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COMPARATIVE RESISTANCE TO AZINPHOSMETHYL IN THE PREDATORY MITE AMBLYSEIUS FALLACIS GARMEN (ACARINA: PHYTOSEIIDAE) AND ITS PREY TETRANYCHUS URTICAE KOCH (ACARINA: TETRANYCHIDAE) IN GREENHOUSE EXPERIMENTS

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COMPARATIVE RESISTANCE TO AZINPHOSMETHYL IN THE PREDATORY MITE *AMBLYSEIUS FALLACIS* GARMEN (ACARINA: PHYTOSEIIDAE) AND ITS PREY *TETRANYCHUS URTICAE* KOCH (ACARINA: TETRANYCHIDAE) IN GREENHOUSE EXPERIMENTS

By

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ABSTRACT

COMPARATIVE RESISTANCE TO AZINPHOSMETHYL IN THE PREDATORY MITE AMBLYSEIUS FALLACIS GARMEN (ACARINA: PHYTOSEIIDAE) AND ITS PREY TETRANYCHUS URTICAE KOCH (ACARINA: TETRANYCHIDAE) IN GREENHOUSE EXPERIMENTS

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Susceptible populations of a phytoseiid predator, Amblyseius fallacis Garmen, and one of its tetranychid prey, Tetranychus urticae Koch, were separately selected for resistance to azinphosmethyl under similar greenhouse conditions.

When a single homogeneous strain was used, resistance failed to develop in the predator after 8 generations. Selection of a predator population of heterogeneity similar to the prey population (consisting of the initial strain hybridized with two additional susceptible strains), however, resulted in appreciable resistance development. A comparison of resistance development in the predator and prey showed a 23.87-fold resistance in A. fallacis following 14 selections vs. a 20.41-fold resistance in T. urticae after 22 selections.

Similar rearing programs, population sizes and selection procedures were maintained in both experiments. Selections were initiated with populations of similar

dosage-mortality line slopes although toxicant ranges used in the two experiments differed. Additional experiments were undertaken to determine the importance of the initial gene frequency (of the major gene responsible for resistance) in the two comparisons.

To my Mother and Father --

for their understanding, support and love

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INTRODUCTION

Public disenchantment with the use of pesticides began in 1962 with Rachel Carlson's "Silent Spring". Increasing public concern over the problems of objectionable pesticide residues, adverse effects on nontarget organisms and direct hazards to the user (Smith 1970) have led to severe limitations on the types and amounts of pesticides available for pest control. Pesticides, however, remain the entomologists most powerful tool (NAS 1969, Metcalf 1975) and their use will most likely increase in the foreseeable future (Mrak Commission 1969).

Problems other than those related to environmental contamination have also been associated with the use of chemical pesticides. Pest resistance resulting in escalation of dosage levels, often accompanies repeated pesticide applications. Secondary pest outbreaks following elimination of natural control by previously unnoticed natural enemies is a similar symptom of the "pesticide syndrome" (Doutt and Smith 1969). The above problems have led to the formulation of the concept of integrated control (Bartlett 1956) and subsequently the concept of pest management (Geier and Clark 1961, Luckmann and Metcalf 1975). Pest

management involves evaluation of all available techniques and their consolidation into a unified program to manage pest populations so that economic damage is avoided and adverse side effects on the environment are minimized (NAS 1969).

Implicit in this concept of pest management is the maximal use of natural enemies for pest control. Much too often the importance of natural enemies in controlling pest populations has been ignored and is emphasized only by its absence when pesticides destroy the effectiveness of natural enemies. Pest resistance is one of the main problems facing pest management, yet natural enemy resistance to pesticides is nearly unknown. Comparing pests and natural enemies, there are more than 268 cases of insecticide resistance known for pest species (Brown 1976) but only 12 reported cases for natural enemies (Croft and Brown 1975, Croft 1977). Three of these twelve resistant natural enemies are parasitoids which developed resistance as a result of laboratory selections - two braconids and an aphelinid. The remaining nine are predators reported to be resistant in the field - seven phytoseiid mites, an anthomyiid and a cocinellid.

The proposed reasons for this major imbalance fall into four main categories:

(1) Previous field and laboratory research has focused on pest species rather than natural enemies. Perhaps

then, at least part of this skewed dichotomy is due to lack of study of natural enemies. Croft and Brown (1975) list five reasons for this differential emphasis and effort.

(a) Greater attention is given to control of direct competitors rather than to conservation of benefactors.

