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EFFECT OF INSECTICIDE RESISTANCE ON THE BIOLOGY OF *MUSCA DOMESTICA.* L. STRAINS

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The study of life history parameters in various insecticide-resistant strains of the house fly, *Musca domestica* L. indicated statistically significant differences in larval and pupal duration and development among the various strains. Relatively non-significant difference was seen in emergence percentage (except strain 7) and sex ratio. The biotic potential of strain 171 was almost half the reference / susceptible strain. The reasons for these differences and their role in the development of insecticide resistance are discussed.
Keywords: biology, biotic potential, fecundity, insecticide resistance

INTRODUCTION

The evolution of insecticide in a field population of the insect species is under the influence of genetical, biologic and ecological factors that vary with species, population and location (Brown and Pal, 1971; Georghiou and Taylor, 1977). Resistance to insecticides is sometimes associated with impaired reproduction. Resistant insects may have a lower fecundity or a slow rate of development (pimental *et al.*, 1951). This fitness disadvantage has been reported for several species of arthropods including the red flour beetle, *Tribolium castaneum* (Herbst) (Bhatia and Pradhan, *1968), Spodoptera littoralis* Boisduval (Moustafa, 1981), the southern house mosquito, *Culex quinquefasciatus* Say and the house fly, *Musca domestica* L. (Roush and Plapp, 1982). In most of these studies, it was not clear if the fitness deficits were associated with metabolic resistance, target site resistance or both. However, the fitness deficits were clearly associated with metabolic resistance to Organophosphorus (OP) insecticides in the mosquito and house fly. The fitness disadvantages have been associated with high levels of glutathione S-tmnsferases in various arthropod groups (Roush and Plapp, 1982), and also with elevated esterase hydrolysis in *Luci/ia cuprina* (Hughes and Rafter, 1985). Presumably, the metabolic cost of producing quantitatively existing detoxifying enzymes is high. However, oxidative detoxication in insecticide resistant *Metaseiulus occidentalts* (Roush and Hoy, 1981), *M domestica* L. (Roush and Plapp. 19820, *Helicoverpa armigera* Hubner (Neal, 1987), possession of malathion-specific carboxylestemse in *Anopheles arabiensis* Say (Lines *et al.*, 1984) an decreased acetylcholinestemse sensitivity to OP and carbamate inhibition in Chironomus riparius (Hoffman and Fisher, 1994) produced no reproductive disadvantages in these insect species.

The populations of insecticide resistant strains, with a Small difference in biotic fitness compared to susceptible strain, can develop the resistance rapidly as compared to populations with fitness disadvantages (Geroghiou, 1972). Higher generation turnover and reproductive potential increase the population growth, which allows the population to increase faster following insecticide treatment (Comins, 1979). The aim of this study was to compare the relative rate of development and biotic potential of susceptible and insecticide-resistant strains of *M domestica* L.

MATERIALS AND METHODS

171. The selection of this strain by pyrethroid was not described when it was received from IACR-Rothamsted Harpenden, Herts, UK. Cooper was a reference susceptible strain for comparison in bioassay and biological parameters with resistant strains.

The populates used for the present studies were not selected with insecticide and the present studies were carried out on flies just received from DPIL.

MEllODS

Rearing of Flies: The house fly strains were reared on a rearing medium consisted of bran, milk powder and yeast mixture (20:2:1) in 400 ml water. A plasticjar (300 ml capacity) full of rearing medium was put in flies rearing cages to get eggs and after 2 days, the jars were removed from rearing cages and covered with nylon mesh held in place with a rubber ring and kept in a rearing room at 24±2°C and 50-55% RH (12:12 hr light and dark period). The emerging flies were used for bioassay and for studying biological parameters,

Materials: House Fly, *M domestica* L Strains:

The source and description of *M domestica* L. strains are given below:

Four strains (l7bb, 594vb, 571ab, and 7) were received from the Danish Pest Infestation Laboratory (DPIL), Lyngby, Denmark

Strain	Origin	Year	Remarks	Lab. Pressure
17bb	Denmark	1950	R to DDT, dieldrin and pyrethroids	DDT
594vb	Denmark	1988	R to OP compounds and to DDT also	azamethiphos
571ab	Japan	1980	collected on Tokyo's city dump, very high OP, R, S tG pyrethroids	fenitrothion
7	Denmark	1948	heterogeneously R to dieldrin, DDT-resistance reverted	None

Lab pressure, means that these strains are exposed to insecticides, to which they are resistant, occasionally or regularly in the laboratory. Year means the time of collection. OP, organophosphorus, R, resistant, S, susceptible.

