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COMMODITY TREATMENT AGAINST INFESTATIONS OF THE CUCUMBER FLY, DACUS (AUSTRODACUS) CUCUMIS FRENCH, IN CUCUMBERS

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SUMMARY

Cucumbers infested with the cucumber fly, *Dacus* (*Austrodacus*) *cucumis* French, were successfully disinfested with 12 g m^{-3} 1, 2-dibromoethane (EDB) over 2 h at 20°C. Complete mortality of more than 30 000 individuals was achieved. This is equivalent to 99·99% mortality at the 95% confidence level, as required for a fruit disinfestation treatment within Australia.

I. INTRODUCTION

The cucumber fly, *Dacus* (*Austrodacus*) *cucumis* French, is sometimes a serious pest of cucurbits in Queensland. Quarantine authorities in Victoria have for many years required measures to prevent its establishment in that State. In the absence of accepted commodity treatments cucurbits have been subject to 100% inspection on arrival in Melbourne, with a consequent increase in costs to both grower and consumer.

The standard of control specified for disinfestation of fruit for fruit fly infestation by the Australian Fresh Fruits Disinfestation Committee for the purpose of inter-State trade is 99·99% mortality for both eggs and larvae. The efficacy of a treatment is to be determined by comparing the survival to the pupal stage in untreated and treated samples. The number of individuals to be tested at any specified level of control is found from the binomial distribution. At the 95% confidence level the specified mortality of 99·99% is achieved after 30 000 individuals have been obtained in the controls, with no survivors in the corresponding treated samples. The total of 30 000 for the controls is obtained by summing the results from a number of tests at a particular dosage.

The purpose of this present work was to demonstrate an acceptable commodity treatment for cucumbers which would eliminate the need for the detailed inspection carried out by the Victorian authorities. The work was carried out under the aegis of the Australian Fresh Fruits Disinfestation Committee.

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II. MATERIALS

The following materials were used at the concentrations indicated:—

EDB—A liquid containing at least 99·99% 1, 2-dibromoethane used at dosages from 0·5 to 12 g m^{-3}.

"Zephiran A"—A concentrate containing 10% v/v alkyldimethyl-benzyl-ammonium chlorides used at 0·05% v/v.

III. METHODS

Cucumbers, purchased from the Brisbane markets at Rocklea, were cleaned by washing in tap water and placed on blotting paper to dry.

In preliminary bioassay tests against mature larvae equal numbers of larval-infested cucumbers were used for treatment and control at any one dosage of fumigant. In the main tests (requiring no survivors in the treated samples but more than 30 000 survivors in the controls) the number of fruit treated at each test was five times that of the control, as laid down by the Fresh Fruits Disinfestation Committee. The number of survivors in the controls at each main test was multiplied by five and the totals for consecutive tests were summed until the grand total exceeded 30 000.

For larval fumigations, cucumbers were infested by injecting 20 ml of a suspension of mature larvae in a rearing medium into the hollowed-out fruit. The larvae were from a laboratory colony originally established by the University of New South Wales, Sydney. The rearing medium comprised:

Boiled pumpkin	100 g
"Torula" yeast	4 g
Hydrochloric acid (Conc.)	0·6 ml
"Nipagin M" (methyl p-hydroxybenzoate)	0·6 g
Water	120 ml

The ingredients were homogenised in a blender. The medium containing the mature larvae was diluted to a suitable consistency with 0·05% Zephiran A immediately before infesting the fruit. Each cucumber was prepared by cutting off one end and drilling out the flesh in the main body of the fruit with a power drill fitted with a 2·5 or 3·2-cm-wide flat wood bit. For ventilation 100 holes were made into the cavity with a 21-gauge needle. The larval suspension was then injected into the cavity from a 50-ml plastic irrigation syringe. The appropriate cut-off end or cap (previously numbered with a felt-tipped pen) was waxed into place with a ring of hot paraffin wax and further secured by strips of clear adhesive tape placed over the end.

