1954 (95% confidence intervals 516-7393) mg/litre higher respectively than the standard population. Assessments with the recommended concentration, one tenth and one hundredth, indicate that one Auckland population had an equally high level of resistance, while a second Auckland population had reduced resistance. Whitefly from outdoor-grown tamarillos and the standard population were equally susceptible.

Keywords: greenhouse whitefly, *Trialeurodes vaporariorum*, buprofezin, resistance, greenhouse tomatoes

INTRODUCTION

Buprofezin is an insect growth regulator which interferes with moulting in the young stages of susceptible insects such as whitefly. When the product was introduced in 1991 for control of whitefly on greenhouse vegetables, a considerable effort went into explaining to growers how the product worked and the need for good spray coverage on the underside of the leaves. The need to use the product no more than three times in one growing season, was also emphasised to minimise the risk of whitefly becoming resistant to the insecticide. Since spring 1993, several greenhouse tomato growers have reported that buprofezin did not seem to give as effective whitefly control compared to when they first used the product. DowElanco and Crop & Food Research investigated the first report of poor whitefly control from a grower of greenhouse tomatoes in Blenheim. It appeared that spray coverage, a frequent problem with pesticide failure, was good. Observations showed that application of buprofezin failed to kill all young whitefly nymphs.

This paper describes the bioassay used to test for greenhouse whitefly resistance to buprofezin and the results of testing whitefly populations from Blenheim and Auckland.

MATERIALS AND METHODS

Adult greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) were allowed to lay eggs for 24 h on the primary leaves of dwarf beans (*Phaseolus vulgaris* cv 'Topcrop'). Separate batches of plants were infested at the same time with whitefly from either a 'test' population or a 'standard' population which had been reared on tobacco at Mount Albert Research Centre, Auckland. The standard population had not been exposed to buprofezin. After removal of the adult whitefly, plants with eggs of the two populations were kept in the same greenhouse unit until the first instar nymphs emerged. A portions of bean leaf (about 1000 mm²) with between 50-200 nymphs, was embedded top-side down on agar in each plastic

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petri dish (90 mm diameter). The leaves were sprayed with buprofezin (Applaud 25W) in a Potter tower with 2 ml suspension at 69 kPa and a 7 second settling time. When the surface of the embedded leaf portion was dry, the lid of the dish was lined with filter paper and closed. The dishes were placed in a cabinet at 25°C and 16 h light and 8 h dark with the agar half upper-most so that honey dew from the nymphs was deposited on the filter paper. Each of the four replicates were placed on a separate shelf. When the nymphs on the water-sprayed control reached fourth instar (7 days), the stage of each nymph was recorded. Nymphs in instars 1 and 2 were scored as dead and those that had developed to instars 3 and 4 were scored as live.

A population from Blenheim and a Mount Albert standard population were each tested at five concentrations of buprofezin in the first test and six concentrations in four subsequent tests (Fig 1.). These data were used to estimate the dose-mortality relationships for each population.

Three Auckland populations and the 'standard' were exposed to a water control and three concentrations of buprofezin, viz. 125 mg/litre (the recommended rate), 12.5, and 1.25 mg/litre. Two populations were from greenhouse tomato crops, in Henderson and Mangere, selected because the growers believed that buprofezin no longer gave control of greenhouse whitefly. A third population was from outdoor tamarillos in a South Kaipara orchard, that used a conventional pesticides including buprofezin, but had no reason to suspect resistance to buprofezin. These populations were compared in two groups.

For comparison between the Blenheim population and the 'standard', a generalised linear model was fitted with binomial errors and probit link with control mortality estimation fitted by maximum likelihood to the proportion "dead" for each population for replicates together and separately. The model fitted was

$$p = C + (1-C) * f(a + b * \log(\text{concentration}))$$

where p is the probability of not reaching instars 3 or 4 (ie. dead), C is the control mortality, a, b are intercept and slope parameters, and f(x) is the inverse probit link function.

For comparison between three Auckland whitefly populations and the 'standard', for each concentration of buprofezin, the mean percent mortality of the test population was compared with the 'standard' using a t-test.

RESULTS

The Blenheim population of greenhouse whitefly was less susceptible to buprofezin than the Mount Albert 'standard' population (Table 1; Fig. 1). The LC₅₀ and LC₉₉ values were 992 (95% confidence interval, 546-1801) and 1954 (95% confidence intervals 516-7393) mg/litre higher respectively, for Blenheim than for the standard.

TABLE 1: The susceptibility of two populations of whitefly to buprofezin when treated as first instars and mortality was assessed when water-treated nymphs reached fourth instar, nymphs at instars one and two were scored as dead.

Population	Log mg/litre (mg/litre)			
	LC ₅₀	sem ¹	LC ₉₉	sem ¹
Blenheim	4.91 (135)	0.19	8.17 (3533)	0.61
Standard	-1.99 (0.136)	0.23	0.60 (1.8)	0.26

¹ sem = standard error of mean

About 50% of the Henderson population in Auckland survived the recommended dose of 125 mg/litre (Table 2) which is similar to the LC₅₀ (135 mg/litre) estimated for the Blenheim population. This suggests that the level of resistance of the two populations is the same. The Mangere population was less resistant than the Blenheim or Henderson populations and the tamarillo population was as susceptible as the standard Mount Albert population (Table 2).

