

INSECTICIDE RESISTANCE IN PHTHORIMAEA OPERCULELLA (Zell.) WITH PARTICULAR REFERENCE TO DDT

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SUMMARY

Strains of *Phthorimaea operculella* (Zell.) from tobacco-growing and potato-growing areas of Queensland have been found resistant to DDT and endrin. No completely susceptible strains of *P. operculella* have been found in Queensland even in areas where little or no insecticide has been used and tolerance of local strains has been referred to a susceptible strain from Victoria. The compound N,N-di-n-butyl-p-chlorobenzene sulfonamide had no influence on DDT-resistant strains. Removal of insecticide pressure did not lower DDT tolerances significantly after 16 generations in culture.

No resistance to azinphos-ethyl has been recorded and attempts to induce resistance to this material have been unsuccessful over 16 generations of selection.

I. INTRODUCTION

The first recommendations of DDT for control of *Gnorimoschema operculella* Zell. (= *Phthorimaea operculella* (Zell.)) in tobacco (Cannon 1946) and potatoes (Caldwell 1946) were in 1946 and until 1957-58 a period of adequate control was maintained. The first claims of reduced efficacy in both crops were made about this time but were discounted generally in favour of poor spray coverage, poor formulation and similar theories, some of which were in fact true. The increased use of aerial and boom spraying and the increase in size of individual plantings, with consequent dependence on these mechanized forms of pesticide application, contributed to the poor coverage and the position was aggravated by the comparatively undeveloped state of particular methods. It also seemed likely that there was an independent upsurge of pest numbers, but concrete evidence of this is lacking. One clear point, however, was that DDT was not giving the spectacular control afforded when it was first introduced. Statements at the time from reliable growers whose application methods had not changed indicated field resistance. A general move away from DDT began and endrin, already in use in tobacco pest

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control (Smith 1954), was the major replacement material in potato fields. Apart from DDT resistance, this material had the advantage of giving higher and faster kills of adults and operated to a greater extent against larvae in mines. Subsequently, and after claims of reduced efficacy of endrin, "Telodrin" (Smith and Saunders 1960) and then azinphos-ethyl (Saunders 1963) have come into use.

The present report covers confirmation of DDT resistance in *P. operculella*, using adults as test material. Necessarily potatoes are considered as well as tobacco. Typical larval responses to DDT are presented for comparison. The status of endrin and of azinphos-ethyl, representing two other groups of insecticides, is defined.

II. MATERIALS AND METHODS

Insect Material.—Adults from field generation larvae only were used to establish resistance levels. Infested leaf material was collected in paper bags and on arrival at the laboratory the larvae were transferred to fumigated potato tubers and allowed to complete development at 25°C. Adults were removed daily, held for 24 hr without feeding and then dosed.

For testing larvae, eggs collected from cotton-gauze tops of 2-lb glass holding bottles were allowed to hatch in plastic bags and then transferred to tobacco plants, from which larvae were removed as required for testing.

A susceptible strain of *P. operculella* kindly provided by Mr. R. van Baer of the Department of Agriculture, Victoria, was used as reference strain in all investigations. No susceptible strain has been found in Queensland.

Insecticides.—Technical grade DDT, endrin and azinphos-ethyl were used for topical testing. A commercial emulsion concentrate containing 25 per cent. *p.p'*-i DDT in toluol was used for dipping tests.

Testing Methods.—All testing was done at 25°C.

Insecticides were applied to adults topically from a microsyringe with a jet needle similar to that described by Hewlett (1954). The solvent used was 1:4 risella oil, dioxane, and the drop size 0.2 μ l. Drops were applied to the ventral surface of the thorax of carbon dioxide anaesthetized subjects, which after treatment were held without feeding for observation at 24 and 48 hr.

Series of graded doses were used for each day's emergence and data then bulked for the whole emergence pattern. Hence the number of individuals treated at each dose may vary, as all insects available were used. Sexes were not separated but results are based on female responses only. Male responses were heterogeneous and though conforming to discriminating dose levels gave unreliable dosage mortality relationships.

