RESISTANCE TO MALATHION AND DICHLORVOS IN STORED-PRODUCT INSECTS IN NEW SOUTH WALES

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ABSTRACT: Malathion resistance has been monitored in New South Wales in *Tribolium castaneum* since 1968, *Sitophilus oryzae* since 1970 and *Rhyzopertha dominica*, *S. granarius* and *T. confusum* since 1971 by exposing adult insects to filter paper impregnated with malathion. Dichlorvos resistance was detected in *R. dominica* in 1972 by the same method, modified to prevent loss of the dichlorvos vapour, and by exposing caged adults to dichlorvos vapour in sealed one-litre flasks.

The survey has shown rapid emergence of resistance problems in *T. castaneum*, *S. oryzae* and *R. dominica* only a short time after malathion resistance was first detected. Resistance to both malathion and dichlorvos was diagnosed in *R. dominica*, though *T. castaneum* and *S. oryzae*, surveyed up to 1973, were susceptible to dichlorvos. There has been one occurrence of malathion-resistant *S. granarius*. Malathion-resistant *T. confusum* occurred in only a few localities.

INTRODUCTION: The climate of eastern Australia is very favourable to the development of insects that infest stored grain and enables them to persist from season to season on wheat-growing farms in New South Wales [1], [2]. Up to 192.6 million bushels per annum of wheat is taken into bulk storage in New South Wales, some 70% of the crop being exported. Smaller quantities of other grains including sorghum, maize, barley, oats and rice are produced for home consumption and export.

Application of malathion to grain on intake at the bulk storages since 1963 helped to prevent insect infestation of the stored grain and safeguarded grain exports. Malathion resistance, which had been recorded earlier in northern Nigeria [3], Florida and Georgia in the United States of America, [4] and Queensland, Australia [5], was detected in rust-red flour beetle, *Tribolium castaneum* (Herbst) in New South Wales in 1968 [6]. As this posed a threat to the continued usefulness of malathion for grain protection, a survey to detect malathion resistance in this insect was continued. The survey was expanded to include rice weevil, *Sitophilus oryzae* (L) in 1970, lesser grain borer, *Rhyzopertha dominica* (F), granary weevil, *Sitophilus granarius* (L.) and confused flour beetle, *Tribolium confusum* Jacq. du Val in 1971. The possible occurrence of resistance to dichlorvos, used as an alternative insecticidal grain treatment in New South Wales, was also investigated with *R. dominica*, *S. oryzae* and *T. castaneum*.

METHODS: Samples of stored-product insects were collected from bulk grain storages, stock-feed mills, flour mills, produce stores, seed-cleaning premises, and farms throughout New South Wales, from rail trucks used to transport bulk grain and from the terminal grain elevators at Newcastle and Sydney. From 1968 to the beginning of 1970 only *T. castaneum* was retained for resistance testing. From 1970 onwards field samples of *S. oryzae*, *S. granarius*, *R. dominica* and *T. confusum* were obtained in addition to *T. castaneum*.

Adult insects from field samples were placed in medium to establish each sample in culture. For the *Tribolium* spp. the medium used was a mixture of finely-ground wholemeal flour and dried yeast powder (12:1 by weight). *Sitophilus* spp. and *R. dominica* were cultured in whole wheat grain brought to approximately 12 per cent moisture content after heat sterilisation.

Insects separated from the medium were tested at 25°C and 55% relative humidity under fluorescent lighting.

Malathion tests - Adult insects were tested by exposure to malathion-impregnated filter paper according to a method developed by Champ [7]. Concentrations of malathion in Risella 17 oil, suitable for discriminating between susceptible and resistant strains of each species, were determined by dose-response testing. Exposure periods were: *T. castaneum* 5 hours; *T. confusum*, *S. oryzae* and *S. granarius* 7 hours; *R. dominica* 24 hours. The discriminating concentrations used were: *T. castaneum* 0.5%; *T. confusum*, *S. oryzae* and *S. granarius* 1.0%; *R. dominica* 2.5%. Tests of *T. castaneum* for malathion specific resistance involving carboxyesterase were made with 0.5% malathion in Risella 17 oil plus 10% triphenylphosphate [8].

