A METHOD FOR DETERMINING THE DOSAGE-MORTALITY CURVE OF MALATHION AGAINST THE PEA APHID, *ACYRTHOSIPHON PISUM* (HARRIS) (HOMOPTERA:APHIDIDAE)[1]

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ABSTRACT

The procedures of a reliable method for establishing the dosage-mortality curve for malathion and the pea aphid, **Acyrthosiphon pisum** (Harris), are described and evaluated. They include the choice of insecticide formulation, the conditions for rearing and collecting, the holding of treated aphids, and the analysis of mortality data. The LD_{50} of actual malathion in acetone solution to the pea aphid is 23.5 nanograms per aphid. The 95% fiducial limits about this estimate are 22.9 and 24.1 nanograms per aphid. The slope, $\pm$ S.E. (n=7), of the log-dosage: probit-mortality line is 5.5 $\pm$ 0.4.

Introduction

The success of the Aphididae as a group is partly due to the evolution of a specialized cycle which is closely adapted to the annual cycle of the host plant. The main features of the aphid cycle are: a thelytokous, or female-producing, spring and summer phase during which one or many generations occur, living usually on herbaceous plants; and a sexual fall generation, usually on a woody plant, which permits gene segregation and recombination, and retains evolutionary potentiality. The viviparous phase allows rapid increase of numbers and ensures that the population at the end of the phase will consist almost entirely of individuals which were adapted to conditions during the summer phase. These characteristics are advantageous in exploiting new ecological niches.

Some species living in mild climates have secondarily lost the sexual fall generation and reproduce entirely by thelytoky. In these species the genetic constitution is presumed to be extremely stable (Suomalainen, 1962; White, 1945). Nevertheless, instances of resistance to insecticides in the Aphididae during the past decade have given reason to doubt this stability. Stern (1962) reported an organophosphate - resistant population of *Therioaphis maculata* (Buckton) in California, an area where the few oviparae present produce only non-viable eggs (Dickson, Laird and Johnson, 1958). Two populations of organophosphate-resistant *Myzus persicae* (Sulzer) have been found in greenhouses, where sexual reproduction is unlikely to have occurred (Dunn and Kempton, 1966; Baerecke, 1962). The mechanisms whereby resistance evolved are unknown, and there is thus interest in them from the academic and applied points of view.

Methods used in the past for toxicological studies on aphids have not been adequate. Dunn and Kempton (1966), in their attempt to trace the decline of organophosphate resistance in a clone of *M. persicae*, were not able to make valid comparisons between generations since different concentrations were used to determine median lethal times. When the same concentrations were used the

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susceptible clone appeared to develop four- and five-fold resistance. The only conclusion they could draw was that resistance declined when selection pressure was removed. The present study was prompted by the increasingly clear need for a precise method for establishing dosage-mortality curves for aphids.

A log-dosage: probit-mortality line (ld-p line), the transformed equivalent of a dosage - mortality curve, provides estimates of the toxicity of the insecticides to individual aphids and by extension to the population. Since these estimates are reference points they must be repeatable, for repeatability is the best indication of reliability. Only with repeatable estimates can investigation begin on the mechanisms whereby resistance evolves.

Procedures of the Method

Rearing Aphids

Colonies of the pea aphid, *Acyrthosiphon pisum* (Harris), used throughout this investigation, were reared on broad bean, *Vicia faba* L., variety Exhibition Long Pod. These were grown in a potting mixture of equal parts of sphagnum moss and fine sand. No additional nutrients were added.

Aphids were reared in insect-proof cages 30.5 cm wide, 45.7 cm high, and 44.5 cm deep. Ventilation was provided by a forced air system delivering filtered air at the rate of 0.17 cu m per minute. The temperature inside the cages was maintained at 21.7 ± 2 C, and the vapor pressure deficit at 12.5 ± 3.5 millibars. Sixteen hours illumination per day was provided by six 2.4 m cool white fluorescent tubes placed 35 cm above the soil surface in the pots. The light intensity ranged from 2100 to 5000 lux, from the soil surface to the ceiling of the cage. Aphids were reared under these conditions for three generations before being treated.

