Comparative Fitness of Malathion-Resistant and Susceptible Indianmeal moth (Lepidoptera: Pyralidae)[1,2]

W. Ross Halliday

Stored Product Insects Research and Development Laboratory, USDA-ARS, P.O. Box 22909 Savannah, GA 31403

J. Entomol. Sci. 25(2): 239-245 (April 1990)

ABSTRACT Three factors contributing to fitness of a species (fertility, fecundity, and development time) were measured in the susceptible Savlab strain and the > 100-fold malathion-resistant Statesboro strain of the Indianmeal moth, *Plodia interpunctella* (Hübner). Both fertility (percent egg hatch) and fecundity (eggs/female) were significantly lower in the resistant strain. Percent egg hatch in the two strains was 96.2% and 73.1% for Savlab and Statesboro respectively. Fecundity was 444 eggs/female for Savlab and 321 eggs/female for Statesboro. Development time did not differ significantly (24.2 ± 0.3 and 23.6 ± 0.2 days for 50% emergence). There was no evidence that these differences arose from non-lethal effects caused by malathion treatment to maintain resistance in the Statesboro strain. The differences, while associated with malathion resistance, cannot be unequivocally attributed to the presence of the resistance gene. When the intrinsic rate of increase (r_m) was calculated using an iterative computer program, Statesboro had a significantly lower rate (0.1734 ± 0.0016) than did Savlab (0.2048 ± 0.0014). Similar measurements were performed with progeny from reciprocal F_1 crosses. While some parameter's values were closer to Statesboro or Savlab an apparent mating incompatibility suggests that using the data from F_1 crosses is premature until this question is resolved.

KEY WORDS *Plodia interpunctella,* Indianmeal moth, malathion, resistance.

Insecticide resistance may be accompanied by a decreased fitness. One way to quantitate this decrease is to measure individual components of fitness: fertility, fecundity, developmental period, and mating success in two strains which differ ostensibly only in the gene(s) for resistance (Roush and McKenzie 1987). One such consequence of decreased fitness would be loss of resistance following cessation of selection pressure, an assumption frequently made in resistance management schemes (Georghiou 1983). It has been found in at least one case that the resistance gene itself does not cause the lowered fitness in malathion-resistant red flour beetle, *Tribolium castaneum* Herbst (Beeman and Nanis 1986).

Malathion has been continually used in grain and peanut storage facilities since its registration in the early 1960's. The inevitable result has been selection for high levels and frequency of resistance in three major pests; red flour beetle; almond moth, *Cadra cautella* (Walker); and Indianmeal moth, *Plodia interpunctella* Hübner

[1] Accepted for publication 5 December 1989.

[2] Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the US Department of Agriculture, nor does it imply registration under FIFRA as amended.

(Speirs and Zettler 1969, Haliscak and Beeman 1983, Arthur et al. 1988, Halliday et al. 1988). Several studies have compared the fitness of susceptible and resistant strains of these stored product pests. For example, Brower (1974) found that a strain of *T. castaneum* resistant to both malathion and DDT produced significantly fewer progeny than did the susceptible strain. In contrast, Beeman and Nanis (1986) inserted the malathion resistance gene of *T. castaneum, Rmal-1,* into a susceptible background by repeated backcrosses and found no differences when they monitored the change in the frequency of *Rmal-1* in the absence of insecticide. This was interpreted as evidence that the resistance gene by itself does not confer a selective disadvantage. Similarly, neither Brower (1973) or Zettler (1977) detected any differences between resistant and susceptible strains of *P. interpunctella* and *C. cautella* when they measured the mean number of progeny produced and time to peak emergence.

Despite this previous lack of correlation between resistance and decreased fitness, this study was undertaken to explain an observed lower rate of adult eclosion in malathion resistant laboratory colonies. This lower survival, if not an artifact, would have implications for resistance management in this species. When preliminary studies revealed consistently lower fertility, the study was expanded to also compare fecundity and development time. These three factors were then used to calculate a summary statistic, the intrinsic rate of increase r_m for the two strains (Andrewartha and Birch 1954).

