Aust. J. Biol. Sci., 1982, **35**, 427–40

Inheritance of Resistance to Chlorpyrifos in the Mt Alford Strain and to Diazinon in the Gracemere Strain of the Cattle Tick (*Boophilus microplus*)

B. F. Stone and N. J. Youlton

Division of Entomology, CSIRO, Long Pocket Laboratories,
Private Bag No. 3, Indooroopilly, Qld 4068.

Abstract

Reciprocal crossing of the Mt Alford (A) strain of the cattle tick *B. microplus* with a susceptible (S) strain and phenotype analysis of F_1, testcross and F_2 progeny showed that high chlorpyrifos resistance in strain A was due to two genes that were complementary and jointly exhibited incomplete dominance. Diazinon resistance in the Gracemere (G) strain appeared to be similarly inherited. The 'average' degree of dominance ('average dominance', D_{av}) of high chlorpyrifos resistance over susceptibility, exhibited by F_1 hybrids from A × S reciprocal crossings, was +0·54 on a −1 to +1 scale and was not significantly different from the parametric value of +0·5 (semi-dominance). The corresponding D_{av} values revealed by G × S crossings were +0·42 for diazinon resistance (significantly less than +0·5) and −0·031 for chlorpyrifos resistance (not significantly different from zero and therefore exhibiting zero dominance/recessivity). Resistance factors for chlorpyrifos in strains A and G for homozygotes were 74 and 35, respectively, and for F_1 hybrids were 25–29 and 5–7, respectively. The resistance factors for diazinon in strain G for homozygotes and F_1 hybrids were 174 and 37–41, respectively.

Introduction

Resistance to chlorpyrifos in the Mt Alford (A) and Gracemere (G) strains of the cattle tick *Boophilus microplus* was reported by O'Sullivan and Green (1971) who showed that these strains resembled the Biarra (B) and Ridgelands (R) strains, respectively (Roulston and Wharton 1967), in their resistance spectrum except that resistance to both chlorpyrifos and diazinon was markedly increased. Schnitzerling *et al.* (1974) further characterized these strains showing that A had moderately greater resistance to bromophos ethyl and dioxathion than B, and G had greater resistance to carbophenothion and coumaphos than R. In addition, strain A possessed acetylcholinesterase (AChE) with decreased inhibitor sensitivity of strain B type, and G possessed AChE sensitivity of R type (Schnitzerling *et al.* 1974). Synganglion ('brain') AChE in adults of strain A also showed the decreased activity and sensitivity to coroxon, which was characteristic of strain B (Stone *et al.* 1976*b*; B. F. Stone, unpublished data). The increased resistance to chlorpyrifos in strains A and G was due to increased detoxication (Schnitzerling *et al.* 1974).

Resistance to each of the acaricides diazinon, dimethoate and fenthion in strain B has been shown to be due to a single incompletely dominant autosomal gene that is almost certainly the allele Dcs^B controlling decreased AChE sensitivity (Wilson *et al.* 1971; Stone *et al.* 1976*a*; B. F. Stone, unpublished data). In strain R, resistance

Table 1. Characteristics of resistant and susceptible strains of *Boophilus microplus*

Resistance to chlorpyrifos, diazinon, bromophos ethyl, dioxathion, carbophenothion, coumaphos and dimethoate is based on data of Schnitzerling *et al.* (1974), that to formothion on Stone (1968*a*), and that to fenthion on Stone *et al.* (1976*a*). Resistance rated as extremely high (EH), very high (VH), high (H), moderate (M), low (L) and negligible (N) according to the classification of Stone *et al.* (1976*a*). Biochemical and genetic characters are summarized in the second half of the table

Strain	Resistance to acaricide								
	Chlor-pyrifos	Diazinon	Bromophos ethyl	Dioxa-thion	Carbo-phenothion	Couma-phos	Dimethoate	Formo-thion	Fenthion
Mt Alford (A)	110× (H)	260× (VH)	5× (L)	55× (H)	109× (H)	—	460× (VH)	—	—
Biarra (B)	6× (M)	47× (H)	4× (L)	18× (M)	72× (H)	43× (H)	420× (VH)	—	290× (H)
Gracemere (G)	51× (H)	220× (VH)	1× (N)	17× (M)	92× (H)	4× (L)	1920× (EH)	—	—
Ridgeland (R)	1× (N)	11× (M)	1× (N)	8× (M)	19× (M)	1× (N)	1040× (EH)	2380× (EH)	—
Mackay (M)	2× (L)	10× (M)	1× (N)	15× (M)	16× (M)	9× (M)	260× (VH)	—	—
Yeerongpilly (susceptible) (S)	1×	1×	1×	1×	1×	1×	1×	1×	1×

Strain	Decreased AChE sensitivity to inhibitors	Presumed gene for sensitivity (not necessarily allelic genes)	Decreased AChE activity	Presumed gene for activity (allelic genes)	Increased detoxication	Presumed gene for detoxication (not necessarily allelic genes)
Mt Alford (A)	Yes (B type)	Dcs^{B}	Yes (B type)	dca^{B}	Yes (A type)	Dtx^{A}
Biarra (B)	Yes (B type)	Dcs^{B}	Yes (B type)	dca^{B}	No	Dtx^{+}
Gracemere (G)	Yes (R type)	Dcs^{R}	Yes (R type)	dca^{R}	Yes (G type)	Dtx^{G}
Ridgeland (R)	Yes (R type)	Dcs^{R}	Yes (R type)	dca^{R}	No	Dtx^{+}
Mackay (M)	No†	Dcs^{+}	Yes (M type)†	dca^{M}	Yes (M type)†	Dtx^{M}
Yeerongpilly (susceptible) (S)	No	Dcs^{+}	No	dca^{+}	No	Dtx^{+}

† See Schnitzerling *et al.* (1974) for details of subsequent changes in strain.

to formothion and almost certainly to its analogue dimethoate and other organophosphorus (OP) compounds is controlled by an incompletely dominant gene that probably forms part of a multiple allelic series including the gene for resistance in strain B and in the Mackay (M) strain (Stone 1968*a*; Stone *et al.* 1976*a*).

The biochemical evidence indicates that strains A and G possess B-type and R-type resistance, respectively, enhanced by detoxication, and in this study the extent to which the inheritance of chlorpyrifos resistance correlates with this finding was investigated.