For first-instar larvae, tobacco plants were dipped in a range of concentrations of diluted DDT emulsion concentrate, dried, and held for 4 hr, after which 50 newly hatched larvae were placed on each plant. Successful entries were recorded 48 hr later. Four replications were used.

For second-instar larvae, 50 first-instar larvae were transferred to each of a series of tobacco plants and allowed to develop to second instar. All larvae not second instar were removed and the infested plants then dipped as for first instar and mortalities recorded at 48 hr. Four replications were used.

Third-instar larvae were removed from tobacco breeding plants, dosed as for adults and held in batches with cape gooseberry (*Physalis peruviana* L.) leaves for observation of mortality at 48 hr. Cape gooseberry leaves were found to be satisfactory and readily available as a medium for holding treated larvae. The insects mined readily and the long petiole enabled isolated leaves to be placed in disposable paper holding cups with the petiole passing through the base of the cup and immersed in a container of water below.

Fourth-instar larvae were removed from tobacco breeding plants, weighed and dosed as for adults. Larvae were held individually with cape gooseberry leaves as for third instars and mortality recorded at 48 hr.

In topical application to third (weight approx. 1.5 mg) and fourth (weight 3-12 mg) instar larvae, the full applied dose (0.2 μ l) was not retained, the surplus draining off onto the holder. All larvae were held identically and the drop placed on the dorsal surface of the thorax. This enabled comparison between strains, expressing dose as DDT content of applied solution.

III. RESULTS

(a) DDT

Adults.—Dose/time relationships (Finney 1962) for DDT for the susceptible strain are given in Table 1.

TABLE 1
DOSE/TIME RELATIONSHIPS FOR DDT AND THE SUSCEPTIBLE STRAIN
OF *P. operculella*

Transformations	Dose (μ g) = $\log x_1 + 2$				Time (hours) = $\log x_2$
Heterogeneity factor 0.56
Degrees of freedom 22
Parameters of regression equation—					
Position -4.140
Dose + Time (b_0) 3.122 \pm 0.292
Dose (b_1) 3.159
Time (b_2) 3.091
$b_1 - b_2$ 0.068 \pm 0.277
Interaction (b_3) 0.578 \pm 0.809
<hr/>					
				Median Dose	95% Mortality Dose
24 hours	0.35 μ g	1.19 μ g
48 hours	0.18 μ g	0.59 μ g

A discriminating dose of 2 μ g was chosen, representing a level at which mortality was complete in the susceptible strain.

Mortality data for the various strains under test are presented graphically in Figures 1, 2 and 3. The total number of individuals used to establish each point is included above each graph.