Determination of the Resistance Level in Various Strains: The resistance level in various strains was determined by treating the 3-day old adult flies (total 50 flies for each concentration in a group of 10 flies) using arnold micro-applicators (Burkard) and a microsyringe (Iml) with canulae (G 36x3") with a series of insecticide concentrations in I-Ill drop of insecticide in acetone to thoracic notum of the adult flies. LD_{30} was calculated on morality (24 hr) based data by probit analysis in a computer program POLO PC (Leora Software, 1987).

Study of Biological Parameters: Larval and pupal duration, development time, generation time, emergence percentage, sex ratio and number of females produced per female were studied. These parameters allowed the intrinsic rate of increase, the biotic potential, the relative biotic potential and the net replacement rate to be calculated.

Development Time, Percentage Emergence and Sex Ratio Study: To study the larval and pupal duration, 300 eggs of each strain were collected 4-6 hr after oviposition and were placed on larval medium in 300ml jars. The larvae hatched in 6-12 hr and from this time point, the larval period was measured up to the appearance of first pupa in the jar. Once the first pupa had been noted then time of pupal duration began. The changing of larval medium was ceased when the pupae were turning to blackish brown colour. The emergence of the first fly was the end of the pupal duration. The emerged adults were sexed and counted daily until the emergence ceased. All the stages of life were timed to within 6 hr of occurrence to obtain as accurate data as possible. Development time is equal to larval+pupal durations. Generation time (T) is the time taken the days from first oviposition to next oviposition.

Fecundity: The fecundity rate for each strain was studied by collecting 5-10 mating pairs of 3-5 day old adult flies of each strain and they were released in a separate, identical and well-ventilated cages. These pairs were provided with sugar and milk+sugar solution (1:1) inpetri dishes. A small plastic dish containing a cotton ball soaked in milk-sugar solution was placed for females to lay eggs. The dishes were examined twice a day (once in the 'morning and then in the afternoon for> 20 days) to count the number of eggs. The dishes for egg laying were changed daily. The average number of eggs per female was calculated.

Net Replacement Rate (1\): It is the number of females produced by one female. To study the net replacement rate, the eggs from known number of females were collected on larval medium and emerged adult flies were placed and sexed to count the number of females. The intrinsic rate of increase(r) measures the rate at which a population is increasing per generation. It is calculated by the following equation: $r = 10g.R_c/T$ where R_o and T equals the net replacement rate and mean generation time respectively. The biotic potential is a function of fecundity and time taken to reach adult stage:

$$\text{Biotic potential} = \frac{\log_e \text{fecundity}}{\text{mean development time of strain}}$$

The relative biotic potential is given below:

$$\text{Relative biotic potential} = \frac{\text{biotic potential of resistant strain}}{\text{biotic potential of susceptible strain}}$$

Statistical Analysis: The studies on above parameters were repeated thrice and were compared among all strains by One Way of ANOVA and significance among the means was calculated by Duncan's Multiple Range Test. Percentage emergence values were subjected to angular transformation before ANOVA. The correlation between resistance ratio and biological parameters was determined by taking together each biological parameter of the strains vs resistance ratios.

RESULTS

Table 1 shows the LD_{30} and insecticide resistance ratios of insecticides in various resistant and susceptible strains.

Table 1. LD!O (ng fly-I) and resistance ratio (RR) of insecticides

Strain	Insecticide	LDso	RR	Strain	Insecticide	LD50
17bb	DDT	2456	85	Cooper	pennethrin	6
571ab	Fenitrothion	1166	232	Cooper	DOT	29
594vb	Azamethiphos	155	22	Cooper	Dieldrin	0.3
7	Dieldrin	13	43	Cooper	Fenitrothion	5
171	Pennethrin	29	5	Cooper	Azamethiphos	7

The mean data (±SE) of various life history parameters studied in strains of *M domesticaL.* are given in Table 2. The Cooper had the shortest (6.3 days) larval duration and had non-significant difference with other strains except 594vb. The pupal duration of 7, 594vb and 171 strains was longer than other strains. The development time of 17bb and Cooper was almost the same.