Materials and Methods

The insects used in this study have been reared in the laboratory for many generations. The susceptible strain, Savlab, has been maintained on a standard artificial diet (Silhacek and Miller 1972) for more than 15 years. The history of the resistant strain, Statesboro, has been described previously (Halliday 1988). Resistance is maintained by topically treating fifth instar feeding larvae with 400 µg malathion which results in a resistance level of > 100-fold. Survivors are reared to adulthood and the eggs used to produce the next generation. At least 200 larvae are treated each generation. Both strains were reared at a density of 30 ± 1 mg eggs per 500 gm freshly prepared media.

To measure development time (DT), approximately 110 freshly laid eggs were reared on 125 gm fresh media. One replication consisted of one test in which ca. 110 eggs were collected and allowed to eclose. Subsequent replications came from different parents. In most instances the age of the eggs was ≤ 6 hrs. Once adults started to emerge, they were removed from each sample and the number recorded every 24 h. Average development time (ADT) was calculated using a weighted mean procedure. ADT is defined as the time (in days) required for 50% of the adults to emerge from a sample. Density did not affect ADT. In experiments in which the number of insects ranged from 10 to 125 per 125 gm media, ADT ranged from 23.4 to 23.8 days. There was no consistent change in ADT with increasing density.

To determine fecundity, larvae of Savlab and Statesboro were isolated according to sex late during the last larval instar, allowed to pupate and eclose. Virgin males and females were added to a 4-liter jar soon after emerging. Mating pairs were isolated singly in 20 ml vials with a wire screen top and kept at 27°C and 55-60% RH. The number of eggs produced by each pair was counted each day until both

parents were dead. Fertility and DT were measured in F_1 progeny from reciprocal crosses between Savlab and Statesboro by isolating late fifth instar larvae and allowing them to emerge and mate with the opposite strain. Eggs were collected and used for measuring percent hatch or DT. Fecundity was measured by collecting fifth instar F_1 larvae of each cross and allowing the adults to mate *inter se*. Eggs were collected and counted from mating pairs which had been isolated singly as above.

Egg hatch (% fertility) was measured by periodically collecting eggs (ca. 125 per replication) produced by a large number (> 10) females as mentioned above and isolating them in petri dishes on black construction paper. The number of unhatched eggs was recorded at various time intervals until the number remained constant. Duplicate or triplicate samples were run for each replication. Cannabalism was a minor problem and was easy to detect since the cannibalistic larvae leave frass where they consume eggs. Eggs kept in a dish with a loose fitting lid were subject to little if any predation, presumably because most hatched larvae escaped before feeding on the remaining eggs.

An experiment investigated the non-lethal effects of the malathion treatment on fecundity and fertility of the Statesboro strain. To eliminate as much variation as possible, these parameters were measured within three separate families. Three virgin mating pairs were isolated separately in vials. The resultant eggs were reared on artificial diet as before at a density of 30 mg eggs per 500 gm media. Midway through the fifth instar, half of each sex were treated with the discriminating dose of malathion and isolated according to sex and treatment. The remaining larvae were likewise isolated, but not exposed to malathion. Mortality of both treatments was monitored. As virgin adults of each sex and treatment eclosed, they were placed with the opposite sex in a 4 L jar to allow mating. Mating pairs were collected individually in vials. The number of eggs per female and the percent egg hatch were recorded for each pair as already described. Data from each family was pooled resulting in a total of three replications since three families were originally used.

Analysis of variance (Proc GLM) was carried out to detect significant differences between strains (SAS Institute 1987). SAS was also used to calculate ADT by a weighted means procedure. The intrinsic rate of increase, r_m, was calculated using the program of Abou-Setta et al. (1986). This program requires knowing the schedule of egg laying (female progeny per female per time interval, m_x) and the age specific survival of females, 1_x. For 1_x, I chose to use the values of 0.88 for Savlab and 0.72 for Statesboro for the following reasons. These two values represent the probability of an individual surviving to eclosion in each strain. Very little mortality occurred in adult females until they had finished laying eggs. Consequently, the survival of females could be considered a function of only the mortality up to eclosion. The r_m was calculated for each female of the two strains since the number of eggs layed by each female every 24 hours was known from the fecundity study. The estimate for 1_x came from experiments in which a known number of eggs were placed on media and the percent emergence was calculated. Thus 1_x takes into account mortality from all sources during egg, larval, and pupal development. Then the average r_m was calculated and ANOVA (Proc GLM) carried out using SAS to get an estimate of the r_m of the population.