Materials and Methods

Ticks

The field history of strains A and G have been described (O'Sullivan and Green 1971; Schnitzerling *et al.* 1974). Initial selection in the laboratory with chlorpyrifos produced populations containing 95% highly resistant individuals; chemical selection was then discontinued for the strains used in these genetic studies. Homogeneity of resistance to chlorpyrifos was then achieved in strain A by the following method. Single pair matings were carried out, followed by breeding only from broods that were highly resistant to chlorpyrifos and were derived from parents with decreased synganglion AChE activity of B type (Stone *et al.* 1976*b*), i.e. male synganglia were removed and tested after mating and female synganglia after mating and oviposition. This was followed by mass rearing within the selected lines, which were combined to form strain A; this strain remained homogeneous. Strain G was rendered homogeneous in a similar fashion, selection for decreased synganglion AChE activity of R type and for high resistance to chlorpyrifos being carried out where appropriate. The acaricide-susceptible Yeerongpilly strain (strain S) of normal wild-type synganglion AChE activity and sensitivity was used as a reference strain. The field history and subsequent laboratory purification of strains R, B and M have been described: R (Roulston *et al.* 1968; Stone 1968*a*), B (Roulston and Wharton 1967; Wilson *et al.* 1971) and M (Roulston *et al.* 1969; Stone *et al.* 1973).

The toxicological, biochemical and genetic characteristics of the strains are summarized in Table 1.

Chemicals

The common names recommended by the International Organization for Standardization (ISO/R 1750–1970) have been used with the exception that the common name cyanophos has been used for 4-cyanophenyl dimethyl phosphorothionate as recommended by the British Standards Institute (BS 1831: 1969 and supplements) and chlorpyrifos for diethyl 3,5,6-trichloropyrid-2-yl phosphorothionate. All chemicals were at least 98% pure.

Cross Notation

The usual notation (Stone 1962*a*, 1962*b*, 1968*a*, 1968*b*) is used to identify the progeny of crosses by their parentage, the female parent being given first: e.g. an A female × S male crossing results in an F_1 AS zygote and an S female × A male crossing in an F_1 AS zygote; F_1 AS crossed with F_1 AS results in F_2 AS, etc. F_1 and F_2 are shown thus: AS, SA, GS, SG; AB, BA, GR, RG; BS, SB; RS, SR; and testcrosses thus: AS/S, S/AS, SA/S, S/SA; GS/S, S/GS, SG/S, S/SG.

The methods used in culturing of tick strains, single pair crossings and analysis of data have been described previously (Stone 1962*a*, 1962*b*, 1968*a*, 1968*c*; Stone *et al.* 1973).

Histochemical Assays

Esterase (principally AChE) activity in synganglia was assayed as described previously (Stone 1968*b*; Stone *et al.* 1976*b*) except as follows. After incubation in 5-bromoindoxyl acetate (IA) medium (Pearse 1960) at 30°C for 6 h and rating of esterase activity, synganglia were rinsed briefly in distilled water, transferred to 'direct colouring' thiocholine (TC) medium (Karnovsky and Roots 1964) and incubated at 30°C for 2·5 h. The enzyme activity was again rated on a 0–5 scale according to intensity of reaction. Synganglia could be classified as type S, 5(IA)/5(TC); A or B, 0–1(IA)/2–3(TC); G or R, 0–1(IA)/0–1(TC); F_1 AS or BS, 3–4(IA)/4–5(TC); F_1 GS or RS, 3–4(IA)/3–4(TC).

Progeny Testing

The responses of parental, F_1, F_2–F_6 and testcross larvae to chemicals were measured using the 'packet' method of Stone and Haydock (1962) now adopted as the FAO test method (Anon. 1975, 1977). Concentrations are expressed as percentage (w/v) in the oil phase.

Dominance

Degree of dominance (D) of resistance was calculated by the formula given by Stone (1968*a*, 1968*c*); the procedure of Misra (1968) was applied for calculation of fiducial limits and significance of D. Where more than one gene controls resistance, the term 'dominance' is used in the sense of 'average dominance' (D_{av}) across all loci (Comstock and Robinson 1952), which appears similar in concept to 'potence ratio' (Mather and Jinks 1977). We preferred to use 'average dominance' as it is a more descriptive term than 'potence ratio'. It is recognized that 'D_{av}' measures the phenotypic relationship of an F_1 to its parents rather than the dominance ratios of the gene pairs that contribute to that relationship.

It is clear that terms such as 'semi-dominance' are well entrenched in the literature that has appeared since 1968 and will continue to be used. Therefore, we now advocate retention of the following series of terms that have been defined (Stone 1968*a*, 1968*c*; Misra 1968; Stone 1981): complete recessivity (D or $D_{av} = -1$), semi-recessivity (D or $D_{av} = -0{\cdot}5$), zero dominance/recessivity (D or $D_{av} = 0$), semi-dominance (D or $D_{av} = +0{\cdot}5$), complete dominance (D or $D_{av} = +1{\cdot}0$).

Genetic Expectations

Since different resistance mechanisms, decreased AChE sensitivity and increased detoxication were known to be jointly responsible for high resistance to chlorpyrifos in both strains (Schnitzerling *et al.* 1974), it seemed likely that at least two different loci may have been involved. In the simplest case, i.e. two loci, the genes may be so closely linked as to be detectable as one factor only, i.e. F_1 AS would have the genotype $Dcs^B\,Dtx^A/+\,+$ and F_1 GS the genotype $Dcs^R\,Dtx^G/+\,+$. At the other extreme they may be unlinked and easily detectable as two factors, i.e. F_1 AS would have the genotype $Dcs^B/+$; $Dtx^A/+$ and F_1 GS the genotype $Dcs^R/+$; $Dtx^G/+$. The genetic expectations for testcross and F_2 progeny may be deduced from a consideration of these proposed genotypes.*

Results

F_1 Progeny of Reciprocal Crosses between Resistant and Susceptible Strains

A × S crossings

Broods of larvae from four A × S crossings and eight S × A crossings were combined after the absence of susceptible larvae was demonstrated by a discriminating dose of 0·16% chlorpyrifos in packets. Susceptible larvae would be expected to appear only if an 'A-type' parent was not homozygous for the high chlorpyrifos resistance factor or factors.