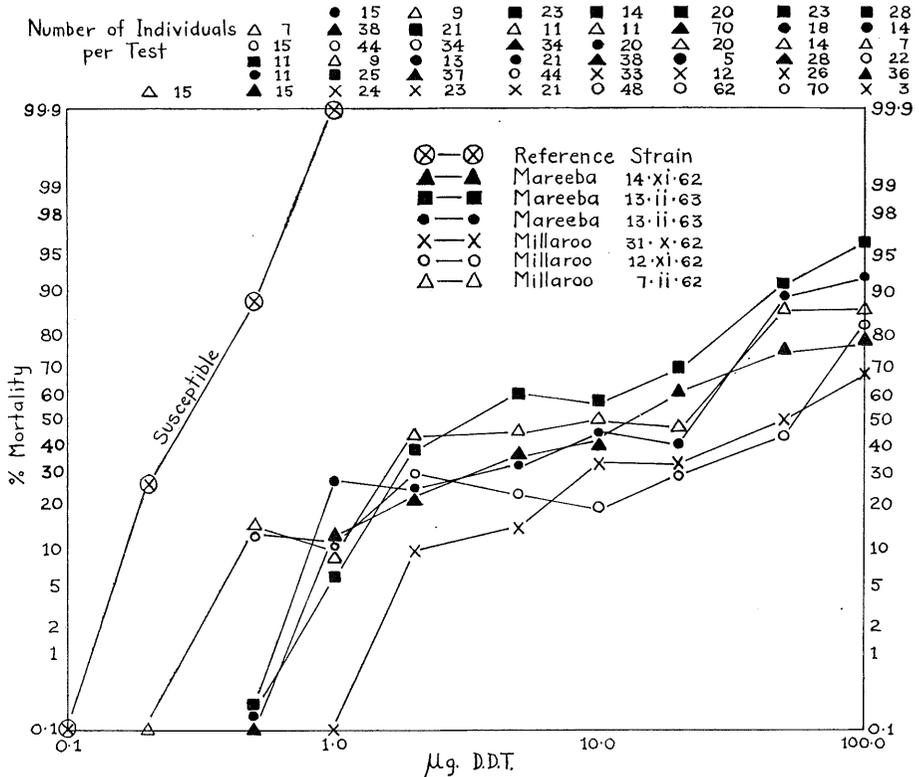


Fig. 1.—Responses to DDT of adult females of 6 strains of *P. operculella* from tobacco in northern Queensland.

No attempt has been made to establish relationships between mortality and dose as this is certainly not linear, and it appears there is little or no mortality change between 2 and 20 μ g. Doses above 100 μ g did not prove practical, as the average female weight is 6.5 mg (1.5 per cent. of body-weight).

The impossibility of defining median doses and resistance factors as such is evident. Attempts to purify resistant strains so that data can be obtained for different genotypes are in progress but to date have met with little success, though single-parent selections have shown greatest promise. Suffice to say *P. operculella* is highly resistant to DDT.

The compound N,N-di-n-butyl-p-chlorobenzene sulfonamide, DDT "Antiresistant" (AR), has been reported to increase the toxicity of DDT to DDT-resistant insects (e.g Anon. 1961). Using the material at the rate of 1 part to 5 parts of DDT, its ineffectiveness in *P. operculella* resistance is

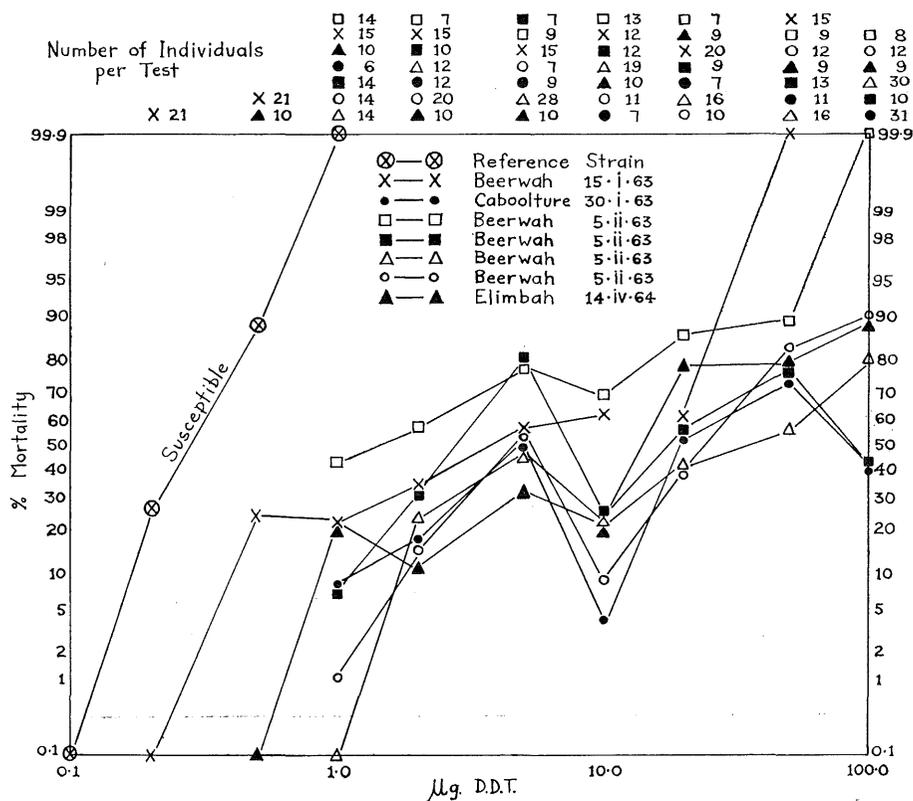


Fig. 2.—Responses to DDT of adult females of 7 strains of *P. operculella* from tobacco in southern Queensland.

demonstrated in Table 2, using three strains from different areas of the Mareeba district of North Queensland. The resistance appears stable; after 16 generations of culture without exposure to insecticide there has been little shift in the proportion of the population responding to particular doses (Table 3).