A simple method was used to provide uniformly large, healthy, apterous aphids by hundreds, which had not been subjected to any adverse condition. Colonies were started by placing 20 nine-day-old aphids in each of two pots containing 12 plants, 2 to 8 cm high. These aphids produced about 440 nymphs during 24 hours, after which the adults were removed.

Collecting Aphids

Aphids were collected when they were 9 ± 0.5 days old. Those not on the plants in the cage were removed and destroyed. Single plants were cut off at the soil line and held over a large glass dish, the inner sides of which were coated with Fluon[3]. Apterous adults were gently brushed from the plant into the dish with a squirrel hair brush. Casts and nymphs were removed from the dish and from the adults. The aphids collected from one or two plants were evenly distributed among the eight petri dishes used to hold them prior to treatment. Sub-samples were taken until 50 aphids for each of seven treatments and a control had been collected. Aphids used in control treatments were weighed before being treated as a check on uniformity and to gauge the effects of handling.

The aphids were then transferred singly from the holding dish with a vacuum pencil to 10-cm arenas, the floors of which were of 12-strand per cm saran screen. Each arena was subdivided into three compartments by Fluon-coated Kodapak[4] cylinders, 4 cm diameter by 1.3 cm high. Fifteen aphids were placed in each arena, 4 in one compartment, 5 in the second and 6 in the third.

[3] A polyterafluoroethylene dispersion manufactured by Imperial Chemical Industries Ltd.

[4] A transparent plastic sheeting obtained from Burnaby Orchids, Burnaby, B.C.

Choice of Insecticide Formulation

Secondary standard malathion[5] of 96% purity was chosen as the test insecticide because it is an organophosphate, the class of insecticide involved in most cases of aphid resistance to insecticides, and because it has low mammalian toxicity. Test solutions were made by diluting a 1% (w/v) solution of the malathion in acetone. Acetone was found by trial to be best for topical application because it spread quickly and evenly, evaporated rapidly, and was non-toxic in the quantity used.

Application of Insecticide

To apply the insecticide to individual aphids, a Yale B-D Luer 0.25 ml glass hypodermic syringe, fitted with a No. 26 square-end, right-angled needle was clamped into the holder of a modified Micro-Metric SB-2[6] syringe micro-buret. The drive spindle of this applicator was fitted with a plywood disc in which 20 equally spaced cogs had been cut. A pawl mounted on the base of the applicator permitted the operator to devote his entire attention to the tip of the needle and the aphids during operation. Turning the disc from one cog to the next advanced the plunger 0.051 mm and delivered a 0.514 ul droplet of insecticide solution. This droplet was transferred by touching the tip of the needle to the dorsum of an aphids abdomen. The insecticide spread at once to cover the entire abdomen. A different random sequence of treatments was used for each replicate.

Holding of Treated Aphids

The aphids were brushed gently into a holding cage containing a young bean plant growing in a 10.3 cm square pot. A base of unpainted fir plywood, with a slot cut to accommodate the plant stem was fitted inside the rim of the pot, on the soil. The slot was sealed with a strip of masking tape and a collar of modelling clay around the base of the plant. The body of the cage, a Kodapak cylinder 9.5 cm diameter by 18 cm high, was fixed into a circular groove cut in the plywood base, using strips of masking tape from the wall of the cage to the sides of the pot. The top of the cage was nylon organdy.

Analysis of Data

At the end of the 48 hour post-treatment holding period the aphids were counted and classified. Any aphid not capable of coordinated movement was classified as dead. The data were analysed by computer, using two probit analysis programs (Finney, 1962). The first, a single line program[7], computed an 1d-p line for each replicate. The second[8] also computed an 1d-p line for each replicate, then tested the lines for parallelism, computed the common slope of the regression from the pooled results, and the relative potency of each replicate.

Evaluation of the Method

The method was judged by the repeatability of the median lethal dosage (LD^{50}) and the slope estimates. Homogeneity of slope estimates is the more critical, since these indicate the variance of response to the insecticide in the treated population. The standard deviation of the slopes of the 1d-p lines, being a measure of variation attributable to the method alone (Hoskins and Craig, 1962), was also used as an indication of reliability.

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5 0, 0 - dimethyl S-(1, 2 - dicarbethoxyethyl) phosphoro dithioate, obtained from Cyanamid of Canada Limited, 1 City View Drive, Rexdale, Ontario.