Dichlorvos tests - (i) Contact tests: Adult insects were tested for dichlorvos resistance by exposure to dichlorvos in Risella 17 oil on filter paper using a method similar to that for malathion tests, modified to reduce loss of the volatile dichlorvos [9]. Concentrations of dichlorvos used for dichlorvos resistance screening tests were: *R. dominica* 0.1%, *T. castaneum* 0.04%, *S. oryzae* 0.1%. Exposure periods were: *R. dominica* and *S. oryzae* 5 hours; *T. castaneum* 2.5 hours.

(ii) Fumigation tests: Adult *R. dominica*, caged in nylon gauze, were exposed to dichlorvos vapour in sealed 1 litre flasks for 60 minutes. The dichlorvos dosage was 0.1 mg/litre as 10 microlitres 1% dichlorvos in ethyl methyl ketone, di-n-octyl phthallate (40:1) solvent mixture, applied to filter paper and introduced immediately to the exposure chamber.

RESULTS AND DISCUSSION: 1. Diagnosis of malathion resistance - (a) *Tribolium castaneum:* Dose-response testing of progeny of *T. castaneum* samples collected when the survey commenced in 1968 demonstrated the applicability of the discriminating concentration of 0.5 per cent malathion for diagnosing malathion resistance, first detected locally in a sample (NTC7) from a Sydney provender mill. Resistance of the order of approximately x30 to x50 at the LC50 was indicated for progeny of samples collected from a number

TABLE I. Responses to Malathion and Dichlorvos of Stored-Product Insects From New South Wales Exposed to Insecticide-Impregnated Paper.

Insecticide and Species	Strain	Exposure Time (hr)	LC50(%)	Resistance Factor	LC99.9(%)	Resistance Factor	Slope	χ^2 (DF)
Malathion								
T. castaneum	NTC138 Susceptible	5	0.18	-	0.39	-	9.3	0.03(2)
	NTC220 Resistant	5	29	160 ± 14	>100	>256	3.4	1.0(4)
R. dominica	NRD83 Susceptible	24	0.45	-	1.6	-	5.6	2.2(4)
	NRD9 Resistant*	24	ca 4.2	ca 9.3	-	-	-	-
	NRD119 Resistant	24	29	65 ± 4	>100	>62	3.9	10.8(3)
S. oryzae	NSO30 Susceptible	7	0.26	-	0.74	-	6.7	4.9(4)
	NSO61 Resistant	7	0.88	3.4± 0.2	3.05	4.1 ± 0.2	5.7	2.6(4)
S. granarius	NSG1 Susceptible	7	0.12	-	0.28	-	8.3	2.8(2)
	NSG4 Resistant	7	0.31	2.6 ± 0.1	1.17	4.2 ±0.3	5.4	3.8(3)
Dichlorvos								
R. dominica	NRD83 Susceptible	5	0.021	-	0.19	-	3.2	4.0(4)
	NRD9 Resistant*	5	0.22	ca 10	1.11	ca 6	4.4	-
	NRD119 Resistant	5	0.33	16 ± 1	0.78	4	8.3	1.1(3)

* Heterogeneous strain.

of localities in the first year of the survey [6]. A sample (NTC220) collected from a bulk grain storage in northern N.S.W. in the fourth year of the survey showed resistance of approximately x160 at the LC50 (Table 1).

(b) *Tribolium confusum:* Samples of *T. confusum* were screened for malathion resistance by exposing adults for 7 hours to the discriminating concentration of 1.0 per cent malathion on paper. In initial tests a small series of doses was used for each sample but numbers of adults obtained were insufficient for fully replicated dose-response tests.

