

Malathion Resistance in Indianmeal Moths (Lepidoptera: Pyralidae) Infesting Stored Corn and Wheat in the North-Central United States¹

RICHARD W. BEEMAN, WILLIAM E. SPEIRS, AND BARBARA A. SCHMIDT

U.S. Grain Marketing Research Laboratory, ARS, USDA,
Manhattan, Kansas 66502, and Department of Entomology,
Kansas State University, Manhattan, Kansas 66506

ABSTRACT

J. Econ. Entomol 75: 950-954 (1982)

Forty-three strains of *Plodia interpunctella* (Hübner) were collected from grain bins on farms in nine midwestern states. Thirty-nine of the 43 strains were more than 17-fold resistant to malathion. None of 10 resistant strains tested were cross-resistant to chlorpyrifos methyl. Of six resistant strains assayed for malathion carboxylesterase activity, all had greatly elevated levels over those of a susceptible strain. The α -monoacid of malathion was the major hydrolysis product in all six resistant strains. When esterase electrophoretograms were prepared of 16 strains, ranging from purely susceptible to purely resistant, a good correlation was observed between resistance level and the staining intensity of certain esterase bands. We conclude that carboxylesterase-type malathion-specific resistance is epidemic in Indianmeal moth populations throughout the U.S. grain belt.

The Indianmeal moth, *Plodia interpunctella* (Hübner), is a serious pest of stored grain in this country (Cotton 1961, McGaughey et al. 1978). Malathion was registered for insect control on postharvest grain in 1958 (Anonymous 1958) and has been the primary residual insecticide for direct treatment of postharvest grain since that time. A number of surveys has revealed widespread resistance to malathion in the Indian meal moth on a worldwide scale (Parkin 1965, Zettler et al. 1973, Champ and Dyte 1976, Attia 1977). Severe resistance, specific to malathion, has recently been reported in Indian meal moths infesting peanut storage facilities in Alabama, Florida, and Georgia (Zettler 1982). Sporadic cases of malathion resistance in this species have been found in North Carolina (Bansode et al. 1981) and California (Armstrong and Soderstrom 1975). The present study provides the first systematic documentation of the extent, severity, and physiological nature of malathion resistance in populations of the Indian meal moth in the U.S. grain belt.

Materials and Methods

Chemicals

Technical-grade malathion (95% pure) and analytical-grade chlorpyrifos methyl were donated by NOVA Products, Inc., and by the Dow Chemical Co., respectively. A crystalline sample of malathion monocarboxylic acid (90% α and 10% β) was a gift from American Cyanamid. [1,2-¹⁴C] malathion (side chain ethyl label), specific activity 4.6 mCi/mmol, was purchased from Amersham.

Sampling

Corn and wheat from randomly selected bins in 26 states were sampled in the summer of 1980 under a

project coordinated by the Agricultural Stabilization and Conservation Service that was designed to assess the quality of farm-stored grain reserves. All samples containing live Indianmeal moths were saved until a total of 43 strains was accumulated from nine states. Within each state, samples containing few insects were pooled until ≥ 20 insects were accumulated for each strain. Most of the resulting strains contained insects from more than one bin, but no two strains contained insects from the same bin. Most of the field strains came from corn, which is the major host grain for the Indian meal moth in the North-Central United States (C. L. Storey, personal communication). The standard laboratory (susceptible) strain has been maintained at the U.S. Grain Marketing Research Laboratory for many years.

Rearing and Bioassay

Before testing, all strains were acclimated to laboratory diet by continuous rearing for at least three generations. The diet consisted of a wheat-based medium fortified with wheat germ, yeast, honey, and glycerol. All testing was completed by the eighth generation.

Wandering stage, 5th instars were treated topically with acetone solutions of insecticides as described by Zettler et al. (1973). All strains were tested by using a discriminating dose of malathion, i.e., a dose known to produce 100% mortality in the standard laboratory (lab-S) strain. Because of preliminary indications that resistance to malathion was severe, we chose a high discriminating dose of 20 μ g (= 4 \times LD₁₀₀ and 17 \times LD₅₀) such that only highly resistant insects would survive. Probit analysis of the data for the lab-S strain was conducted by the method of Finney (1952). All doses are expressed as μ g per larva.