6 Manufactured by Micro-Metric Instrument Co., Cleveland, Ohio.

7 Adapted by P. M. Morse and E. A. Reimer, Statistical Research Service, Canada Department of Agriculture, from the original program by M. J. Garber, U.S.D.A. Users Library No. 1620-06.0.093.

8 Taken from the program by R. J. Daum and C. Givens, U.S.D.A. Users Library No. 1620-06.0.085.

Discussion

The most important factors in establishing the dosage-mortality curve were: formulation of the insecticide, and the procedures of rearing, collecting, treating, and holding the aphids after treatment. Repeatable results obtained with this method are shown by the homogeneity of the LD^{50} and slope estimates from replicate to replicate (Table 1). Figure 1 gives an ld-p line, with 95% fiducial limits, calculated from the pooled data by Bliss' (1952) method and demonstrates the homogeneity of the data.

Various methods of exposing *A. pisum* to malathion were investigated before a suitable one was developed. During the investigation the insecticide formulation was changed and the component procedures were progressively refined.

Exposing the aphids on glass surfaces which had been sprayed with acetone solutions of malathion did not give satisfactory results. Mortality is affected by the length of time the aphid is withheld from its normal environment; and the relationships between dosage and the length of exposure, and the concentration of the deposit, are not linear (Hoskins and Craig, 1962). The estimates obtained in these tests varied widely, and the standard deviations of the slopes were too large.

Solutions and emulsions were sprayed directly on the aphids in a Potter tower (Potter, 1952). Estimates of the LD^{50} were homogeneous once the procedures of rearing, collecting and post-treatment holding had been refined. Neverthless the slopes of the ld-p lines varied widely, and their standard deviations were still too large.

Hoskins and Craig (1962) set out 10 criteria which should be satisfied if a treatment procedure is to have general and specific applicability. These were used as guiding principles. The criteria, in descending order of importance, are: 1. constant relation of dose to dosage; 2. precise measurement of dosage; 3. quantitative evaluation of effect; 4. normality of environment; 5. constancy of environment; 6. sensitivity to variation; 7. reproducibility of results; 8. wide applicability; 9. representativity of population; 10. simplicity and rapidity.

TABLE 1. Mortality of 9-day-old **A. pisum** to which 0.5 microliter drops of actual malathion in acetone solution were applied on the abdominal dorsum. Aphids kept on live plants for 48 hours before mortality assessment. Statistics obtained by probit analysis.

Malathion ng/Aphid	Percent mortality in replicate						
	1	2	3	4	5	6	7
0	2	2	7	4	0	0	2
15	4	6	3	18	6	4	6
20	36	44	20	42	38	52	36
25	60	66	76	78	54	75	78
30	72	82	70	70	80	66	74
35	84	82	76	92	90	86	86
40	86	88	92	74	90	84	88
50	96	100	82	96	94	87	96
Statistics:							
LD 50 (ng/Aphid)	24.54	23.01	25.69	22.08	23.58	22.91	23.16
95% Fiducial Limits							
Upper	26.21	24.50	27.75	24.01	25.07	24.77	24.71
Lower	22.88	21.39	23.40	19.85	22.02	20.86	21.49
Slope	5.86	6.24	5.13	4.51	6.13	4.59	5.97
±S.E.	0.58	0.62	0.64	0.53	0.58	0.50	0.60