(c) *Sitophilus oryzae:* Malathion resistance in *S. oryzae* in Australia was first indicated in samples collected from the Newcastle terminal elevator, tested by Dr. B. R. Champ, CSIRO, in 1970. Small samples of weevils collected at the terminal and from produce stores at nearby centres were tested by exposure to malathion-impregnated paper doses of 0.5, 1.0 and 2.0 per cent malathion. Survival of weevils after exposure to the highest dose indicated possible resistance in samples from the grain terminal and from a produce store at Newcastle.

Replicated dose-response tests were performed with progeny of local samples to obtain response data for susceptible strains e.g. NS030, a produce store in the Sydney metropolitan area (Table 1). The discriminating concentration of 1.0 per cent malathion based on these data was used to screen samples for malathion resistance. Data for one of the resistant strains subjected to progeny testing - NS061 collected from a disused grain bin at Sydney terminal elevator in 1971 - is presented in Table 1.

(d) *Sitophilus granarius:* One instance of malathion-resistant *S. granarius* occurred at a flour and stock-feed mill at Sydney in January, 1971. This provided material for determining the response of the resistant strain (NSG4) in comparison with a susceptible strain (NSG1) given in Table 1. Tests on samples collected from other localities were performed using the discriminating concentration.

(e) *Rhyzopertha dominica:* In initial dose response tests in 1971, *R. dominica* adults ex cultures were exposed for 7 hours on papers impregnated with malathion at serial concentrations. From the response of susceptible strains a tentative discriminating concentration of 3.5% malathion in non-volatile oil was derived. This was used to screen a number of samples; survival of insects in one sample (RD9) collected at the Sydney terminal elevator indicated possible malathion resistance. Following advice from Dr. B. R. Champ, CSIRO, Canberra these samples were re-tested using a 24 hour exposure period, and the resistance confirmed. Samples were thereafter screened by 24 hr exposure to 2.5 per cent malathion on filter papers. Responses of typical malathion susceptible and malathion resistant strains from NSW are given in Table 1.

2. Distribution of malathion resistance in New South Wales - (a) *Tribolium castaneum:* The widespread occurrence of malathion-resistant *T. castaneum* in N.S.W. is shown in Figure 1. Instances of resistance detected in 1968-69 in this insect [6]