Carboxylesterase Assay

Fifty 5th instars (25 of each sex) were homogenized in 37.5 ml of 0.067 M sodium phosphate buffer pH 7.2 in a glass-Teflon homogenizer. Homogenates were diluted with an equal volume of buffer and centrifuged at

¹Contribution No. 82-151-j, Dept. of Entomology, Kans. Agric. Exp. Stn., Manhattan. Cooperative investigation between the Kans. Agric. Exp. Stn. and the USDA. Mention of a proprietary product does not constitute a recommendation or an endorsement by the USDA. Received for publication 23 November 1981.

Table 1. Resistance to malathion and cross-resistance to chlorpyrifos methyl in field strains of the Indianmeal moth^a

Strain ^b	Mortality (%) ^c		R-factor ^d	
	Malathion	Chlorpyrifos methyl	Malathion	Chlorpyrifos methyl
NE-1	0 ± 0		>17	<2.2
IA-1	0 ± 0	97.3 ± 0.9	>17	<2.2
MN-1	0 ± 0.1	100 ± 0	>17	<2.2
IA-2	0.4 ± 0.7		>17	
SD-1	0.4 ± 0.4	94.4 ± 9.7	>17	<2.2
IA-3	0.6 ± 0.9	100 ± 0	>17	<2.2
ND-1	0.7 ± 1.2		>17	
MN-2	1.2 ± 2.1		>17	
SD-2	1.6 ± 1.7	98.8 ± 2.0	>17	<2.2
NE-2	1.7 ± 2.3	95.2 ± 5.6	>17	<2.2
MO-1	2.3 ± 2.0		>17	
NE-3	2.7 ± 0.7		>17	
KS-1	2.9 ± 3.6	98.7 ± 2.3	>17	<2.2
ND-2	3.6 ± 3.7		>17	
MN-3	3.8 ± 4.5		>17	
IA-4	4.0 ± 3.7		>17	
MN-4	4.1 ± 6.2		>17	
ND-3	5.2 ± 6.5		>17	
MN-5	5.2 ± 4.0	97.7 ± 4.0	>17	<2.2
MN-6	5.3 ± 4.6		>17	
IA-5	5.3 ± 3.9		>17	
NE-4	5.3 ± 4.8		>17	
ND-4	5.5 ± 5.5		>17	
WI-1	6.4 ± 5.1		>17	
MO-2	9.0 ± 5.7	96.2 ± 6.6	>17	>2.2
IA-6	11.3 ± 8.1		>17	
IA-7	15.2 ± 9.4		>17	
MN-7	16.2 ± 7.8		>17	
MN-8	17.0 ± 8.1	100 ± 0	>17	<2.2
SD-3	22.3 ± 2.4		>17	
ND-5	22.7 ± 5.6		>17	
ND-6	22.8 ± 6.9		>17	
IA-8	23.1 ± 6.4		>17	
IA-9	27.6 ± 12.0		>17	
WI-2	32.1 ± 1.3		>17	
MN-9	37.9 ± 10.5		>17	
IL-1	38.2 ± 12.8		>17	
WI-3	44.1 ± 10.9		>17	
MN-10	44.4 ± 13.9		>17	
SD-4	60.2 ± 5.2			
MN-11	68.1 ± 4.9			
MN-12	91.5 ± 7.3			
MN-13	100 ± 0			
SD-5	100 ± 0			
Lab-S	100 ± 0		≡1.0	≡1.0

^aLarvae were treated with a single discriminating dose of insecticide. The doses used were 20 µg/larva (4 × LD₁₀₀) for malathion and 0.5 µg/larva (~LD₉₉) for chlorpyrifos methyl. See text for probit analysis of data for the Lab-S strain.

^bStrain identifications indicate the state of origin.

^cValues for percent mortality are means (±SD) of three independent determinations, using 30 to 60 larvae each (either sex). Values were corrected for mortality in the controls by using Abbott's formula.