Results and Discussion

Table 1 lists the results of the non-lethal effects experiment. Survival following the discriminating dose was uniformly high ranging from 95.4 to 100% for the treated larvae and ranging from 94.3 to 96.4% for the non-treated larvae. In terms of mortality, the Statesboro strain is unaffected by the discriminating dose.

Of more interest was whether adults or their resulting progeny exhibited any non-lethal effects due to the malathion treatment. Analysis of variance was used to detect differences in fertility between replications and between treatments. There was no difference in fertility in any comparison (Table 1). ANOVA produced F values of 0.13, 0.11, and 1.86 when fertility of treated and non-treated females was compared for replications 1, 2, and 3. When the data from all three replications was pooled, an ANOVA gave an F value of 0.60 (1 df, $P > 0.446$).

When effects of malathion treatment on fecundity were investigated, similar results were found (Table 1). Fecundity did not decrease in response to malathion treatment. As shown in Table 1, in only one of the three replications was fecundity lower in the malathion-treated samples. ANOVA produced values of 0.12, 1.32, and 3.29 for comparisons within each replication. When the data is pooled, the value of the F statistic is 1.48, indicating there is no significant difference in the means of the two treatments. The results of the sublethal portion of this experiment strongly suggest that treating Statesboro larvae with malathion to ensure homozygosity, does not adversely affect fertility or fecundity.

Average development times (ADT) for Savlab and Statesboro were 24.2 and 23.6 days respectively (Table 2). One way analysis of variance did not reveal a significant difference in the ADT. These values are considerably lower than those of Zettler (1977) in which the time to peak emergence was measured, not the time to 50% emergence. In that study he allowed the mated moths to continuously lay eggs over several days. This may lengthen the time span during which eclosion occurs. This would mask any slight differences in DT. Had the schedule of egg laying been different, it would not have been detected. A delayed DT is one of the classical pieces of evidence used to argue for a lower fitness in homozygous resistant insects (Ferrari and Georghiou 1981, Roush and Plapp 1982). It does not appear to play a role in malathion resistance in this species.

Mean percent egg hatch (fertility) in Statesboro was significantly lower (73.1%) than that of the Savlab strain (96.2%) (Table 2). Fertility of the Savlab strain has been reported to range from 92.0% (Flaherty et al. 1973) to 96.0% (Press and Flaherty 1972).

Statesboro produced an average of 320.5 eggs per female while Savlab produced 444.2 eggs per female (Table 2). Analysis of variance demonstrated this difference to be significant (df = 1, F = 70.05, $P < 0.0001$). Fecundity of the Savlab strain has been reported to be much lower. Zettler (1977) and Press and Flaherty (1972) observed 198 and 240 eggs per female. Their low values might have arisen from rearing the larvae at higher densities than in this study.

Reciprocal F_1 crosses were carried out to determine whether the differences observed for Savlab and Statesboro were genetically dominant or overdominant (heterosis). Due to some unknown factor(s), successful mating, as defined by the production of eggs in a pattern similar to that of Savlab or Statesboro, was quite low. For ♀ Statesboro x ♂ Savlab mating success was 26.7% (4 of 15). For ♀ Savlab x ♂ Statesboro the success rate was 50.0% (12 of 24). The low success rates are not

Table 1. Comparison of survival, fertility (mean ± SE) and fecundity (mean ± SE) of the malathion-resistant Statesboro strain of *Plodia interpunctella* treated or not treated with a discriminating dose of malathion (400 μg/larva).