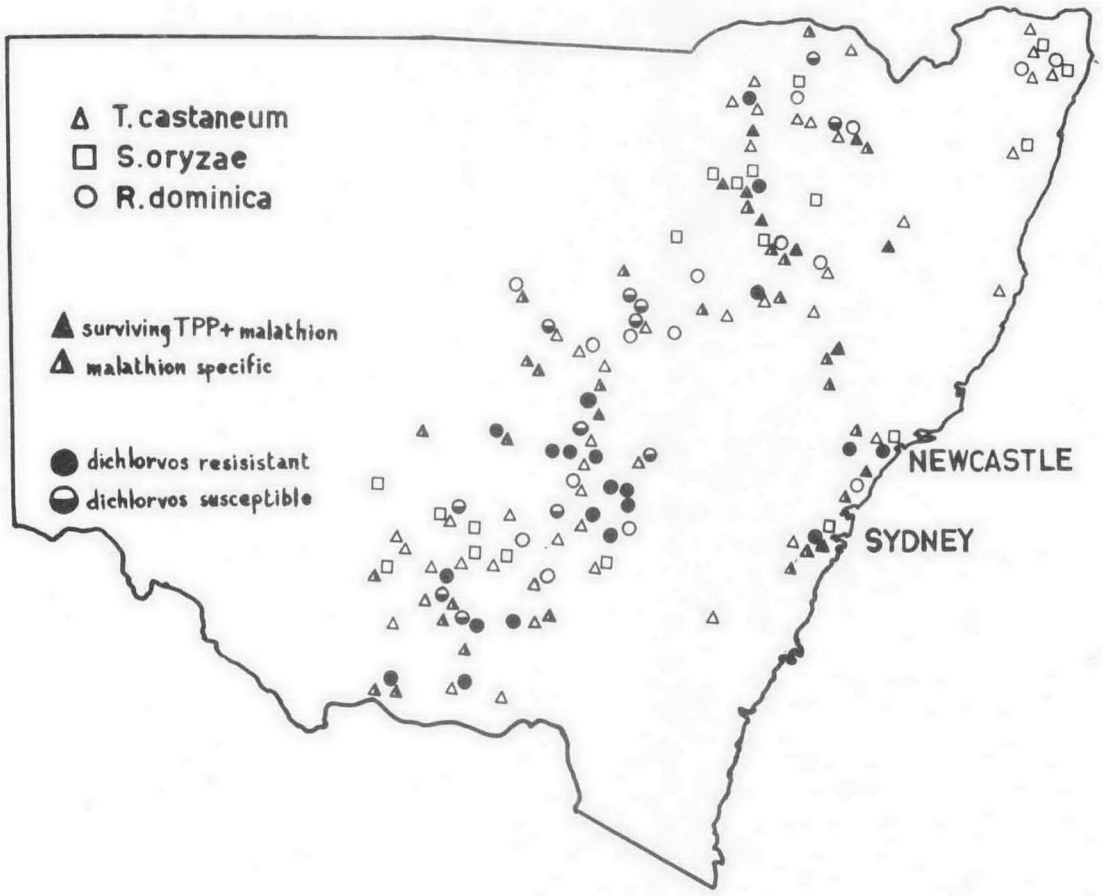

FIGURE 1. Collection localities in New South Wales of samples diagnosed malathion resistant 1968-1974.

were collected at stock-feed mills and produce stores in the Sydney metropolitan area and at 3 country centres in northern N.S.W. One resistant sample was taken from a rail truck of wheat delivered to the Sydney terminal elevator from a bulk grain storage in southern N.S.W. in 1968. One of 6 samples obtained from rail trucks in 1969/70 was malathion resistant. In 1970/71 malathion resistance was diagnosed from the Sydney terminal elevator and from bulk grain storages at 6 country centres. Subsequent samples from bulk storages throughout the grain handling system eventually were predominantly malathion resistant, as were samples from stock feed mills and produce stores at Newcastle, and Sydney and most country centres, from infestations of wheat, barley, maize, oilseeds and pelleted stock-feed. Malathion-resistant *T. castaneum* occurred on stock and poultry farms north of Sydney in 1970. The first instance of malathion resistance in farm stored grain was detected in central N.S.W. in 1971. Samples collected from wheat farms during surveys in 1968, 1970 and 1972 [1], [2], were susceptible to malathion. Few other occurrences of malathion resistance on grain farms were noted until 1973/74 when malathion-resistant *T. castaneum* was collected from 10 farms.

Tests with malathion plus triphenyl phosphate indicated

that the resistance detected during the first year of surveying this insect in New South Wales was malathion specific [6]. In later samples from 9 localities in northern N.S.W., from the Sydney metropolitan area and from one locality in central N.S.W., a proportion of the beetles survived exposure to malathion plus triphenyl phosphate. These occurrences are shown in Figure 1.

(b) *Tribolium confusum:* Malathion resistant *T. confusum* was detected in 1971 at 3 flour mills in the Sydney metropolitan area, and at one mill in southern N.S.W. In 1972, resistant confused flour beetles were collected from stock feed pellets at stables on the outskirts of Sydney. Susceptible samples were collected from many country centres and from Newcastle.

(c) *Sitophilus oryzae:* Malathion resistant *S. oryzae* was first detected at the Newcastle terminal elevator in May, 1970. Following this, samples for testing from produce stores and stock feed mills in the Newcastle and Sydney metropolitan areas and at centres where malathion resistant *T. castaneum* occurred earlier. Systematic surveying of similar premises was undertaken in southern, south-western and mid-western N.S.W. and samples were obtained from bulk grain storages.