^dThe resistance factor is the ratio of the LD₅₀ value for the field strain to that for the Lab-S strain.

10,000 × g for 10 min. The supernatant was used as the enzyme source. Carboxylesterase assay was carried out in 1 ml of enzyme solution containing 50 µg (0.001 µCi) of [¹⁴C]malathion. Assay tubes were incubated for 30 min at 30°C. The reaction was stopped with 100 µg of triphenylphosphate and 125 µg of nonradioactive malathion monoacid. We developed a rapid and efficient technique for separating malathion from its carboxylester hydrolysis products by using disposable, reversed-phase C-18 Sep-Pak cartridges, purchased from Waters Associates, Inc. The cartridge was prewetted with 2 ml

of acetonitrile, followed by 5 ml of 0.1 M phosphate buffer pH 7.0. The reaction mixture was then loaded onto the cartridge, followed by 2 ml of phosphate buffer. Malathion monoacids were then eluted with 10 ml of 25% acetonitrile in phosphate buffer. Finally, malathion was eluted, and the cartridge was regenerated with 5 ml of acetonitrile, followed by 5 ml of buffer. An aliquot of the 25% acetonitrile eluate was counted by liquid scintillation spectroscopy (LSC).

The α- and β-monoacids were separated by high-performance liquid chromatography (HPLC) and quantified

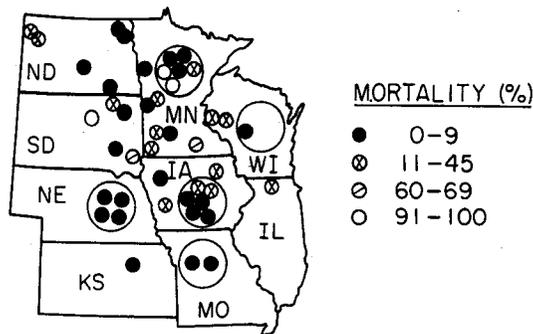


FIG. 1.—Distribution of malathion resistance in populations of the Indian meal moth in the North-Central United States. Data points enclosed by large circles indicate pooled strains from many counties or from scattered localities within the state. Data points not enclosed by large circles indicate the county of origin or the county contributing the largest number of samples in the case of composite strains from a few adjacent counties. Data for percent mortality are from Table 1.

by LSC. An aliquot of the Sep-Pak fraction containing the monoacids was acidified to pH 1.5 to 1.9 with 1 N HCl and extracted with an equal volume of chloroform. The chloroform phase was spiked with an additional increment of nonradioactive monoacids for subsequent peak identification on HPLC. The chloroform was then evaporated under N_2 , and the residue was redissolved in 50% (vol/vol) aqueous acetonitrile containing 0.05% (vol/vol) acetic acid for HPLC analysis. A Waters model 6000A pump was used in conjunction with a Waters model 710B injection system, a Hitachi model 100-10 UV-vis spectrophotometer, and a Waters model 730 data module. We used a Bio-Rad ODS-10 reverse-phase column (25 cm by 4 mm) in series with a 3-cm precolumn of the same composition. The mobile phase (50%, vol/vol aqueous acetonitrile containing 0.05%, vol/vol, acetic acid) was pumped at a flow rate of 1 ml/min. The detector was set at 235 nm. Under these conditions the retention times of the α - and β -monoacids were 5.5 and 6.3 min, respectively, and base-line separation was achieved. Peaks corresponding to the α - and β -monoacids were collected separately and quantified by LSC.

Electrophoresis

Zone electrophoresis was performed on polyacrylamide gels by using a Bio-Rad model 220 dual vertical gel slab cell. We used a system of nondenaturing gels based on the procedure of Laemmli (1970). The 6% acrylamide running gels pH 8.8 were overlaid with 3% stacking gels pH 6.8. The 1.5-mm gels were electrophoresed at 4°C at a constant current of 10 mamp per gel stacking and 30 mamp per gel running. After electrophoresis, the gels were stained for α -naphthyl acetate esterase by soaking at room temperature in 0.1 M phosphate buffer, pH 6.0, containing 0.05% (wt/vol) Fast Red TR salt (Sigma Chemical Co.) and 0.0093% (wt/vol) α -naphthyl acetate, the latter added with 1.3 ml of 50% acetone per 100 ml.