TABLE 2

A COMPARISON OF DDT AND DDT + "ANTIRESTANT", USING DDT-RESISTANT STRAINS OF *P. operculella* FROM MAREEBA

Dose (µg DDT)	Percentage Mortality at Indicated Dose				
	5	10	20	50	100
Strain 21A DDT	71	57	70	100	100
DDT + AR	81	43	64	100	100
Strain 21B DDT	45	30	40	89	92
DDT + AR	23	60	58	94	95
Strain 21c DDT	14	61	65	80	93
DDT + AR	60	64	50	80	85

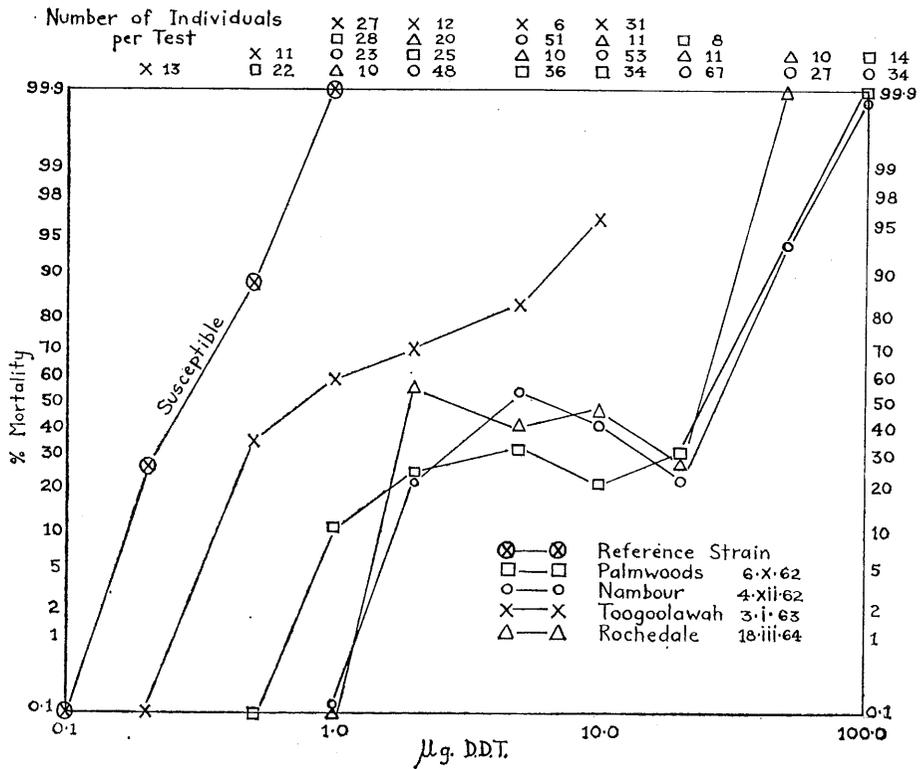


Fig. 3.—Responses to DDT of adult females of 4 strains of *P. operculella* from potatoes in southern Queensland.

TABLE 3

MORTALITY OF ADULT FEMALES OF A DDT-RESISTANT STRAIN OF *P. operculella* FROM MILLAROO AFTER 7 AND 16 GENERATIONS IN CULTURE WITHOUT EXPOSURE TO INSECTICIDE

(Number of individuals used in each test is given for each mortality level)

(Dose μg DDT)				50	100
Generation 1	44 (70)	82 (22)
Generation 7	94 (20)	100 (14)
Generation 16	83 (24)	79 (24)

Larvae.—A DDT-resistant strain from Millaroo, North Queensland, was used in these comparisons.