Another instance was found at a produce store at Newcastle in June, 1970. Susceptibility of samples from other premises indicated that the resistant infestation at this produce store may have originated from the Newcastle terminal elevator, the source of wheat for this firm. Malathion resistance was later diagnosed from 2 flour mills at Sydney and from the Sydney terminal elevator. Samples from 23 country centres, from 3 premises at Newcastle and from 5 premises in the Sydney Metropolitan area were malathion susceptible to June 1971.

In the following year further instances of malathion-resistant *S. oryzae* were detected at bulk grain storages and provender mills at several country centres indicated in Figure 1. Samples from 34 localities were susceptible.

The incidence of living *S. oryzae* in samples collected from bulk grain storages declined thereafter and all samples tested from new localities were susceptible in 1972/73. This pest continued to occur frequently in samples from farms and private grain handling premises and in November, 1973 the first instance of malathion-resistant *S. oryzae* detected at a grain farm was collected in southern New South Wales. Ten other samples from farms in 1973/74 were susceptible. Resistant *S. oryzae* was also collected at produce stores at 3 centres on the north coast in 1974, at one private grain handling depot at a northern inland centre and again at the Newcastle terminal elevator.

(d) *Sitophilus granarius:* Malathion resistance was diagnosed in *S. granarius* collected in January, 1971 at a flour and stock-feed mill in Sydney where malathion-resistant *T. castaneum* and later *R. dominica* and *S. oryzae* also occurred. This was the only instance of malathion resistance detected in *S. granarius* in New South Wales. Eleven samples of *S. granarius* from farms, one sample from another flour and stock-feed mill and 3 samples from

private bulk-grain storages at country centres were malathion susceptible

(e) *Rhyzopertha dominica:* Malathion resistance was detected in *R. dominica* in May, 1971 in a sample collected from the Sydney terminal elevator in March, 1971, possibly the first instance of resistance in this species diagnosed anywhere in the world. Other instances of malathion-resistant *R. dominica* were detected from grain storages at 17 country centres in 1971/72 and at Newcastle terminal elevator. Samples from 35 localities in New South Wales were malathion susceptible.

In 1972/73 *R. dominica* was the predominant insect in samples from bulk grain storages and widespread malathion resistance in this pest in the grain-handling system was evident. Samples from both Sydney and Newcastle terminal elevators and from wheat storages at 15 country centres were diagnosed malathion resistant, also a sample from a privately-operated bulk barley storage. Samples from grain storages at 9 country centres were malathion susceptible. The pattern of occurrence of malathion resistant samples was repeated in 1973/74 at 17 country centres and both terminal elevators; samples from bulk grain storages at only 4 localities were malathion susceptible.

Malathion resistance was detected in this pest on farms in New South Wales for the first time late in 1973. Three instances of malathion resistant *R. dominica* have been recorded from farms now while there were susceptible samples from 5 farms in 1973/74. Resistant samples also occurred at produce stores at 2 centres on the far north coast, at one location between Newcastle and Sydney and at one grain depot in the northwest of the State. The locations at which resistance has been recorded in New South Wales are shown in Figure 1.

3. Worsening of the malathion resistance problem, 1968-1974 - The number of samples diagnosed resistant, suspect-resistant and susceptible to malathion is represented diagrammatically in Figure 2 for the 3 major pest species, *T. castaneum*, *R. dominica* and *S. oryzae*. The proportion of resistant samples increased for all 3 species as the survey progressed.

Considering suspect-resistant and resistant samples collectively as the resistant portion of the samples tested, the following percentages illustrate the rapidity with which the malathion resistance problem emerged in New South Wales.

For *T. castaneum* the resistant portion increased from 38% for the first year's samples to a consistently high level averaging 78% for the next 4 years and reaching almost 96% in 1973/74. For *R. dominica* the resistant portion increased steadily from 27 per cent in 1970/71 to 81 per cent in 1973/74. For *S. oryzae* the resistant portion was relatively constant, in the range from 24% to 29% for the 4 years from 1969/70, increasing to 52% in 1973/74.