Results and Discussion

Survey of Resistance

Resistance to malathion was found to be widespread and severe in field-collected populations of the Indian-meal moth (Table 1). Of 43 strains tested, 26 (representing 8 states) were almost fully resistant (> 90% survival at the discriminating dose used). Thirty-nine of the 43 strains were more than 17-fold resistant at the LD_{50} level, and only two strains suffered 100% mortality, not including the standard laboratory (lab-S) strain. For malathion against the lab-S strain, $LD_{50} = 1.17 \mu\text{g}$ (1.06–1.29), slope = 3.32, $LD_{95} = 3.65 \mu\text{g}$ (2.93–5.06, and $5 \mu\text{g} = LD_{100}$ (95% confidence intervals given in parentheses). Figure 1 illustrates the geographic distribution and resistance levels of the strains tested.

The calculated resistance factor of $\times 17$ is a minimum value. Since malathion resistance in the Indian meal moth is usually extreme (Zettler 1982), the true value is probably much higher for most strains. NE-1 was the only field strain tested at higher doses. It was virtually immune to malathion (2% mortality at a dose of 320 μg) and had a resistance factor of >273.

Cross-resistance to chlorpyrifos methyl was negligible. Ten strains (representing six states) highly resistant to malathion were tested at an $\sim LD_{95}$ dose (0.5 μg) of chlorpyrifos methyl. Mortality was >94% for all strains (Table 1). For chlorpyrifos methyl against the lab-S strain, $LD_{50} = 0.23 \mu\text{g}$ (0.19–0.26), slope = 6.21, and $LD_{95} = 0.42 \mu\text{g}$ (0.34–0.76).

Survey of Carboxylesterase

At least two types of malathion resistance are common in nature, namely a nonspecific type, which extends to many other organophosphate insecticides, and a specific type. The latter results from increased activity of a malathion-degrading carboxylesterase enzyme in the resistant strains and usually confers cross-resistance to only a few substances closely related to malathion (Matsumura and Brown 1961, Townsend and Busvine 1969). Since none of the malathion-resistant strains of the Indian meal moth were highly cross-resistant to chlorpyrifos methyl, we suspected that carboxylesterase-type resistance might be the predominant type in this species.

We screened six field strains from four states for malathion carboxylesterase activity. The strains ranged from partially to totally resistant (0 to 44% mortality at the discriminating dose used). A highly resistant laboratory strain (lab-R), known to possess malathion-specific resistance of the carboxylesterase type (Beeman and Schmidt 1982) was included for comparison. The lab-R strain and all six field strains had greatly elevated carboxylesterase levels over those of the standard laboratory (sensitive) strain (Table 2). The α -monoacid was the major hydrolysis product in all seven resistant strains. In contrast, the susceptible strain produced about equal amounts of the α - and β -products (Table 2). Preferential hydrolysis of the α -ester is characteristic of this type of malathion resistance in other insect species (Welling and Blaakmeer 1971). These results suggest that carboxy-

Table 2.—Malathion carboxylesterase activity in malathion-resistant strains of the Indianmeal moth

Strain	Mortality (%) ^a	Malathion carboxylesterase (relative activity) ^b	Monoacid ratio (α/β) ^b
Lab-S	100	1.00	1.1
WI-3	44	5.06	3.4
SD-3	22	8.22	7.3
NE-2	2	12.6	4.7
IA-3	1	8.30	8.1
IA-1	0	10.6	6.7
MN-1	0	12.0	8.1
Lab-R	7	12.1	6.7

^aData for percent mortality refer to a 20- μ g dose of malathion (see Table 1).

^bData for malathion carboxylesterase activity and monoacid ratio are single determinations, each based on a homogenate of 50 pooled larvae.

lesterase-type malathion-specific resistance may be the major or sole type present in Indianmeal moths in the North-Central United States.