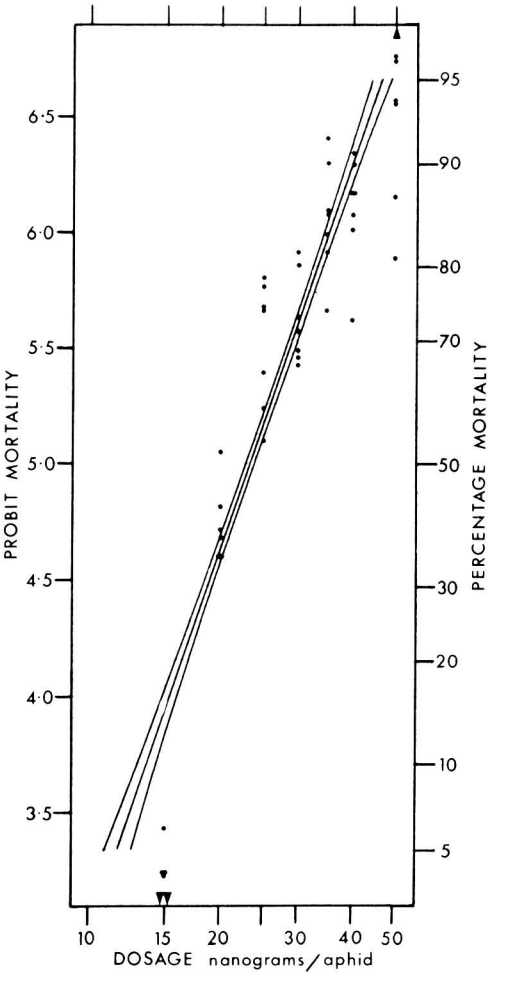

Fig. 1. Relationship between malathion dosage and mortality of **Acyrthosiphon pisum** (Harris). Bliss' (1952) method of partially weighted means was used to compute the the single 1d-p line and the 95% fiducial limits. Points represent the individual observations, adjusted for control mortality, given in Table 1.

There are no means as yet to satisfy the first criterion in which dose is defined as the amount of toxicant which reaches the site of action, and dosage as the amount of toxicant applied. Neither the mode nor the site of action of the organophosphate insecticides is known. Nevertheless, the relation is measured indirectly by the standard deviation of the slope; since these values are small (Table 1), it can be assumed that the criterion is satisfied.

The eighth criterion can be satisfied only by further development. With minor modifications to suit the requirements of different species, the method developed here should be widely applicable.

The last two criteria are difficult to satisfy fully. A laboratory clone cannot represent the population because of restrictions imposed by a controlled environment and by the size of the clone; it is impossible that the total gene pool of the species be

represented. Although the method is simple, requiring little more than patience and a steady hand, it is not rapid.

The six remaining criteria are fully satisfied. Numbers 4 and 5 are of great importance because aphids are particularly sensitive to environment. Photoperiod, temperature, and population density have been shown to be instrumental in morph determination in *Megoura viciae* Buckton (Lees, 1959), *Aphis craccivora* Koch (Johnson, 1965, 1966b), and *Brevicoryne brassicae* (Linnaeus), (Lamb and White, 1966). The condition of the host plant certainly affects morph determination in *A. craccivora* (Johnson, 1966a) and probably does so in other species. Cytological studies by Uichanco (1924) on *Dactynotus (=Macrosiphum) tanaceti* (Linnaeus) have shown that ovulation begins while the mother is still an embryo. Lees (1961) states that the sex ratio can be modified by the temperature in the grandmother's environment. The morph is determined by the maternal physiology while the aphid is an embryo in its mother's ovariole (Lees, 1961) and the mother's physiology is influenced by environmental conditions to which she is subjected. The mother's or grandmother's physiological state may well influence susceptibility to an insecticide in a daughter or granddaughter.

Conditions of light, temperature, vapor pressure deficit, host plant condition, and population density were held constant from replicate to replicate and from generation to generation. The order of treatments was randomized in each replicate to avoid the possibility of an interaction of treatment time with a daily rhythm of susceptibility, as shown for *Anthonomus grandis* Boheman by Cole and Adkisson (1964), and *Tetranychus urticae* (Koch), by Fisher (1967).

Significance of the Dosage-Mortality Curve

The average weight of the aphids used for determination of the seven ld-p lines of Table I was 4.1 + 0.11 mg, based on seven samples of 50 aphids each. The average LD_{50} computed was 23.5 ng per aphid, or 5.8 ug per g of body weight. This value indicates high toxicity, but comparisons with other insecticides against *A. pisum*, or with malathion against other insects, have not been possible because no reference has been found which gives the necessary information.

The slope of the ld-p line (Fig. 1) is relatively steep, indicating that there is little variation of response of the aphids to the insecticide. The probability of the aphids being able to discriminate between dosages decreases as the range of dosage is narrowed. Even though there are deviations from the computed ld-p lines, these are not truly aberrant since the dosages used to establish the lines varied within very narrow limits. The steep slope, and lack of consistent or major deviation from the ld-p lines give no indication of the presence of a pre-adapted resistance mechanism in the clone; there appears to be little chance of a resistant population developing, even after repeated selection with the insecticide.

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