The mortalities of strains A and S and the two F_1 progenies (i.e. F_1 AS and F_1 SA) from the reciprocal crosses were compared (Fig. 1) after testing in chlorpyrifos packets over the full dose range (F_1 AB and F_1 BA mortalities are included in the figure because of simultaneous testing and comparison with mortalities of A and B—see Accessory Publication). The corresponding LC_{50} values, slopes of *ld-p* lines and resistance factors are shown (Table 2). The results eliminate the possibility of sex linkage of high chlorpyrifos resistance as the LC_{50}'s for F_1 AS and F_1 SA were not significantly different and F_1 SA clearly did not contain the 50% of completely susceptible (XO type) larvae that would result from sex linkage. The four *ld-p* lines were

* If clarification or more detail is required, additional information is deposited as an Accessory Publication with, and copies may be obtained from, the Editor-in-Chief, 314 Albert Street, East Melbourne, Vic. 3002.

parallel. High chlorpyrifos resistance was semi-dominant as the D_{av} value for each of the hybrids (F_1 AS and F_1 SA) was not significantly different from the parametric value of +0·5.

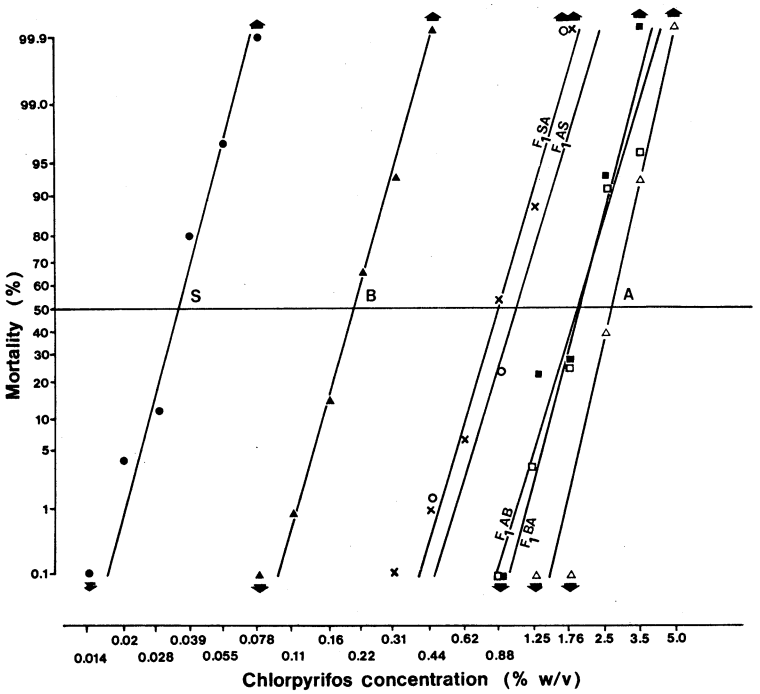

Fig. 1. Mortality of *B. microplus* larvae of the following types enclosed in chlorpyrifos packets: A (△), F_1 AB (■), F_1 BA (□), F_1 AS (○), F_1 SA (×), B (▲), and S (●). Each percentage mortality is based on 100–200 larvae.

Table 2. LC_{50} values, slopes of *ld-p* lines, resistance factors and 'average dominance' for A, F_1 AS, F_1 SA and larvae exposed in chlorpyrifos-impregnated packets

95% fiducial limits are shown in parentheses

Type	LC_{50} (% w/v)	Slope of *ld-p* line[A]	Resistance factor	'Average dominance'
A	2·59 (2·29–2·86)	11·6	74·1 (64·7–84·4)	
F_1 AS	1·03 (0·867–2·42)	8·61	29·4[B] (24·4–35·7)	+0·57 (+0·48 to +0·66)
F_1 SA	0·879 (0·806–0·977)	8·85	25·1[B] (22·2–28·5)	+0·50 (+0·47 to +0·52)
S	0·0350 (0·0318–0·0385)	10·1	1	

[A] Slopes not significantly different.
[B] Resistance factors not significantly different.

G × S crossings

A similar procedure to the A × S crossing was adopted and the mortalities of G, F_1 GS, F_1 SG and S were compared, together with LC_{50}'s, resistance factors and

degrees of dominance (Table 3) after exposure in chlorpyrifos packets and in diazinon packets. The D_{av} values calculated for F_1 GS and F_1 SG larvae tested with chlorpyrifos were close to zero and this was sufficiently unusual in our experience of resistance genetics of *B. microplus* to warrant further examination of the data. Therefore, the data were pooled in order to obtain a better approximation to the mean value of D_{av} for chlorpyrifos resistance. The mean value was $-0{\cdot}031$, which is not significantly different from 0, and hence this resistance was neither dominant nor recessive (Fig. 2*a*).

Table 3. LC_{50} values, slopes of *ld-p* lines, resistance factors (r.f.) and average dominance (D_{av}) for G, F_1 GS, F_1 SG and S larvae exposed in chlorpyrifos- or diazinon-impregnated packets

95% fiducial limits are shown in parentheses

Tick type	LC_{50} (% w/v)	Slope of *ld-p* line	Resistance factor	'Average dominance'
		Exposure to chlorpyrifos		
G	0·587 (0·552–0·625)	9·47[A]	35·3 (24·1–52·0)	
F_1 GS	0·119[B] (0·0641–0·567)	3·08[A]	6·76[B] (4·50–10·3)	+0·10[B] (−0·15 to +0·36)
F_1 SG	0·0867[B] (0·0747–0·100)	5·14[A]	5·24[B] (3·67–7·49)	−0·075[B] (−0·16 to +0·0075)
S	0·0167 (0·0142–0·0195)	5·91[A]	1	
		Exposure to diazinon		
G	2·79 (2·60–3·01)	10·5[A]	174 (149–202)	
F_1 GS	0·591 (0·545–0·643)	6·84[A]	36·7 (31·4–42·8)	+0·40 (+0·36 to +0·44)
F_1 SG	0·653 (0·577–0·743)	5·12[A]	41·0 (35·2–47·7)	+0·44 (+0·36 to +0·49)
S	0·016 (0·014–0·018)	6·28[A]	1	

[A] Slopes significantly different due to greater slopes for strain G type.
[B] LC_{50} values and chlorpyrifos-resistance factors not significantly different. Pooled data: LC_{50} = 0·0937 (0·0776–0·113), r.f. = 5·69 (4·06–8·03), D_{av} = −0·031 (−0·013 to +0·070).