Table 2. Comparison of biological parameters of various strains of *M. domestica* resistant and susceptible to insecticides

Strains	Larval duration (days)	Pupal duration (days)	Development time (days)	Generation Time (days)	Emergence (%)	Fecundity	Sex ratio	R_o
7	7.0±0.5a	11.0±0.6a	18.0±1.1a	32.0±1.0a	42.3±1.2b	119±O.6d	1:1.5±O.5n.s.	11.0±1.2d
571ab	7.3±O.3a	7.6±O.7ab	15.0±0.6ab	27.6±O.9ab	66.4±4.0a	73±O.4e	1:1,7±O.1	37.7±2.4c
Cooper	6.3±O.6ab	7.0±O.6b	13.3±O.9b	22.0±0.9b	82.2±4.4a	310±2.3a	1;1,4±O.1	176.0±6.6a
17bb	7.7±O.6a	5.7±O.7b	13.3±O.9bc	21.7±O.9bc	66.8±IO.a	151±O.5c	1:1,3±O.3	56.7±1O.2c
171	6.7±O.6ab	12.0±1.1a	18.7±1.2a	23.0±1.2a	56.2±2.6a	68±O.7e	1:1,4±O.1	26.3±2.Ocd
594vb	5.7±O.6a	1O.3±O.7a	16.0±1.0ab	22.7±1.0ab	60.3±5.0a	261±O.Ob	1:1.2±O.1	lO1,3±4,3b

Development time: egg to adult; Generation time: egg to egg; ~, Net replacement rate: Number of females per female; Insecticides to which strains are resistant; RR, resistance ratio. *Means with the same letters are not significantly different at 5% level of significance; n.s.: Non-significant.

The generation time of strain 7 was the longest (18 days) as compared to other strains. The lowest percentage emergence (42$) was observed in strain 7, while 17bb and 571ab shared the same percentage emergence. The Cooper registered comparatively higher fecundity rate than the resistant strain, fecundity being the lowest in strain 7. The non-significant difference in low fecundity rate between 571ah and 171 strains may indicate the low reproductive potential of selected pairs behaving differently from the general population. All the strains were at par in sex ratio. The net replacement rate was the lowest in strain 7 and highest in 594vb strain. The intrinsic rate of increase (r), biotic potential an relative biotic potential of all strains are given in Table 3. The lowest rate of r was recorded in strain 7, although the fecundity was high in this strain as compared to other resistant strains. The closer biotic potential to the Cooper strain was shown by 17bb and 594vb strains, however, the latter two strains showed high relative biotic potential. Correlation coefficient values between RR and biological parameters of the resistant strains are given in Table 4. All the biological parameters of the resistant strains were non-significantly correlated with resistance ratios.

DISCUSSION

The results of this study validate Roush and Plapp's (1982) findings on four organophosphate-resistant strains of *M domestica* for that resistant strains showed a decrease in biotic potential relative to a susceptible strain (Orlando Regular strain). The decrease was associated with reduced fecundity in all resistant strains and increase in two of them. An increase in development time and a subsequent increase in generation time indicate that the resistant strains require long development time to produce detoxifying enzymes.

Table 3. Intrinsic rate of increase, biotic potential, and relative biotic potential of various strains of *M. domestica* resistant to insecticides

Strain	Intrinsic rate of increase (r)	Biotic Potential	Relative biotic potential
7	0.08	0.26	0.60
571ab	0.13	0.29	0.65
Cooper	0.23	0.44	1.00
17bb	0.18	0.37	0.84
171	0.14	0.23	0.52
594vb	0.20	0.35	0.80

Table 4. Correlation values between resistance ratio and biological parameters

	Weight fly"	T	DT'	Fecundity	n_d	RR
Weight fly"	1.00					~.55
T	.0.58	1.00				0,33
DT'	.0.66	0.48	1.00			~.39
Fecundity	0.71	~.47	.0.54	1.00		0,17
R_o	0.76	.0.59	.0.65	0.93	1.00	0.29
Survival	0,54	~.69	~.79	0.46	0.73	0,32
Sexratio	~.39	0.71	.0.02		~.35	0.54

R_o Net Replacement Rate; T_1 means Generation Time; DT, Development Tiime; AA, Resistance Ratio, n=3; P=0.05.