4. Diagnosis of dichlorvos resistance - (a) *Rhyzopertha dominica:* Dichlorvos resistance was first diagnosed in *R. dominica* in October, 1972 by dose-response testing of cultures maintained for malathion resistance investigations. The dichlorvos resistance

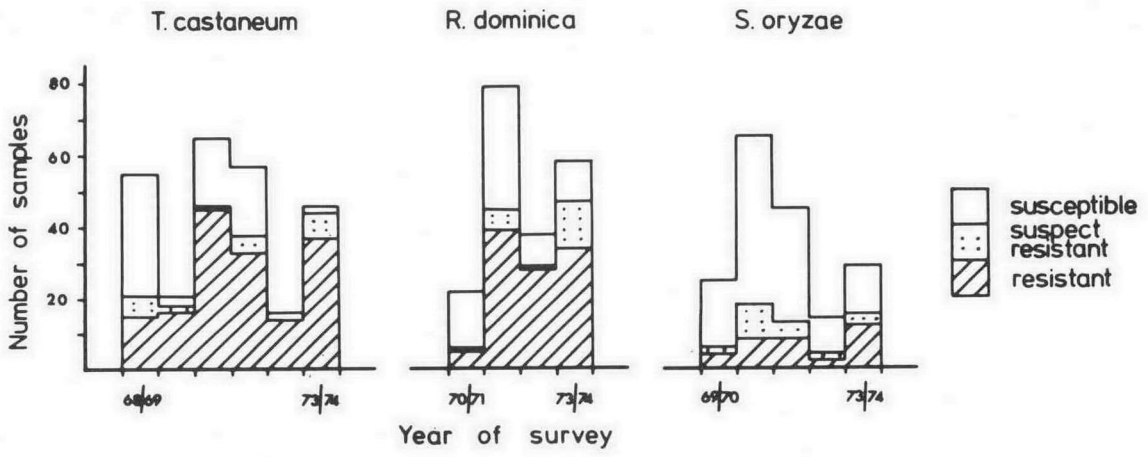

FIGURE 2. Malathion resistance diagnosed by discriminating doses in New South Wales.

of malathion-resistant *R. dominica* progeny of a sample (NRD9) collected from the Sydney terminal elevator in March, 1971, was evaluated, in repeated dose-response testing on dichlorvos-treated paper, in comparison with a malathion-susceptible field strain (NRD83). Dichlorvos resistance of the order of x10 approximately at the LC50 was detected in NRD9 (Table 1), the response indicating that this dichlorvos resistant strain is probably heterogeneous (Fig. 3). The dose-response line shown in Fig. 3 for the homogeneous resistant strain (NRD119) was obtained with a sample collected from a grain storage bin at Sydney terminal elevator in April, 1973. Response data for this strain is given in Table 1.

Contact screening: Following the detection of dichlorvos resistance in this species the resistance status of *R. dominica* cultures was investigated by exposure to selected concentrations of dichlorvos on filter paper. All cultures diagnosed dichlorvos resistant were all malathion-resistant also.

Fumigation test: An alternative rapid method of diagnosing resistance to dichlorvos in *R. dominica*, based on exposure of caged insects to the fumigant effect of dichlorvos in sealed 1 litre flasks was investigated. Results were obtained for 14 samples of *R. dominica*. The fumigation test proved useful, though not infallible, for indicating dichlorvos resistance. Several attempts to obtain dose-response data for a dichlorvos susceptible strain by this method were not completely successful, though the method appears to have potential as a simple rapid diagnostic test for dichlorvos resistance in stored-product insects.

(b) *Tribolium castaneum:* Dose-response tests with dichlorvos treated papers for 2 cultures of *T. castaneum*, one formed of combined samples diagnosed malathion specific resistant and the other of combined samples surviving malathion plus triphenyl phosphate, indicated that the LC50 of both cultures approximated 0.015 per cent dichlorvos. This response is similar to that of malathion susceptible cultures to dichlorvos. Another 63 samples of *T.*

castaneum, screened with a single dose of dichlorvos were assessed as dichlorvos susceptible.