D_{av} values for diazinon resistance (Fig. 2*b*) were significantly different from the parametric value of +0·5 (semi-dominance, $t = 4{\cdot}80$, $P < 0{\cdot}001$, and $t = 2{\cdot}16$, $P < 0{\cdot}05$ for F_1 GS and F_1 SG, respectively). In the case of chlorpyrifos resistance, there appeared to be poor separation (Fig.2*a*) of the response of the hybrid phenotype from that of either parental phenotype. It is known from previous studies (see Stone 1972 and 1981 for references) that this separation would be insufficient for clear genetic interpretation of testcross and F_2 data. In contrast, there was a very high level of resistance to diazinon and clear separation of hybrid and wild-type susceptible phenotypes (Fig. 2*b*). Therefore, subsequent genetic studies on strain G (involving crosses with S and testcrosses back to S) were carried out using diazinon as the diagnostic chemical.

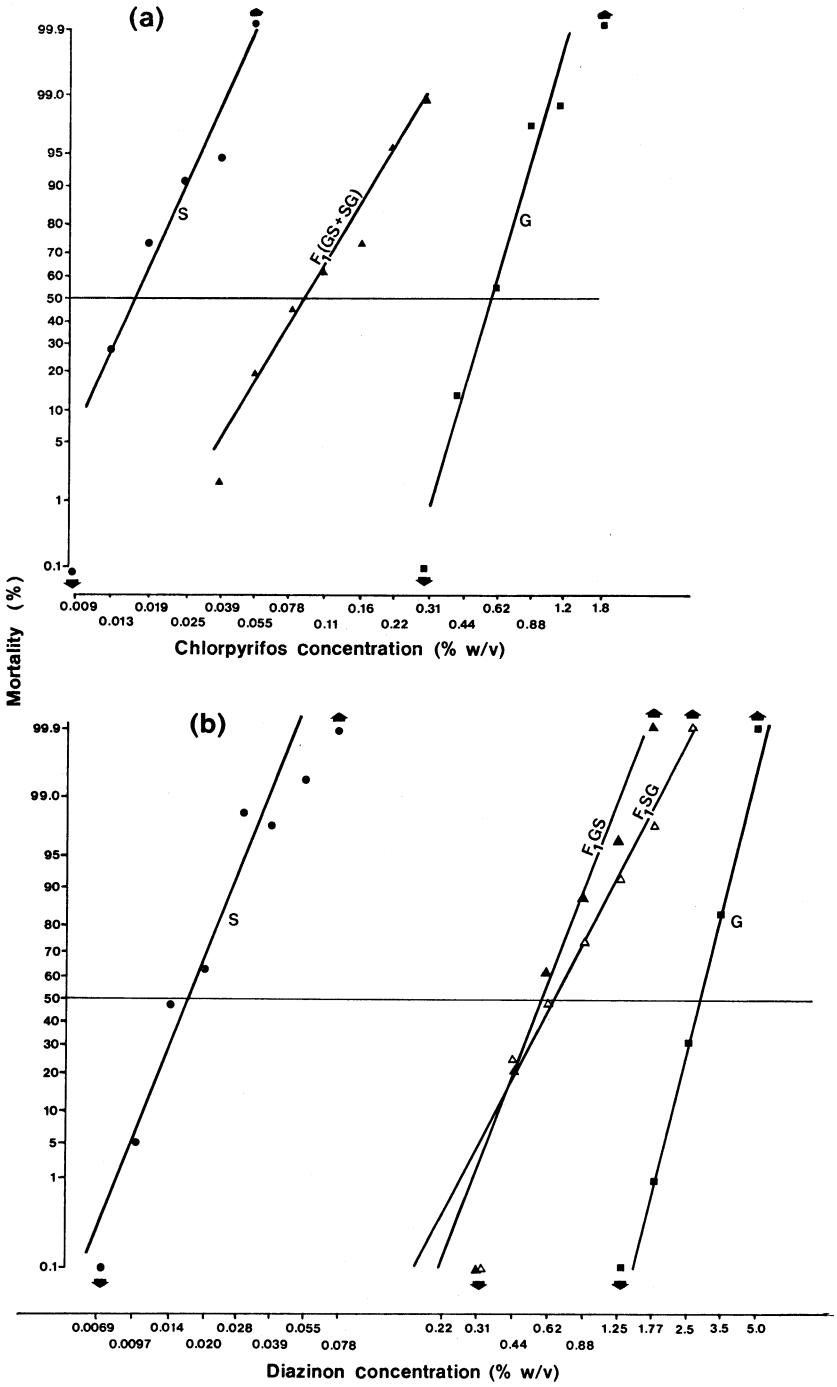

Fig. 2. Mortality of *B. microplus* larvae of the following types enclosed in chlorpyrifos (*a*) or diazinon (*b*) packets: (*a*) G (■), F_1 (FS+SG) (▲), and S (●); (*b*) G (■), F_1 GS (▲), F_1 SG (△), and S (●). Each percentage mortality is based on 100–200 larvae.

Testcrosses

(F_1 AS and SA) × S

Samples from 12 broods of testcross larvae (four AS/S, seven S/AS and one SA/S) were exposed in 0·1 % chlorpyrifos packets to determine, in each brood, the proportion of larvae present that had negligible resistance to chlorpyrifos (see Accessory Publication and Fig. 1). In nine broods mortalities ranged from 70 to 77%, and in three broods they ranged from 66 to 69%.