Moorfield and Kearns (1957) found that flies pupating last contained 40% more DDTase enzyme in a DDT-resistant strain of house fly than those that were first to pupate. In contrast, the present data show the lowest pupal duration in DDT-resistant (1Thb) strain. There is a possibility of males emerging earlier than females that could have affected the pupal duration in IThb but Roush and Plapp (1982) observed that the males and females had similar development period. The variation in temperature oflarval medium might have played a role in early emergence of flies. Roush and Plapp (1982) explained the variation in development time due to difference in temperature in the larval medium, which warmed as a result of bacterial fermentation. However, small difference in values among replicates cannot justify this argument, because the susceptible strain is subjected to the same temperature variation; a plausible explanation is still required. The warming of larval medium due to bacterial fermentation can occur in all replicates of resistant and susceptible strains where equal number of eggs were placed / incubated in each replicate.

It has been noted that small changes in development time could have much greater effect on reproductive potential than small changes in fecundity (Lewontin, 1965). The development time in the present study consists of larval and pupal durations; increase in one period was offset by the other period. Though longer development time was observed in all resistant strains than Cooper except IThb. As the pupal duration in IThb was studied only in single generation so it was difficult to explain the value obtained when development and generation times were similar in IThb and Cooper. The strain IThb is selected with DDT once a year (at 60-70% mortality) and the study was carried out with this yearly selection of IThb but little difference was found between biotic potential and intrinsic rate of increase between lZbb and Cooper. In other words, similar biotic potential between the IThb an Cooper strains also explains the selection of an energetically efficient resistance mechanism in IThb strain, that results in population fitness equaling or exceeding the fitness of the Cooper strain. The energetically efficient resistance mechanism could be the elevated enzyme activity or intensitivity of target (Ho:ffman and Fisher, 1994). Possibly selection for resistance mechanism in IThb had no effect on the reproductive potential, compared to Cooper strain. The strain 7, resistant to dieldrin and DDT, seems to be at fitness disadvantage, despite the high fecundity rate which had been shown previously by Knutson (1959) and Georghiou (1965), they found that application of dieldrin to the house flies caused an increase in fecundity over the untreated control. This may' explain the stimulatory effect of dieldrin on egg production. The pre-exposed high fecundity of strain 7 compared with other strains cannot be explained on the basis given above, the most likely explanation for such an observation can be sought in heterogeneous nature of this strain to dieldrin resistance.

The results of fecundity deficits in 171 strain resistant to pyrethroids match with that ofCampanhola *et al., (1991)* who found lower egg production in ICI strain of *Heliothis virescens* L. Reduced fecundity resulting from resistance appears to be the most common fitness cost (Carriere *et al.*, 1994). The strain 571ab, resistant to organophosphate and susceptible to pyrethroid, shared the non-significant difference in fecundity rate with 171 strain, which is resistant to pyrethroid. The fecundity deficiency in strain 571ab can be associated with metabolic resistance in this strain. The association of fitness with a resistance mechanism appears to vary in the presence of cross resistance mechanism where strains are resistant to more than one insecticide, because strains may differ in fitness for reasons unrelated to resistance (Roush and Daly, 1990). A relationship between log LC_{50} and mean daily progeny production in the absence of insecticide exposure was negative for esfenvalerate and positive for methomyl an non-significant for endosulfan or oxydemeton-methyl (Hollingworth et al., 1997).

This study suggests that the major fitness cost of resistance to one insecticide can obscure relationship between fitness

and resistance to another insecticide.

The present data show that intrinsic rate of increase (r) except for strain 7, biotic potential and relative biotic potential appear to be the same in most of the strains, but levels of those parameters were less than those of Cooper strain. The lowest relative biotic potential in strain 171 may explain the possibility of heterogeneity in the population. However, r, biotic potential and relative potential values obtained for a single generation cannot extrapolate genetic potential of a strain. At present, the data show that all these strains, in spite of differences in life history stages can be used for further selection without much loss in overall fitness.

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