Percentage successful entries of newly hatched first-instar larvae into tobacco plants dipped in DDT emulsion at the concentrations indicated are given in Table 4 together with the mortality of second-instar larvae present

in leaf mines when dipped as for first-instar larvae. Differences in tolerance between the strains are marked for newly hatched larvae but are obscured for second-instar larvae by the poor penetration of DDT into leaf tissue.

TABLE 4

COMPARISON OF THE DDT SUSCEPTIBILITY OF DDT-SUSCEPTIBLE AND DDT-RESISTANT FIRST AND SECOND INSTAR LARVAE OF *P. operculella*

Dipping Concentration (% DDT)	0	0.001	0.002	0.005	0.01	0.025	0.05	0.1	0.2
First Instar (% Successful entries)—									
Susceptible	96	42	16	3	1	0	0	0	0
Resistant	81	50	73	52	35	32	14	8.5	2.5
Second Instar (% Mortality)—									
Susceptible	59	83	87	93	100
Resistant	43	60	56	84

A comparison of the DDT susceptibility of third- and fourth-instar larvae is given in Table 5. Doses are expressed as DDT content ($\mu\text{g}/\mu\text{l}$) of applied toxicant. Methods used for fourth-instar larvae were essentially similar to those of Way (1954) for individual mortality records of varying weight larvae. All fourth-instar larvae used were in the feeding phase. Differences between strains in tolerance to DDT are again evident.

TABLE 5

COMPARISON OF THE DDT SUSCEPTIBILITY OF DDT-SUSCEPTIBLE AND DDT-RESISTANT THIRD AND FOURTH INSTAR LARVAE OF *P. operculella*

Instar	Third		Fourth		Larval Weight (mg)
	Susceptible	Resistant	Susceptible	Resistant	
Heterogeneity factor ..	1.80	1.17	0.73	1.65	..
Degrees of freedom ..	4	6	18	34	..
Parameters of regression equation—					
Position	1.763	0.920	3.666	4.863	..
Dose	1.569 ± 0.439	1.279 ± 0.195	1.631 ± 0.297	0.818 ± 0.168	..
Weight	-3.206 ± 0.997	-2.263 ± 0.524	..
Median concentration ($\mu\text{g}/\mu\text{l}$)	0.58	7.74	0.25 1.22 2.81	1.54 16.0 37.9	3 7 11
95% mortality ($\mu\text{g}/\mu\text{l}$)	6.47	149	2.57 12.4 28.6	157 1,640* 5,700*	3 7 11
Transformations ..	Dose ($\mu\text{g}/\mu\text{l}$) = $\log\left(\frac{x}{5}\right) + 3$		Dose ($\mu\text{g}/\mu\text{l}$) = $\log\left(\frac{x_1}{5}\right) + 2$ Weight (mg) = $\log x_2$		

* By extrapolation

(b) Endrin

For probit analysis of endrin data, all doses (μg) have been transformed $\log x+3$ and times (hours) $\log x$.

Dose/time relationships for endrin for the susceptible strain and a resistant strain from Millaroo are given in Table 6. The greater heterogeneity and flattening dose time—mortality regression of the resistant strain are to be expected. A discriminating dose of $0.1 \mu\text{g}$ was chosen, representing a level at which mortality was complete in the susceptible strain.

TABLE 6
COMPARISON OF ENDRIN SUSCEPTIBILITY OF A SUSCEPTIBLE STRAIN
OF *P. operculella* AND A STRAIN FROM MILLAROO

Strain	Susceptible	Resistant
Heterogeneity factor	1.05	1.79
Degrees of freedom	44	28
Parameters of regression equation—		
Position	-6.819	-6.126
Dose + Time (b_0)	2.518 ± 0.510
Dose (b_1)	4.317 ± 0.365	2.879
Time (b_2)	3.730 ± 0.335	2.144
b_1-b_2	0.587 ± 0.254	0.735 ± 0.600
Interaction (b_3)	0.081 ± 0.654	-2.642 ± 1.914
Median dose—		
24 hours	$0.035 \mu\text{g}$	$1.09 \mu\text{g}$
48 hours	$0.019 \mu\text{g}$	$0.55 \mu\text{g}$
95% mortality dose—		
24 hours	$0.084 \mu\text{g}$	$4.91 \mu\text{g}$
48 hours	$0.046 \mu\text{g}$	$2.46 \mu\text{g}$

Comparisons of strains of *P. operculella* from various areas of northern and southern Queensland are included in Table 7. The data indicate resistance to endrin occurring in the areas listed. Resistance factors in parentheses have been derived for median doses, as unique factors are not possible because of significant changes in line slopes.