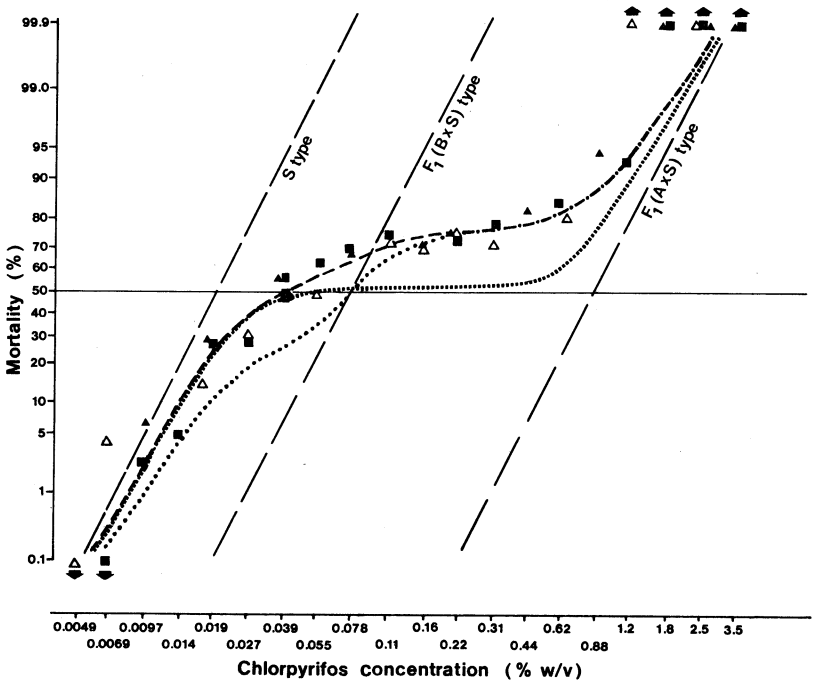

Fig. 3. Mortality of *B. microplus* larvae of the following types enclosed in chlorpyrifos packets: AS/S (■), AS/S+S/AS (▲), SA/S+S/SA (△). If two unlinked *complementary* (– – –) or *additive* (· · · ·) genes control resistance, expected mortalities are calculated on the basis of 25% each of the phenotypes F_1 AS or SA, F_1 BS or SB, S (*complementary*), F_1 BS or SB (*additive*), and S; if one gene or two closely linked genes (■■■■■), mortalities are calculated as 50% F_1 AS or SA and 50% S types. Each percentage mortality is based on 100–200 larvae.

AS/S larvae were then tested over the full range of concentrations and the *ld-p* line showed a clear inflexion indicating that about 75% of these larvae were more susceptible than their F_1 hybrid parents. There were insufficient S/AS larvae to test over the full range because of small numbers in some broods. However, on combining S/AS larvae in roughly equal numbers with the remaining AS/S larvae, a *ld-p* line very similar to that for AS/S larvae alone was obtained. SA/S and S/SA larvae were also combined, again with similar results (Fig. 3). Clearly there was a marked departure from the 50 : 50 ratio of susceptible homozygotes to resistant heterozygotes that would be expected if chlorpyrifos resistance were monofactorial or if resistance were due to two closely linked genes. The ratio obtained differed also from the 25 : 75

ratio expected if two unlinked additive genes were responsible (Fig. 3) as observed and expected mortalities differed significantly (χ^2 test, $P < 0{\cdot}001$) below 0·16% chlorpyrifos. Over the same range of concentrations, there was no significant difference between observed and expected mortalities calculated on the basis of 75 susceptible phenotypes to 25 more resistant phenotypes. Thus, a ratio of 75 : 25 fitted the observed data best and could be explained on the basis of two dominant, unlinked, complementary genes. It was concluded that there was reasonable agreement between the observed mortalities and those expected if there were four phenotypes, as described in the case of unlinked genes (see Accessory Publication), assuming that the +/+; Dtx^A/+ genotype had negligible resistance.

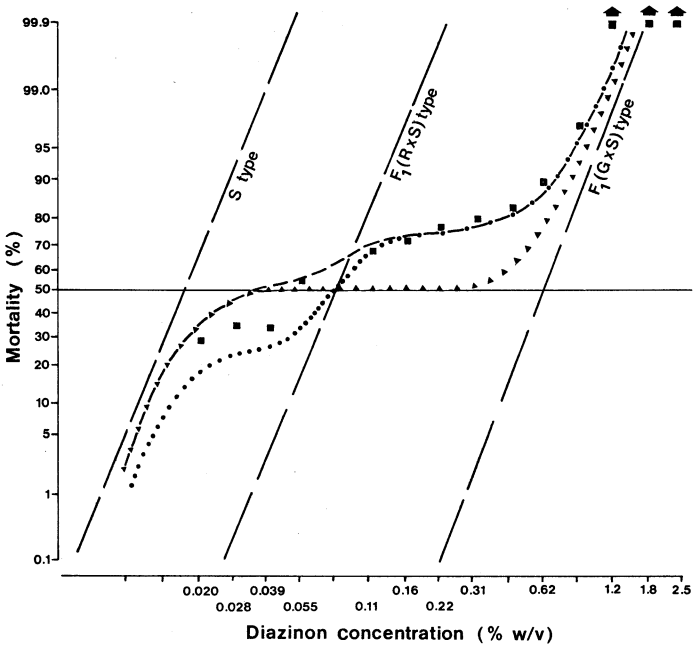

Fig. 4. Mortality of *B. microplus* larvae of SG/S (■) type enclosed in diazinon packets. If two unlinked *complementary* (– – –) or *additive* (· · · ·) genes control resistance, expected mortalities are calculated on basis of 25% each of the phenotypes F_1 GS or SG, F_1 RS or SR, S (*complementary*), F_1 RS or SR (*additive*), and S; if one gene or two closely linked genes (▲▲▲), mortalities are calculated as 50% F_1 GS or SG and 50% S types. Each percentage mortality is based on 100–200 larvae.