Numerous studies have demonstrated a close genetic association in insects between esterase-related organophosphate resistance and aliesterases which hydrolyze naphthyl acetate or methyl butyrate. Aliesterase activities in resistant strains may be either higher (Ozaki and Kasasai 1970, Beranek 1974, Georghiou and Pasteur 1980) or lower (Franco and Oppenoorth 1962, Togby et al. 1976) than those in susceptible strains, depending upon species. Naphthyl acetate esterases can be conveniently visualized on electrophoretic gels by rapid and specific staining procedures (Hubby and Lewontin 1966, de Stordeur 1976). The stained gels, or "zymograms," can thus serve as biochemical indicators of esterase-related organophosphate resistance.

Figure 2 shows esterase zymograms of 16 field strains of the Indian meal moth from 8 states. The strains ranged from purely resistant to purely susceptible (0 to 100% mortality after a discriminating dose of malathion). At least 11 bands of esterase activity were resolved on the gels. The bands fell into four mobility groups, labelled A to D in Fig. 2. The most resistant strains had generally fewer and fainter esterase bands than the most suscep-

tible strains. In particular, a good correlation was observed between resistance and the staining intensities of group A esterases. Ten of the 11 susceptible or partially susceptible strains tested (22 to 100% mortality after a discriminating dose of malathion) had intensely staining esterase bands at A2 and A4. Strain SD-4 (60% mortality) was the single exception. In sharp contrast, none of the seven highly resistant strains (0 to 9% mortality) showed intense staining at positions A2 and A4, although the resistant strain MN-2 had an intense band at A1. These observations are consistent with our finding (Beeman and Schmidt 1982) that malathion-resistant Indianmeal moth larvae have less total α -naphthyl acetate esterase activity in vitro than susceptible larvae.

It is apparent from the present survey and from that of Zettler (1982) that after 20 years of use of malathion as the primary protectant of stored grain and peanuts, malathion-specific resistance in the Indianmeal moth is widespread in this country. We conservatively estimate that over half of the Indian meal moths infesting grain storage facilities in the North-Central United States may carry genes for carboxylesterase-type malathion-specific resistance. It must be emphasized, however, that there is no evidence for a causal relationship between the use of malathion as a stored-grain protectant, and the present-day existence of widespread resistance in the Indian meal moth. It is conceivable that such resistance was already predominant in this species before the existence of malathion.

Results from our laboratory (unpublished data) indicate that this type of resistance is under the control of a single dominant gene (or closely linked set of genes), and is therefore an all-or-none phenomenon. This implies that the "partially resistant" strains described herein probably do not contain partially resistant individuals, but instead consist of a mixture of susceptible and highly resistant moths.

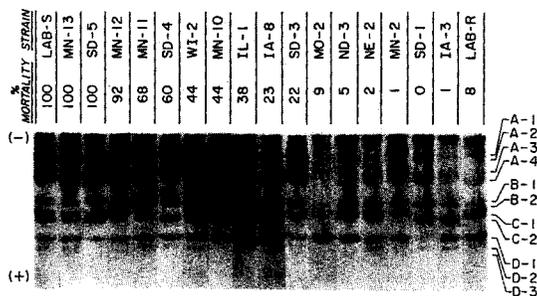


FIG. 2.—Esterase zymograms of malathion-resistant and -susceptible strains of the Indianmeal moth. Data for percent mortality refer to a 20- μ g dose of malathion (Table 1). Each zymogram is from a homogenate of 40 pooled 5th instars (20 of each sex).

Acknowledgment

We thank C. L. Storey of this research laboratory for making the survey samples available. We also thank W. Blodgett and J. Wilson for collecting the strains.