	Survival				Fertility					Fecundity				
	Treated		Untreated		Treated		Untreated			Treated		Untreated		
Replication	% Survival	(n)	% Survival	(n)	% Hatch	(n)	% Hatch	(n)	F	Avg. # eggs/female	(n)	Avg # eggs/female	(n)	F
1	100.0	(30)	96.4	(28)	80.5 ± 5.5	(6)	83.3 ± 5.3	(7)	0.13	378.7 ± 42.3	(6)	395.9 ± 28.3	(7)	0.12
2	95.4	(55)	94.3	(53)	85.6 ± 3.4	(6)	83.6 ± 4.6	(7)	0.11	365.3 ± 34.2	(7)	313.5 ± 29.7	(8)	1.32
3	95.5	(22)	94.7	(19)	88.4 ± 6.1	(6)	96.8 ± 0.8	(6)	1.86	470.3 ± 11.6	(6)	419.6 ± 23.8	(7)	3.29
TOTAL	96.3	(107)	95.0	(100)	84.8 ± 2.9	(18)	87.4 ± 2.8	(20)	0.60	402.7 ± 20.8	(19)	373.5 ± 18.3	(22)	1.48

Table 2. Comparison of biological parameters associated with fitness in malathion-susceptible (Savlab) and -resistant (Statesboro) Indianmeal moth.

	Parameter*					
Strain	n	Development Time (Days)	n	Fertility (Percent)	n	Fecundity (Eggs/Female)
Savlab	14	24.2 ± 0.3 A	7	96.2 ± 0.7 A	38	444.2 ± 7.0 A
Statesboro	5	23.6 ± 0.2 A	10	73.1 ± 5.6 B	81	320.5 ± 9.6 B

* Values in columns followed by the same letter are not significantly different (P > 0.05, Duncan's Multiple Range Test).

due to unsuccessful spermatophore transfer in mated but non-egg-laying females. Five of these females were examined for the presence of spermatophores and all contained at least one. It is not known whether sperm were successfully transferred to the spermatheca from the spermatophore.

There was great variation in fecundity of the F_1 progeny. Egg production in F_1 (♀ Savlab × ♂ Statesboro) was 350.0 ± 32.0 (SEM) for a sample size of 12. Egg production in F_1 (♀ Statesboro × ♂ Savlab) was slightly lower, 337.0 ± 52.8 (n = 4). Duncan's Multiple Range Test (SAS Institute, 1987) revealed these values were not significantly different from Statesboro but were different from Savlab. Fertility in F_1 eggs was high; 96.1 ± 0.30% (n = 2 replications) for ♀ Savlab × ♂ Statesboro and 91.5 ± 2.0% (n = 2 replications) for ♀ Statesboro × ♂ Savlab. Percent adult emergence in the two F_1 crosses followed the pattern of the maternal parent. For F_1 (♀ Savlab × ♂ Statesboro) there was 88.2 ± 1.8% emergence (n = 3 replications). For the opposite cross, F_1 (♀ Statesboro × ♂ Savlab) there was a 75.6 ± 7.3% emergence (n = 3 replications). DT, in days, for the F_1 progeny were 22.0 ± 0.10 for F_1 ♀ Savlab × ♂ Statesboro and 22.4 ± 0.10 for F_1 ♀ Statesboro × ♂ Savlab. No calculations of r_m were carried out for the F_1 strains since it was felt that the F_1 data were too preliminary in nature due to the unresolved question of the low mating success.

The intrinsic rate of increase, r_m, was used as a summary statistic. Savlab's r_m under these conditions was 0.2048. Statesboro's r_m was 0.1734. Analysis of variance revealed there was a significant difference (df = 1, F = 80.14, $P < .0001$) between strains. Thus, fitness of Statesboro is approximately, 85% that of Savlab, a reduction similar to that (79%) found by Ferrari and Georghiou (1981) for an organophosphate resistant strain of *Culex quinquefasciatus* Say. Resistant individuals in a field population exhibiting decreased fitness should, in theory, be at a severe disadvantage in the absence of insecticide. Over time, the result would be a decrease in the frequency of the resistance gene. If lower fitness were not associated with the resistance gene itself, but was a characteristic of the population, one might expect population levels to be lower in grain bins infested with the lower fitness moths since they would produce fewer viable eggs.