$(F_1\ SG) \times S$

In a similar fashion to corresponding data for progeny of (F_1 AS and SA) × S test crosses, the *ld-p* line for S/SG larvae showed evidence of a plateau at 75% between the doses just producing 100% mortality in strain S and 0% mortality in the F_1 hybrid (0·039 and 0·22% diazinon, Fig. 4); F_1 GS adults were not available for the corresponding testcrossing. The inflexion was not as clearcut as with AS/S, S/AS and SA/S testcross larvae but it was apparent that 75% of the S/SG larvae were killed and, therefore, were more susceptible than the most susceptible F_1 GS or SG

larvae. This excluded the possibility that one gene or two closely linked genes controlled diazinon resistance as a 50 : 50 ratio would be expected. There was also a significant difference between mortalities observed at 0·020, 0·028, 0·039 and 0·055% diazinon (Fig. 4) and those expected in the case of two additive genes [$\chi^2_{(1)} = 6{\cdot}54$ ($P < 0{\cdot}02$), 8·90 ($P < 0{\cdot}01$), 9·02 ($P < 0{\cdot}01$) and 35·81 ($P < 0{\cdot}001$) respectively; $\chi^2_{(4)} = 60{\cdot}27$ ($P < 0{\cdot}001$)]. In the test of the hypothesis that two complementary genes were involved, the observed and expected mortalities (Fig. 4) differed significantly only at 0·028 and 0·39% [$\chi^2_{(4)} = 5{\cdot}55$ ($P < 0{\cdot}02$) and 21·30 ($P < 0{\cdot}001$) respectively; $\chi^2_{(4)} = 27{\cdot}46$ ($P < 0{\cdot}001$)]. Thus, although not meeting fully the requirements of the complementary gene hypothesis, the data agree somewhat better with that hypothesis than with the additive gene hypothesis (see Accessory Publication and Fig. 4).

Double Backcrossings (SA/S × S and AS/S × S) with Selection

These confirmed the findings of the first testcross and indicated that one backcrossing with selection did not increase the proportion of ticks that were more susceptible to chlorpyrifos than the F_1 hybrid. This helped to exclude polygenic inheritance (see Accessory Publication for data).

Double and Triple Backcrossing to S without Selection

The result supported the simplest interim conclusion from the initial testing, which was that two unlinked genes complement one another to produce high resistance to chlorpyrifos in strain A (see Accessory Publication for data). If gene Dcs^B were one of these two genes in strain A, it should be possible to isolate Dcs^B/Dcs^B; +/+ homozygotes from testcross progeny, which should have 1/4 $Dcs^B/+$; +/+ genotypes (see next section).

Isolation of Dcs^B/Dcs^B; +/+ Genotypes from Strain A

Several $Dcs^B/+$; +/+ broods of larvae (see Accessory Publication) were combined, fed and the adults mated. The larval progeny were selected in 0·025% dimethoate packets to remove S-type larvae. The adults were mated and the progeny tested for B × B or B × S matings using diagnostic doses of dimethoate. This was repeated for three generations and larval batches apparently derived from B × B matings were combined. Progeny testing of five broods of larvae in 0·44% chlorpyrifos packets showed that all broods were more susceptible than A or F_1 A × S larvae, thus confirming the absence of A or A × S phenotypes. Further testing in 0·625% cyanophos packets showed that at least 70% of these larvae were B homozygotes, cyanophos being chosen because of its diagnostic value in clearly identifying B types (Stone *et al.* 1976*a*). Testing in 0·04% cyanophos packets indicated that no S larvae were present. Synganglia from 13 females and 1 male of the next generation, obtained by combining the larval batches, were assayed histochemically for AChE activity by the 'two-substrate' method. Enzyme activity was rated 0–1 (IA), 1–2 or 2–3 (TC) and therefore these adults were confirmed as being of the A or B type. As resistance testing had shown clearly that no A homozygotes were present, the adults must have been of B type. Almost certainly, homozygosity had been achieved in the previous generation also.

F_2 Progeny of Reciprocal Crosses of A × S Crossings

There was a definite inflexion in the *ld-p* line over the range 0·078–0·31% chlorpyrifos; this suggested the presence of about 40% 'susceptibles' (Fig. 5). Again, this is a departure from expectation for one gene (25% susceptibles) or for two dominant additive genes (6·25% susceptibles). If two dominant complementary genes controlled resistance, it would be expected that 7/16 (43·75%) of F_2 progeny would be less resistant than heterozygotes from A × S crossings (see Accessory Publication).

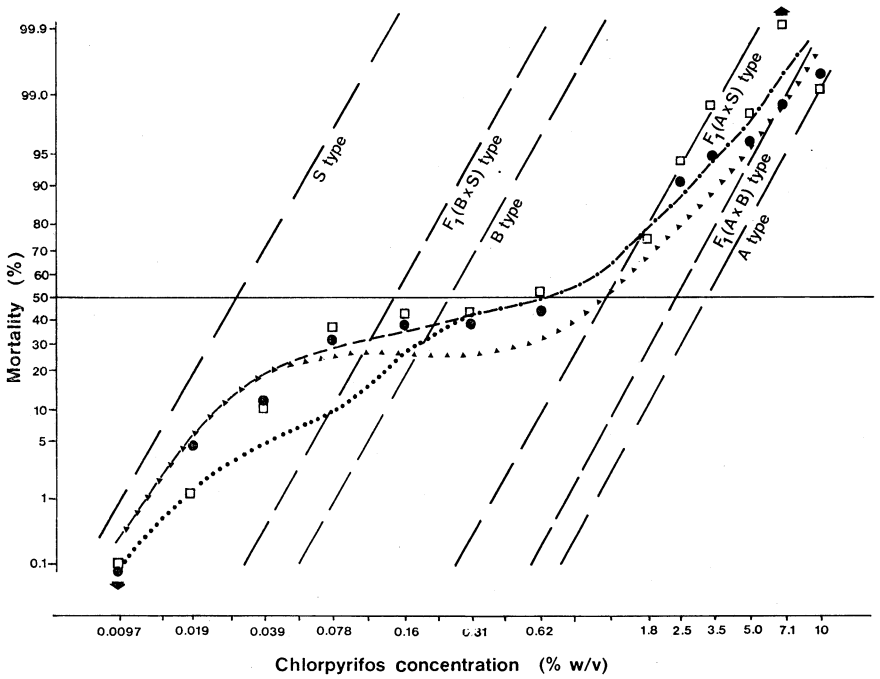

Fig. 5. Mortality of *B. microplus* larvae of the following types enclosed in chlorpyrifos packets: F_2 AS (□), F_2 SA (●). Expected *ld-p* lines for F_2 progeny calculated according to possible phenotype composition (see Accessory Publication). If two unlinked complementary (– – –) or additive (· · · ·) genes control resistance, 56·25% of the phenotypes were expected to be at least as resistant as phenotype F_1 AS or SA, 18·75% (*complementary*) or 37·5% (*additive*) at least as resistant as F_1 BS or SB and 25% (*complementary*) or 6·25% (*additive*) as susceptible as S. ▲▲▲ One gene or two closely linked genes. Each percentage mortality is based on 100–200 larvae.