TABLE 7

COMPARISON OF THE SUSCEPTIBILITY TO ENDRIN OF STRAINS OF *P. operculella* FROM SOUTHERN AND NORTHERN QUEENSLAND

Strain		Date Collected	Median Dose (μg)			Regression Coefficient	Heterogeneity Factor and Degrees of Freedom	Comparisons with Reference Strain				Maximum Dose Tested any Survival (μg) and Corresponding % Mortality	Minimum Dose 100% Kill (μg) and No. of Individuals Used
Locality	Host		Upper Limit (0.05)	Estimate	Lower Limit (0.05)			Test	Position	Slope	Factor		
Reference	0.025	0.019	0.007	3.0 ± 0.9	0.98 (6)	0.05 (90)	0.10 (10)
Southern Queensland—													
Beerwah	Tobacco	15.i.63	0.38	0.31	0.23	3.7 ± 0.6	0.71 (4)	χ^2	42*	$8 \times 10^{5*}$	(16.3)	1.0 (97)	2.0 (19)
Beerwah	Tobacco	5.ii.63	1.31	0.25	0.054	2.0 ± 0.6	2.30 (4)	t	4.82*	0.90	19.0	1.0 (88)	2.0 (14)
Beerwah	Tobacco	5.ii.63	0.36	0.35	0.35	2.5 ± 0.9	2.58 (4)	t	4.52*	0.42	20.4	0.5 (67)	1.0 (7)
Caboolture	Tobacco	30.i.63	0.45	0.26	0.16	2.0 ± 0.4	0.93 (3)	χ^2	38.5*	$1 \times 10^{6*}$	(13.7)	1.0 (90)	N.A.
Elimbah	Tobacco	14.iv.64	0.65	0.54	0.45	4.1 ± 0.7	0.97 (3)	χ^2	49.9*	$9 \times 10^{11*}$	(28.4)	1.0 (81)	2.0 (10)
Palmwoods	Potato	6.x.62	..	0.70	(36.8)	0.5 (38)	1.0 (5)
Nambour	Potato	4.xii.62	0.51	0.084	0.064	1.6 ± 0.7	0.68 (2)	χ^2	16.5*	$7 \times 10^{5*}$	(4.4)	0.25 (82)	N.A.
Goomeri	Potato	8.i.63	..	0.23	(12.1)	0.5 (90)	N.A.
Rochedale	Potato	18.iii.64	0.38	0.28	0.16	3.2 ± 0.7	0.7 (3)	χ^2	25.7*	$7 \times 10^{5*}$	(14.7)	0.5 (82)	1.0 (16)
Northern Queensland—													
Mareeba	Tobacco	31.x.62	1.82	0.41	0.18	1.6 ± 0.5	3.07 (5)	t	5.22*	1.36	33.0	1.0 (86)	2.0 (7)
Mareeba	Tobacco	14.xi.62	1.18	0.58	0.36	2.0 ± 0.4	1.38 (4)	t	7.97*	0.99	38.8	1.0 (58)	2.0 (17)
Mareeba	Tobacco	13.ii.63	0.54	0.26	0.13	2.9 ± 0.8	3.48 (6)	t	5.51*	0.08	14.2	0.5 (90)	1.0 (13)
Mareeba	Tobacco	13.ii.63	0.41	0.33	0.26	3.7 ± 0.3	0.23 (4)	χ^2	58.7*	$9 \times 10^{5*}$	(17.4)	0.5 (70)	1.0 (13)
Mareeba	Tobacco	13.ii.63	0.39	0.35	0.31	4.3 ± 1.8	1.70 (2)	t	4.24*	0.61	16.7	0.5 (60)	1.0 (11)
Millaroo	Tobacco	12.xi.62	2.47	0.41	0.21	1.5 ± 0.3	1.78 (4)	t	7.56*	1.53	33.6	1.0 (90)	N.A.