REFERENCES CITED

- Anonymous.** 1958. Tolerances and exemptions from tolerances for pesticide chemicals in or on raw agricultural commodities. Food and Drug Administration. Fed. Register 23: 6417.
- Armstrong, J. W., and E. L. Soderstrom.** 1975. Malathion-resistance in some populations of the Indian meal moth infesting dried fruits and tree nuts in California. *J. Econ. Entomol.* 68: 505-507.
- Attia, F. I.** 1977. Insecticide resistance in *Plodia interpunctella* (Hübner) (Lepidoptera:Pyralidae) in New South Wales, Australia. *J. Aust. Entomol. Soc.* 16: 149-152.
- Bansode, P. C., W. V. Campbell, and L. A. Nelson.** 1981. Toxicity of four organophosphorus insecticides to a malathion-resistant strain of the Indian meal moth in North Carolina. *J. Econ. Entomol.* 74: 382-384.
- Beeman, R. W. and B. A. Schmidt.** 1982. Biochemical and genetic aspects of malathion-specific resistance in the Indian meal moth (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 75: 945-949.
- Beranek, A. P.** 1974. Esterase variation and organophosphate resistance in populations of *Aphis fabae* and *Myzus persicae*. *Entomol. Exp. Appl.* 17: 129-142.
- Champ, R. B., and C. E. Dyte.** 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Prod. Prot. Ser. NO. 5. 279 pp.
- Cotton, R. T.** 1961. Moth pests of cereal products and their control. Northwest. Miller 265: 36-39.
- Finney, D. J.** 1952. Probit analysis, 2nd ed. Cambridge University Press, London. 38 pp.
- Franco, M. G., and F. J. Oppenoorth.** 1962. Genetical experiments on the gene for low alioesterase activity and organophosphate resistance in *Musca domestica* L. *Entomol. Exp. Appl.* 5: 119-123.
- Georghiou, G. P., and N. Pasteur.** 1980. Organophosphate resistance and esterase pattern in a natural population of the southern house mosquito from California. *J. Econ. Entomol.* 73: 289-292.
- Hubby, J., and R. C. Lewontin.** 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. *Genetics* 54: 577-594.
- Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227: 680-685.
- Matsumura, F., and A. W. A. Brown.** 1961. Biochemistry of malathion resistance in *Culex tarsalis*. *J. Econ. Entomol.* 54: 1176-1185.
- McGaughey, W. H., E. B. Dicke, and J. H. Schesser.** 1978. Indian meal moth infestation of farm-stored wheat in Kansas. *Ibid.* 71: 503-506.
- Ozaki, K., and T. Kassai.** 1970. Biochemical genetics of malathion resistance in the smaller brown planthopper, *Laodelphax striatellus*. *Entomol. Exp. Appl.* 13: 162-172.
- Parkin, E. A.** 1965. The onset of insecticide resistance among field populations of stored-product insects. *J. Stored Prod. Res.* 1: 3-8.
- de Stordeur, E.** 1976. Esterases in the mosquito *Culex pipiens pipiens* L.: formal genetics and polymorphism of adult esterases. *Biochem. Genet.* 14: 481-493.
- Togby, A. H., G. E. Nasrat, H. Nafei, and A. Z. El-Abidin Salam.** 1976. Insecticide resistance. VI. The inheritance of parathion resistance in *Drosophila melanogaster* strains with special references to esterases. *Egypt. J. Genet. Cytol.* 5: 300-312.
- Townsend, M. G., and J. R. Busvine.** 1969. The mechanism of malathion-resistance in the blowfly *Chrysomya putoria*. *Entomol. Exp. Appl.* 12: 243-267.
- Welling, W., and P. T. Blaakmeer.** 1971. Metabolism of malathion in a resistant and a susceptible strain of houseflies, pp. 61-75. In A. S. Tahori [ed.], *Insecticide resistance, synergism, enzyme induction*. Proc. 2nd Int. IUPAC Congr. Pesticide Chemistry. Vol. II. Gordon and Breach Science Publ. Ltd., London. 279 pp.
- Zettler, J. L.** 1982. Insecticide resistance in stored-product insects infesting peanuts in the southeastern United States. *J. Econ. Entomol.* 75: 359-362.
- Zettler, J. L., L. L. McDonald, L. M. Redlinger, and R. D. Jones.** 1973. *Plodia interpunctella* and *Cadra cautella*: resistance in strains to malathion and synergized pyrethrins. *Ibid.* 66: 1049-1050.