For egg treatments the cucumbers were pricked 30 times with a 21-gauge needle along one face marked with a felt-tipped pen. They were then infested by exposing them overnight, with the marked face uppermost, to adult flies in large cages at 25°C. In a preliminary test equal numbers of egg-infested cucumbers were used in both the treated and the control. In the main tests (requiring no survivors in the treated samples, but more than 30 000 survivors in the controls) the number of fruit treated at each test was five times that of the control. As with the main tests for larvae the number of survivors in the controls at each main egg test was multiplied by five and consecutive totals were summed until the grand total exceeded 30 000.

The required number of ventilation holes for larval infestation was decided on after initial replicated experiments. In these experiments the oxygen concentration in the air cavity in fruit prepared as described was checked with a Carle 6500 thermistor-detector gas chromatograph at 2·5 to 3 h and 26·5 to 27 h after larval infestation. The instrument was fitted with an oxidation gas-analysis package comprising 122 cm parallel silica gel and molecular sieve columns. A hole was made with a 21-gauge needle into the air cavity in a prepared cucumber held in the upright position and a sample of air was withdrawn immediately through the same hole with a 100 µl syringe for determination by gas chromatography. The area of sample traces from infested and uninfested cucumbers (peak height x width at half height) in mm^2 on a 1-mV recorder was compared with the standard for 100 µl of atmospheric air corresponding to 21% oxygen. Larval survival in these experiments was determined from pupal counts.

Details of the fumigation chamber, the method of fumigation and airing were as given previously (Swaine *et al.* 1975). Cucumbers for fumigation were packed into commercial half-bushel (10 kg) wooden boxes. Fruit for larval fumigation was kept at 20°C and larval suspensions were at that temperature when injected into the hollowed-out fruit. Cucumbers infested with larvae were so placed in the boxes that the replaced cap was uppermost in the chamber, thereby preventing it falling off and the larvae being lost during the course of fumigation. Fruit for egg fumigation was held several hours at 20°C prior to fumigation at that temperature. After fumigation and airing cucumbers infested with larvae were placed on organdie-covered plastic boxes in rearing cages (Swaine *et al.* 1975). Cucumbers infested with eggs were removed from the boxes in which they had been fumigated and placed on the rearing medium described previously, and held in rearing cages to obtain survivors as pupae. The effect of a fumigation was determined by reference to the number of pupae obtained from the control fruit held in rearing cages.

Inorganic bromide residues in cucumbers fumigated with EDB were determined by X-ray fluorescence (Getzendaner *et al.* 1968).

IV. RESULTS AND DISCUSSIONS

The results of the preliminary bioassay with EDB against mature larvae of *D. cucumis* in cucumbers (table 1) were analysed by the standard probit method detailed by Finney (1971). This method has been used previously for fumigation bioassay involving separate controls for each dosage level by Shaw and Lopez (1954) and by Seo *et al.* (1971). The modified procedure suggested by Wadley (1949), which is based on an assumed Poisson distribution and on a bioassay using a single control for all dosages, is not appropriate to our data. Our regression equation for the log dose/probit mortality line was $Y = 2 \cdot 19X + 5 \cdot 84$, giving a 99·99% mortality at 9·7 g m^{-3} EDB over 2 h at 20°C. The 5% fiducial limits for this dosage ranged from 6·0 to 21·0 g m^{-3} EDB. In the main series of tests with larvae, and for which a minimum of 30 000 survivors in the controls was required, a dosage of 12 g m^{-3} was selected. There were no survivors in the treated fruit from any of the seven tests at this dosage, whilst 40 886 pupae were obtained in the controls.

In a preliminary test using 4 g m^{-3} EDB over 2 h at 20°C against infestations of eggs, there were no survivors in the treated fruit but 12 831 pupae were obtained from the controls. For the main tests with eggs a dosage of 8 g m^{-3} over 2 h at 20°C was selected and in two tests there were no survivors in the treated fruit but 33 174 in the controls.