Discussion

The inheritance of high chlorpyrifos resistance in strain A and of diazinon resistance in strain G has been shown to be controlled by two unlinked genes in each strain. These are the first reported instances of resistance to acaricides being controlled by more than one gene in ticks. Resistance in *B. microplus* in Australia to DDT, BHC–dieldrin, formothion, diazinon, dimethoate and fenthion has been shown to be due to a single gene in each strain (see Stone 1972, 1981 for reviews). In Africa, lindane resistance in *Amblyomma variegatum*, BHC resistance in *Rhicephalus appendiculatus* and toxaphene–BHC–dieldrin resistance in *R. evertsi evertsi* were shown also to be due to a single gene in each strain (Lourens 1979, 1980; Lourens and Tatchell 1979).

The genetic conclusion that two genes control high chlorpyrifos resistance in strain A is not altogether surprising in view of the biochemical evidence that two mechanisms operate in this strain (Schnitzerling *et al.* 1974). Although detoxication of diazinon was not studied in strain G, merely chlorpyrifos detoxication, a similar detoxifying mechanism probably causes enhanced diazinon resistance in strain G. It seems likely that the gene controlling decreased AChE sensitivity in strain A was Dcs^B (Stone *et al.* 1976*a*) since $Dcs^B/Dcs^B;+/+$ homozygotes were isolated after two backcrossings followed by selection and inbreeding. It was assumed that the gene complementary to Dcs^B controlled increased detoxication (i.e. gene Dtx^A) but it is not known why it should be strongly active only when coupled with Dcs^B. It is probable that a pair of complementary genes in strain G controls the two biochemical mechanisms proven for this strain, decreased AChE sensitivity of R type and increased detoxication. As no attempt was made to isolate the presumed $Dcs^R/Dcs^R;+/+$ homozygotes from strain G, direct evidence is lacking. Although the possibility remains that the two genes are additive rather than complementary, the existing data tend to favour complementarity in strain G as in strain A.

Degree of dominance was calculated, using the formula of Stone (1968*a*, 1968*c*), which was originally applied to cases of monofactorial inheritance. Although Arnold and Whitten (1976) used the formula for genetic analysis of OP resistance in the Australian sheep blowfly *Lucilia cuprina* where resistance was controlled by two major loci on different chromosomes, they applied the formula to the expression of individual genes in isolated substrains. However, it also appears reasonable to use it where more than one factor controls resistance, providing interpretation as outlined in the Materials and Methods is applied. Heather (1979) used the formula of Stone (1968*a*, 1968*c*) in studies on malathion resistance in the rice weevil *Sitophilis oryzae* and concluded that the formula could be used to measure 'average dominance' (Comstock and Robinson 1952) across all loci if more than one gene controlled malathion resistance. In this present paper dealing with resistance in *B. microplus*, the phenotypic expression of the interaction of both resistance genes in F_1 hybrids has been evaluated in terms of degree of dominance of resistance over susceptibility. This may be analogous to applying the theoretical 'potence ratio' of Mather and Jinks (1977).

It is not known why high chlorpyrifos resistance was semi-dominant in strain A but neither dominant nor recessive in strain G whereas diazinon resistance was much closer to semi-dominance, although significantly less dominant than chlorpyrifos resistance. The augmentative interaction of the gene Dcs^B with the presumed detoxication gene (Dtx^A) was multiplicative. This phenomenon in resistant insects was discussed by Plapp (1970). A resistance factor of 5·8 towards chlorpyrifos in the homozygote carrying Dcs^B only (B type) was boosted 12-fold to 74 in a homozygote carrying Dtx^A as well (see Accessory Publication). In the heterozygote, Dcs^B alone would have increased resistance to chlorpyrifos less than fourfold but resistance was boosted perhaps sevenfold in the heterozygote carrying both resistance genes (Tables 1 and 2; Accessory Publication). We are postulating that $+/+$; $Dtx^A/+$ genotypes had no appreciable resistance. This would appear to be the simplest explanation that fits the data (Figs 3 and 5).

This multiplicative interaction is in contrast with the very slight augmentative interaction demonstrated when strains B, M and R of *B. microplus* were crossed in all possible ways; in these strains the resistance genes were almost certainly allelic (Stone *et al.* 1976*a*). In each of the strains A and G, the resistance genes were clearly

not allelic within the strains. In a similar way, crossing strains A and B, G and R has not produced phenotypes that are more resistant than their most resistant parent either as F_1 hybrids or recombinants in F_2 progeny (B. F. Stone, unpublished data). In the F_2–F_6 progeny of A × M crosses, no larvae were detected that were more resistant than the parental strains to various chemicals including chlorpyrifos, dioxathion, ethion, cyanophos and phosmet (B. F. Stone, unpublished data).

The characteristics of the original strain M are described in Table 1 but it should be noted that this strain, which originally owed its resistance to detoxication only, subsequently acquired an additional mechanism, decreased AChE sensitivity of R type. The acquisition of the additional mechanism occurred during laboratory culturing and coincided with a reduction but not elimination of the capacity of strain M for detoxication. The resistance status of the strain was unchanged. Thus, the results of testing F_2–F_6 progeny from A × M crossings are of particular interest since strain M would presumably contribute its own detoxifying gene and progeny were tested as far as the F_6 without detecting individuals more resistant to the above chemicals than existed in the parental A strain.

Acknowledgments

The assistance of Dr M. J. Whitten, Division of Entomology, CSIRO, Canberra, in suggesting certain procedures and his valued advice and encouragement are gratefully acknowledged. Thanks are due to Mr J. D. Kerr for some of the statistical analyses.

References

Anon. (1975). Report of the FAO Expert Consultation on Research on Tick-borne Diseases and their Vectors, Rome, 1975, pp. 14–17.

Anon. (1977). Report of the Second FAO Expert Consultation on Research on Tick-borne Diseases and their Vectors, Rome, 1977, pp. 16–18.

Arnold, J. T. A., and Whitten, M. J. (1976). The genetic basis for organophosphorus resistance in the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera, Calliphoridae). *Bull. Entomol. Res.* **66**, 561–8.