* Significant at 0.05 level

N.A.= Not available

(c) Azinphos-ethyl

For probit analyses of azinphos-ethyl data, all doses (μg) have been transformed $\log x+3$ and times (hours) $\log x$.

Dose/time relationships for azinphos-ethyl for the susceptible strain and a DDT-resistant strain from Millaroo are given in Table 8. The greater contribution of the dose parameter to mortality changes is evident. Symptoms of intoxication appear within an hour at higher doses, and after 12 hr some flattening of the time curve can be expected. A discriminating dose of $0.2 \mu\text{g}$ was chosen, representing a level at which survival rarely could be expected in a susceptible strain. In fact, a dose of $0.1 \mu\text{g}$ was used, as this proved satisfactory for most samples. If survivals were recorded, further samples were examined where possible.

TABLE 8
COMPARISON OF AZINPHOS-ETHYL SUSCEPTIBILITY OF A DDT-SUSCEPTIBLE
AND A DDT-RESISTANT STRAIN OF *P. operculella*

Strain	DDT Susceptible	DDT Resistant
Heterogeneity factor	0.61	0.99
Degrees of freedom	14	29
Parameters of regression equation—		
Position	-8.787	-9.006
Dose (b_1)	6.760 ± 1.299	6.405 ± 0.802
Time (b_2)	2.054 ± 0.391	1.959 ± 0.321
b_1-b_2	4.706 ± 1.233	4.446 ± 0.764
Interaction (b_3)	-3.513 ± 4.942	3.512 ± 2.267
Median dose—		
24 hours	$0.042 \mu\text{g}$	$0.058 \mu\text{g}$
48 hours	$0.034 \mu\text{g}$	$0.047 \mu\text{g}$
95% mortality dose—		
24 hours	$0.073 \mu\text{g}$	$0.105 \mu\text{g}$
48 hours	$0.059 \mu\text{g}$	$0.085 \mu\text{g}$

Comparisons of strains of *P. operculella* from various areas of northern and southern Queensland are included in Table 9. There is no evidence to indicate any resistance to azinphos-ethyl. The strains under test had significantly flatter regression lines than the reference strain but the only significant change in position was in the strain from Rochedale, which was less tolerant.