FIGURE 1: Nymphal mortality (mean for each of five tests) of two populations of greenhouse whitefly after treatment of first instars with buprofezin. Mortality was assessed when water-treated controls reached the fourth instar. Nymphs remaining at instar 1 and 2 were recorded as dead. Mortality above the dotted line equals 100 percent.

TABLE 2: Percent mortality of whitefly nymphs treated with buprofezin as first instar nymphs on bean leaves. Mortality was assessed when water-treated nymphs had reached the fourth instar; nymphs at instars 1 and 2 were scored as dead.

Population	Concentration of buprofezin (mg/litre)			
	0	1.25	12.5	125
Henderson	7 (390) ¹	18* (276)	11* (357)	56* (462)
Standard	15 (209)	75 (331)	99 (285)	100 (227)
Mangere	16 (318)	11** (184)	97 (229)	100 (403)
Tamarillo	11 (426)	98 (423)	100 (349)	100 (437)
Standard	12 (471)	97 (469)	100 (522)	100 (510)

*, ** significantly different from the standard population, $P < 0.05$, $P < 0.01$.

¹ Total number treated in brackets.

DISCUSSION

This is the first documented case of greenhouse whitefly resistance to buprofezin. Reduced susceptibility of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) to buprofezin was found after two applications of the pesticide to cotton in Israel (Horowitz and Ishaaya 1992) where LC_{50} and LC_{90} values increased by 1.4- to 2.1-fold and 2.6- to 2.9-fold, respectively. In Israel, the recommendation was for buprofezin to be used no more than twice a year and only during a 1-month period. This appeared to have prevented resistance developing to a level where field control is lost (Horowitz 1993). Resistance of *B. tabaci* to buprofezin had occurred in ornamental crops in the Netherlands (Katagiri, Nihon Nohyaku, pers. comm.) where it was associated with 20 applications in one crop season.

In New Zealand, the current label recommendation for greenhouse whitefly is to use no more than three applications of buprofezin during any crop season. Our observations indicated that this has usually been observed. However, even when the

product was used according to label recommendation, it was sometimes applied when whitefly numbers per plant were very high. It is unlikely that resistant whitefly have transferred between the two geographic regions. One explanation for the occurrence in two widely separate geographic areas of such a high level of resistance in less than 3 years is that the resistant genotype was already present throughout the country at a very low level before the full registration and widespread use of the product.

Unlike Israeli cotton growers, it is not practical in New Zealand to restrict use of buprofezin to 1 month of the year on greenhouse crops. It is now recommended that buprofezin is not used more than twice within any 12-month period and that it is used as a mixture with an adulticide, eg. methomyl. In areas with resistant whitefly, it is possible that the level of resistance may decline to a negligible level if buprofezin is not used for 2 or 3 years. This hope is supported by the finding of a much lower level of resistance in the Mangere population (Table 2) where buprofezin had not been used for 9 months and where there were nearby sources of whitefly.

Because buprofezin kills juvenile whitefly when they moult, bioassays must allow time for treated whitefly to pass through to the next nymphal stage. The Israeli tests with *B. tabaci*, involved dipping leaves of cotton seedlings into buprofezin suspension and assessing whitefly mortality when eggs laid on the water-dipped control leaves had reached fourth instar. Healthy seedlings ensured leaf quality was maintained for the full period of whitefly growth. The disadvantage of using seedlings is that it is difficult to apply a known dose of pesticide to the leaves.

Spraying leaf discs with a Potter tower enables accurate doses of insecticide to be applied but it is more difficult to maintain the leaf discs in good condition to support healthy growth of whitefly or other insects. While leaf portions embedded on agar remained alive and whitefly nymphs completed development, there was high and variable mortality in the water-treated controls which suggests that leaf, and hence nutritional, quality was not adequate for the time (7 days at 25°C) to develop from first to fourth instar. The time needed to keep a leaf portion on agar could be reduced by treating late instar nymphs. However, it is difficult to obtain uniform batches of late instar whitefly. This is critical because the toxicity of buprofezin decreases with nymphal stage; first instar being very sensitive (LC_{50} 0.6 mg/litre) and fourth instar least sensitive (LC_{50} 16 mg/litre) (Anon 1985). It was easiest to obtain newly emerged first instar nymphs.

It would be desirable to improve the bioassay so that control mortality was reduced to less than 10%. However, the level of resistance found was so large that there were clear differences between populations, even when only three concentrations of pesticide were compared (Table 2). If there is a need to test other whitefly populations for resistance to buprofezin, a test using three concentrations, 1.25, 12.5 and 125 mg/litre is recommended.

The difference in susceptibility of first instar nymphs to buprofezin, presents an opportunity to test progeny from mating adults from resistant and susceptible populations and hence study the genetics of the resistance mechanism. These studies could assist development of resistance management strategies for greenhouse crops and tamarillos.

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