Comstock, R. E., and Robinson, H. F. (1952). Estimation of average dominance of genes. In 'Heterosis'. (Ed. J. W. Gowen.) pp. 494–535. (Iowa State College Press: Ames.)

Heather, N. W. (1979). Aspects of the biology of malathion-resistant *Sitophilus oryzae* (Linnaeus) (Coleoptera : Curculionidae). Ph.D. Thesis, University of Queensland.

Karnovsky, M. J., and Roots, L. (1964). A direct-colouring thiocholine method for cholinesterases. *J. Histochem. Cytochem.* **12**, 219–21.

Lourens, J. H. M. (1979). Genetic basis for organochlorine resistance in *Amblyomma variegatum* and information on the susceptibility of *A. lepidum* to organochlorine acaricides. *J. Econ. Entomol.* **72**, 790–3.

Lourens, J. H. M. (1980). Inheritance of organochlorine resistance in the cattle tick, *Rhicephalus appendiculatus* Neumann (Acari : Ixodidae) in East Africa. *Bull. Entomol. Res.* **70**, 1–10.

Lourens, J. H. M., and Tatchell, R. J. (1979). Studies on acaricide resistance in *Rhipicephalus evertsi evertsi* Neumann (Acarina : Ioxdidae) in East Africa. Identification and inheritance of a resistance factor to organochlorines. *Bull. Entomol. Res.* **69**, 235–42.

Mather, K., and Jinks, J. L. (1977). 'Introduction to Biometrical Genetics.' (Chapman and Hall: London.)

Misra, R. K. (1968). Statistical tests of hypotheses concerning the degree of dominance in monofactorial inheritance. *Biometrics* **24**, 429–34.

O'Sullivan, P. J., and Green, P. E. (1971). New types of organophosphorus-resistant cattle ticks (*Boophilus microplus*). *Aust. Vet. J.* **47**, 71.

Pearse, A. G. E. (1960). 'Histochemistry, Theoretical and Applied.' 2nd Edn. (J. and A. Churchill: London.)

Plapp, F. W., Jr (1970). On the molecular biology of insecticide resistance. In 'Biochemical Toxicology of Insecticides'. (Eds R. D. O'Brien and I. Yamamoto.) pp. 179–92. (Academic Press: New York.)

Roulston, W. J., Schnitzerling, H. J., and Schuntner, C. A. (1968). Acetylcholinesterase insensitivity in the Biarra strain of the cattle tick *Boophilus microplus* as a cause of resistance to organophosphorus and carbamate acaricides. *Aust. J. Biol. Sci.* **21**, 759–67.

Roulston, W. J., Schuntner, C. A., Schnitzerling, H. J., and Wilson, J. T. (1969). Detoxification as a mechanism of resistance in a strain of the cattle tick *Boophilus microplus* (Canestrini) resistant to organophosphorus and carbamate compounds. *Aust. J. Biol. Sci.* **22**, 1585–9.

Roulston, W. J., and Wharton, R. H. (1967). Acaricide tests on the Biarra strain of organophosphorus resistant cattle tick *Boophilus microplus* from southern Queensland. *Aust. Vet. J.* **43**, 129–34.

Schnitzerling, H. J., Schuntner, C. A., Roulston, W. J., and Wilson, J. T. (1974). Characterization of the organophosphorus-resistant Mt Alford, Gracemere and Silkwood strains of the cattle tick *Boophilus microplus*. *Aust. J. Biol. Sci.* **27**, 397–408.

Stone, B. F. (1962*a*). The inheritance of DDT-resistance in the cattle tick, *Boophilus microplus*. *Aust. J. Agric. Res.* **13**, 984–1007.

Stone, B. F. (1962*b*). The inheritance of dieldrin-resistance in the cattle tick, *Boophilus microplus*. *Aust. J. Agric. Res.* **13**, 1008–22.

Stone, B. F. (1968*a*). Inheritance of resistance to organophosphorus acaricides in the cattle tick, *Boophilis microplus*. *Aust. J. Biol. Sci.* **21**, 309–19.

Stone, B. F. (1968*b*). Brain cholinesterase activity and its inheritance in the cattle tick (*Boophilus microplus*) strains resistant and susceptible to organophosphorus acaricides. *Aust. J. Biol. Sci.* **21**, 321–30.

Stone, B. F. (1968*c*). A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to chemicals. *Bull. W.H.O.* **38**, 325–6.

Stone, B. F. (1972). The genetics of resistance by ticks to acaricides. *Aust. Vet. J.* **48**, 345–50.

Stone, B. F. (1981). A review of the genetics of resistance of acaricidal organochlorine and organophosphorus compounds with particular reference to the cattle tick *Boophilus microplus*. In 'Tick Biology and Control'. (Eds G. B. Whitehead and J. D. Gibson.) pp. 95–102. (Tick Research Unit, Rhodes University: Grahamstown, Republic of South Africa.)

Stone, B. F., and Haydock, K. P. (1962). A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Canestrini). *Bull. Entomol. Res.* **53**, 563–78.

Stone, B. F., Nolan, J., and Schuntner, C. A. (1976*b*). Biochemical genetics of resistance to organophosphorus acaricides in three strains of the cattle tick, *Boophilus microplus*. *Aust. J. Biol. Sci.* **29**, 265–79.

Stone, B. F., Wilson, J. T., and Youlton, N. J. (1973). Inheritance of dimethoate resistance in the Mackay strain of the cattle tick (*Boophilus microplus*) in Australia. *Aust. J. Biol. Sci.* **26**, 445–51.

Stone, B. F., Wilson, J. T., and Youlton, N. J. (1976*a*). Linkage and dominance characteristics of genes for resistance to organophosphorus acaricides and allelic inheritance of decreased brain cholinesterase activity in three strains of the cattle tick, *Boophilus microplus*. *Aust. J. Biol. Sci.* **29**, 251–63.

Wilson, J. T., Stone, B. F., and Wharton, R. H. (1971). Inheritance of diazinon resistance in the Biarra strain of the cattle tick, (*Boophilus microplus*) in Australia. *Aust. J. Agric. Res.* **22**, 169–75.

Manuscript received 14 January 1982, accepted 8 June 1982