The data in this paper show that a malathion-resistant strain of the Indianmeal moth has significantly lower fitness than does a susceptible strain. Contributing factors to this were lower fertility and lower fecundity. In the absence of repeated backcrosses to the susceptible strain, it is not known whether this decreased

fitness is due to the resistance gene or other factors such as inbreeding, chronic diseases in one but not the other strain, or the origin of each strain.

References Cited

Abou-Setta, M. M., R. R. Sorrell, and C. C. Childers. 1986. LIFE 48: A basic computer program to calculate life table parameters for an insect or mite species. Fla. Entomol. 69: 690-697.

Andrewartha, H. G. and L. C. Birch. 1954. The distribution and abundance of animals. Chicago Univ. Press, Chicago, IL. 782 pp.

Arthur, F. H., J. L. Zettler, and W. R. Halliday. 1988. Insecticide resistance among populations of almond moth and Indianmeal moth (Lepidoptera: Pyralidae) in stored peanuts. J. Econ. Entomol. 81: 1283-1287.

Beeman, R. W. and S. M. Nanis. 1986. Malathion resistance alleles and their fitness in the red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol. 79: 580-587.

Brower, J. H. 1973. Gamma radiation sensitivity of malathion-resistant strains of the Indianmeal moth. J. Econ. Entomol. 66: 461-462.

Brower, J. H. 1974. Radio-sensitivity of an insecticide-resistant strain of *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 10: 129-131.

Ferrari, J. A. and G. P. Georghiou. 1981. Effect of insecticidal selection and treatment on reproductive potential of resistant, susceptible and heterozygous strains of the southern house mosquito. J. Econ. Entomol. 74: 323-327.

Flaherty, B. R. J. W. Press, and P. T. M. Lum. 1973. Fecundity of *Plodia interpunctella* (Hübner) adults after exposure to sublethal dosages of carbon dioxide. J. Ga. Entomol. Soc. 8: 141-145.

Georghiou, G. P. 1983. Management of resistance in arthropods. In *Pest Resistance to Pesticides,* ed. G. P. Georghiou & T. Saito. pp. 769-792. New York: Plenum. 809 pp.

Haliscak, J. P. and R. W. Beeman. 1983. Status of malathion resistance in five genera of beetles infesting farm-stored corn, wheat, and oats in the United States. J. Econ. Entomol. 76: 717-722.

Halliday, W. R. 1988. Tissue specific esterase and malathion carboxylesterase activity in larvae of malathion-resistant *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 24: 91-99.

Halliday, W. R., F. H. Arthur, and J. L. Zettler. 1988. Resistance status of red flour beetle (Coleoptera: Tenebrionidae) infesting stored peanuts in the Southeastern United States. J. Econ. Entomol. 81: 74-77.

Press, J. W. and B. R. Flaherty. 1972. Reduction of fecundity and egg hatch in three stored-product Phycitid moths after repetitive sublethal carbon dioxide exposures. Environ. Entomol. 2: 147-148.

Roush, R. T. and F. W. Plapp, Jr. 1982. The effects of insecticide resistance on the biotic potential of house flies. J. Econ. Entomol. 75: 708-713.

Roush, R. T. and J. A. McKenzie. 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. 32: 361-380.

SAS Institute. 1987. SAS/STAT™ Guide for Personal Computers, Version 6 Edition. SAS Institute, Cary, NC.

Silhacek, D. L. and G. L. Miller. 1972. Growth and development of the Indianmeal moth, *Plodia interpunctella* (Lepidoptera: Phycitidae) under laboratory mass rearing conditions. Ann. Entomol. Soc. Am. 65: 1084-1087.

Speirs, R. D. and J. L. Zettler. 1969. Toxicity of three organophosphorus compounds and pyrethrins to malathion-resistant *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 4: 279-283.

Zettler, J. L. 1977. Fecundity of malathion-resistant *Plodia interpunctella* and *Ephestia cautella* (Lepidoptera: Phycitidae). J. Ga. Entomol. Soc. 12: 333-336.