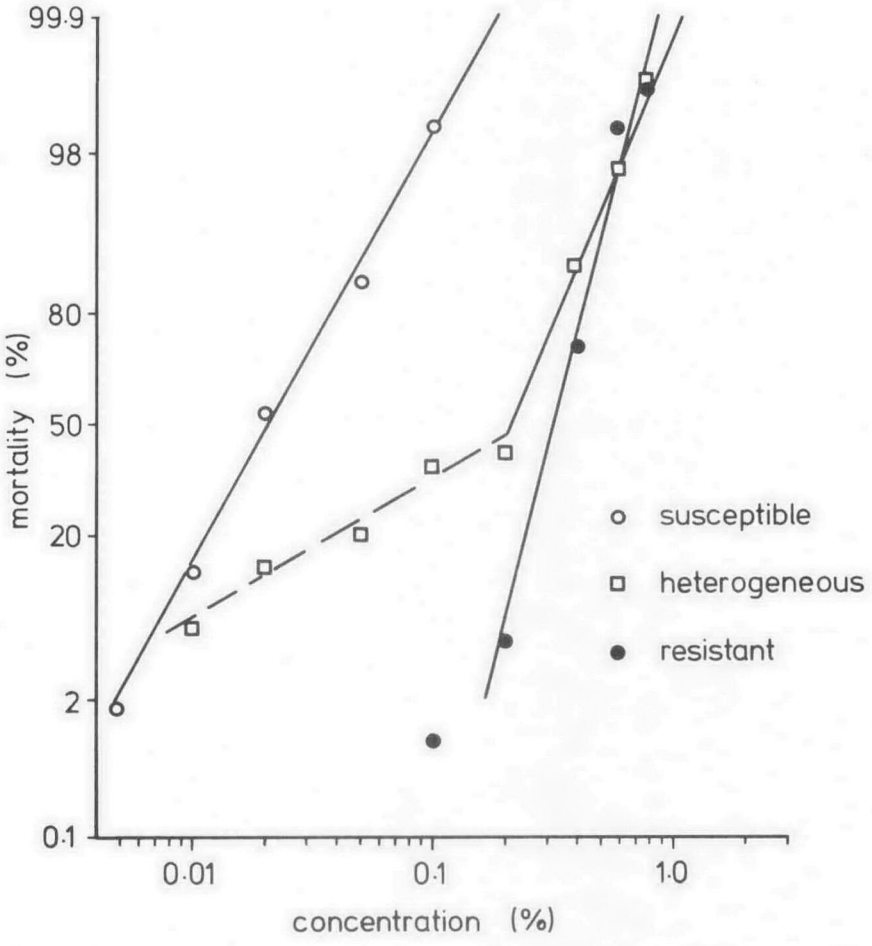

FIGURE 3. Dichlorvos resistance in *Rhyzopertha dominica* from New South Wales.

(c) *Sitophilus oryzae:* Dose response tests with dichlorvos treated papers for malathion resistant and susceptible cultures of *S. oryzae* indicated LC50 values of 0.018 per cent and 0.014 per cent dichlorvos respectively. Exposure of 22 samples of *S. oryzae* to a selected dose of dichlorvos indicated that they were all dichlorvos susceptible.

5. Distribution of dichlorvos-resistant *Rhyzopertha dominica* in New South Wales - Dichlorvos resistance (Fig. 1) was indicated initially in malathion-resistant *R. dominica* collected in 1971/72 from bulk grain storages at 12 country centres, from both Newcastle and Sydney terminal elevators and from a provender mill in Sydney, adjacent to the Sydney terminal elevator. Samples from 21 localities in New South Wales were dichlorvos susceptible, including both malathion-resistant and susceptible strains from bulk grain storages and farms. *R. dominica* collected at another 8 country

centres was diagnosed dichlorvos resistant subsequently. These were malathion resistant samples originating from bulk grain storages, a poultry farm and a privately-operated grain depot.

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