TABLE 9

COMPARISON OF THE SUSCEPTIBILITY TO AZINPHOS-ETHYL OF STRAINS OF *P. operculella* FROM SOUTHERN AND NORTHERN QUEENSLAND

Strain		Date Collected	Median Dose (μg)			Regression Coefficient	Heterogeneity Factor and Degrees of Freedom	Comparisons with Reference Strain			Maximum Dose Tested any Survival (μg) and Corresponding % Mortality	Minimum Dose 100% Kill (μg) and No. of Individuals Used
Locality	Host		Upper Limit (0.05)	Estimate	Lower Limit (0.05)			Test	Position	Slope		
Reference	0.044	0.037	0.030	6.8 ± 0.6	0.02 (2)	0.05 (77)	0.075 (14)
Southern Queensland—												
Beerwah	Tobacco	15.i.63	0.026	0.020	0.014	3.3 ± 0.3	0.15 (2)	χ^2	2.8	2.9×10^{11} *	0.05 (88)	0.01 (15)
Beerwah	Tobacco	5.ii.63	0.029	0.016	0.008	3.4 ± 1.0	1.5 (5)	t	-4.1	3.7	0.05 (89)	0.1 (15)
Caboolture	Tobacco	30.i.63	0.045	0.030	0.021	5.1 ± 0.3	0.03 (1)	χ^2	0.53	2.6×10^{11} *	0.05 (86)	0.1 (9)
Elimbah	Tobacco	14.iv.64	..	0.035	..	3.3 ± 1.7	2.1 (2)	t	0.80	2.66*	0.075 (70)	0.1 (20)
Palmwoods	Potato	6.x.62	0.041	0.024	0.007	3.3 ± 1.0	1.7 (2)	χ^2	0.99	2.9×10^{11} *	0.1 (91)	N.A.
Nambour	Potato	4.xii.62	0.035	0.024	0.013	3.0 ± 0.6	0.59 (2)	χ^2	0.67	2.9×10^{11} *	0.05 (17)	0.1 (22)
Rochedale	Potato	18.iii.64	0.028	0.021	0.009	3.9 ± 1.1	0.85 (3)	χ^2	9.3*	2.0×10^{10} *	0.02 (40)	0.035 (23)
Northern Queensland—												
Mareeba	Potato	14.xi.62	..	0.024	..	2.1 ± 0.5	1.4 (1)	χ^2	0.08	2.9×10^{11} *	0.1 (91)	N.A.
Mareeba	Tobacco	13.ii.63	0.038	0.030	0.022	4.6 ± 1.0	2.3 (10)	t	-1.52	2.14	0.075 (97)	0.75 (23)
Mareeba	Tobacco	13.ii.63	0.037	0.032	0.026	5.7 ± 1.0	0.83 (3)	χ^2	0.65	2.8×10^{11} *	0.05 (89)	0.75 (23)
Millaroo	Tobacco	12.xi.62	..	0.038	..	3.1 ± 0.8	1.4 (1)	χ^2	0.35	2.9×10^{11} *	0.1 (88)	0.1 (17)

* Significant at 0.05 level

N.A. = Not available

INSECTICIDE RESISTANCE IN *PHTHORMAEA*

The first strain from Mareeba had the flattest mortality curve recorded. An attempt was made to select from this a strain resistant to azinphos-ethyl. Data in Table 10 show the responses over 15 generations, indicating no significant change in tolerance.

TABLE 10
MORTALITY RESPONSES DURING 15 GENERATIONS OF SELECTION FOR AZINPHOS-ETHYL RESISTANCE IN *P. operculella*

(Numbers in parentheses are total females tested. Groups in italics indicate test batches from which survivors were used as parents for following generation. Generations 1-10 were selected on females only. Generations 11-16 were selected on males and females by isolation of individual pupae before emergence)

Generation Number	Dose (μg)					
	0.01	0.02	0.035	0.05	0.1	0.2
1	<i>13 (16)</i>				91 (23)	
2		53 (119)				
3		20 (50)				
4		20 (107)				
5		26 (79)	43 (46)	55 (49)	90 (60)	95 (82)
6				49 (143)	85 (80)	
7				60 (111)		
8			From undosed individuals	100 (6)		
8				62 (53)		
9				27 (86)		
10				47 (160)		
11			67 (9)	100 (10)		
12			17 (6)			
13			4 (24)	15 (13)		
14			17 (23)		No progeny	
15			17 (12)	57 (23)		
16				25 (4)	No progeny	
16				75 (28)	100 (14)	

IV. DISCUSSION

The results do not represent all samples tested; other areas, e.g. Central Queensland (potatoes), have been examined and the picture is similar.

The general occurrence of DDT resistance throughout Queensland indicates that its effects must have been operating for some time before the first samples were tested in 1962. It would not be possible to determine precisely when and where resistance first appeared. Similarly, its manner of development is obscure. A definite possibility, however, lies in the treatment of seed potatoes in storage with a 2 per cent. DDT dust. This was first recommended for use in 1947 (Cannon 1947) and since has been more or less in general use on farms. *Prima facie* it appears that selection pressure would operate more effectively under these conditions than under conditions of use of DDT against field populations. The movement of both seed and table potatoes throughout the State would provide ready dissemination of new strains.

The presence of a lower level endrin resistance is also general. This appears to be a separate resistance and strains have been examined from outside Queensland which are susceptible to DDT but show a small but significant increase in tolerance to endrin. Separation of these resistances and other resistances—e.g. to DDD and lindane, both of which are present—will be considered in further reports together with corresponding tolerances of immature stages.

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