TABLE 1

BIOASSAY OF EDB FUMIGATION OVER 2 h AT 20°C AGAINST MATURE LARVAE OF *Dacus (Austrodacus) cucumis* FRENCH IN CUCUMBERS

Dose of EDB ($g\ m^{-3}$)	Number of Larvae in Control	Mortality in Treated (%)
0·5	2 250	46·36
1·0	1 788	80·98
2·0	2 187	97·76
3·4	2 778	99·75
4·0	2 907	99·93
5·0	2 913	99·42
6·0	3 244	99·20
8·0	14 716	99·95
9·0	6 536	99·95
10·0	8 152	100
12·0	10 856	100

Log dose/probit mortality regression

$Y = 2{\cdot}9120X + 5{\cdot}8418$

LD 99·99 = 9·7 g m^{-3} EDB

TABLE 2

EFFECT OF DIFFERENT NUMBERS OF VENTILATION HOLES ON OXYGEN CONCENTRATION INSIDE HOLLOWED-OUT CUCUMBERS ARTIFICIALLY INFESTED WITH LARVAE OF *Dacus (Austrodacus) cucumis* FRENCH AND ON THE CORRESPONDING SURVIVAL OF LARVAE

No. of Holes per Cucumber	Mean Oxygen Concentration (%) in Sealed Fruit Cavity at Specified Time after Infesting				Mean No. of Pupae	Mean No. of Dead Larvae	Mean Pupation (%)
	2·5 to 3 h		26·5 to 27·0 h				
	Infested	Not Infested	Infested	Not Infested			
0	1·3	19·1	0	15·9	0	196·3	0
20	2·4	18·7	0	16·6	6·3	136·0	4·4
40	2·4	18·7	10·0	19·2	89·3	39·3	69·4
80	8·0	19·2	16·3	18·0	151·3	1·7	98·9
160	8·3	19·2	16·3	20·2	142·3	3·0	97·9
320	13·7	19·9	15·3	19·9	113·7	1·0	99·1

Results are means of 3 tests.

Holes made with a 21 guage needle into the air cavity of hollowed-out fruit containing 20 ml of pumpkin medium with or without larvae.

Oxygen concentration in atmospheric air—21%.

TABLE 3

INORGANIC BROMIDE RESIDUES IN CUCUMBERS FUMIGATED WITH 12 g m^{-3} EDB OVER 2 h AT 20°C

Treatment	No. of Days after Fumigation	Inorganic Bromide Residues (ppm in Fresh Weight)	
		Test 1	Test 2
Control	0	1·5	1·4
Treated	0	2·7	2·4
Treated	1	2·5	2·6
Treated	3	2·9	2·9

The need for ventilation holes in the fruit infested artificially with larvae is shown by the results in table 2. Poor pupation is clearly correlated with low oxygen concentration in the fruit cavity. Comparison of the oxygen concentrations in larval-infested and in uninfested fruit shows that oxygen depletion was due mainly to the larvae, which used up the contained oxygen in respiration. Where there was significant survival (40 to 320 holes) increase in oxygen concentration at 26·5 to 27·0 h was probably due to the pinned holes being enlarged by the mature larvae as they emerged through them to pupate outside the fruit, thus allowing air to enter from the atmosphere. On the basis of these results it was decided to adopt 100 holes per fruit.

Data on inorganic bromide residues in treated fruits are given in table 3. The residues reached a maximum of 2·9 ppm inorganic bromide after 3 days and were, therefore, well below the maximum level of 20 ppm inorganic bromide recommended by the Australian National Health and Medical Research Council.

Since the work was completed, quarantine authorities in Victoria have concluded that *D. cucumis* is unlikely to establish itself in Victoria and this avoids the immediate need for this disinfestation treatment in Australia.

Based on the work reported here, fumigation with EDB at 12 g m^{-3} over 2 h at 20°C has been accepted by the Australian Fresh Fruits Disinfestation Committee as a satisfactory method of disinfesting cucumbers for *D. cucumis*. The method is, therefore, available should quarantine considerations demand its use.

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