(b) The assumption is often made that natural enemies respond to insecticides in the same way as pests and thus their study is unnecessary.

(c) Much greater monetary resources are made available for studying the responses of pests as compared to those of predators and parasites.

(d) Predators and parasites are often more difficult to rear or culture in the large numbers required for detailed experimentation.

(e) There is a lack of standardized toxicological test methods for natural enemies similar to those developed for pest evaluations.

(2) The appearance of a resistant pest species is often more apparent than that of a resistant natural enemy. Damage caused by resistant pest species makes them more visible and usually results in their investigation. Natural enemy abundance is quite dependent on host or prey levels and thus resistance development among natural enemies could be overlooked if host or prey species were not especially abundant.

(3) A third possibility is that natural enemy and pest species may actually differ in their "intrinsic" abilities to develop resistance. Gordon (1961) hypothesized that "the extraordinarily high and generalized tolerance of the larval feeding stages of relatively polyphagous holometabolous insects to contact insecticides is probably the result of selection for endurance of prolonged and varied biochemical stresses associated with a diversity of their natural food plants." Kreiger et al. (1971), working with lepidopterous larvae, found a correlation between range of host plants and activity of aldrin epoxidase in midgut tissues (polyphagous > oligophagous > monophagous). They concluded that the detoxification of secondary plant substances is the chief function of the mixed function oxidase (MFO) system in the midgut of lepidopterous larvae. Brattsten and Wilkinson (1977) confirmed that in the southern armyworm moth, Spodoptera eridma (a broadly polyphagous insect), MFO enzymes are induced by secondary plant substances and that this induction "proceeds with enough speed to provide the animal with increased protection against these potentially offensive dietary factors".

Can this principle be extended to include natural enemies? Is there a natural ordering of MFO activity in insects (as reflected in inherent tolerances to certain insecticides) related to the degree of biochemical and metabolic specialization (polyphagous > oligophagous >monophagous

> predators > parasites)? Plapp and Bull (1977) studied the toxicity of several insecticides to the tobacco budworm, Heliothis virenscens (F.), one of its predators, Chrysopa carnea (Stephens), and one of its parasites, Carpoletis sonorensis (Carlson). They determined the order of toxicity to organophosphates (detoxified mostly by oxidases) as parasite > predator > budworm. Certainly much more data is needed to determine if this principle may be extended to other pests, predators and parasites.

Differences also exist between pest and natural enemy species due to their different modes of life. Secondary poisoning (from their prey or host) may complicate natural enemy resistance development. Additionally, pest species are often less mobile than natural enemy species (due to the need for natural enemies to search for hosts or prey, especially following insecticidal treatment), and thus natural enemies may come in contact with more insecticide. Croft and Brown (1975) however, stated that "there is little experimental evidence published to date indicating that the behavior of these natural enemies confers a greater exposure to insecticides."

(4) The most compelling hypothesis explaining the paucity of resistant natural enemies involves their density dependence upon their hosts or prey. In order for a natural enemy population to exist, sufficient hosts or prey must be present. In the presence of pesticide applications, natural

enemies are faced not only with the stress imposed by the selecting chemical but additionally with the stress of a limited food supply. Thus one might expect natural enemies to develop resistance only after their host or prey species have done so. It is significant to point out that in all known cases of natural enemy resistance, "resistance in the principal prey (the pest) has preceded the resistance similarly developed by the predator" (Croft 1977; all cases of parasite resistance reported were the result of laboratory selections).

Complicating this problem of natural enemy resistance development is the necessary simultaneous maintenance of both an adequate food supply and continued exposure to the selecting chemical. Commonly, as soon as a pest species develops resistance, and thus becomes abundant enough for its natural enemy to reproduce, the pesticide is changed to one that again controls the pest. Thus the natural enemy is faced with a food shortage as well as the stress of pesticide exposure.

Among the documented cases of natural enemy resistance is one phytoseiid mite species, Amblyseius fallacis Garmen, which when combined with one of its principal prey species, Tetranychus urticae Koch, the two-spotted spider mite, presents an ideal model pest/natural enemy system. These two mite species are remarkably similar in most physiological, biological and behavioral characteristics as well

as in their magnitude and spectrum of resistance to organophosphates (Croft 1977). In addition, they are quite comparable in most ecological aspects, occupying nearly identical habitats throughout their life histories. They thus provide an outstanding opportunity to investigate the development of resistance in a pest/natural enemy system as well as to test the hypothesis that the dependence of natural enemies upon their host or pest species is a primary deterrant to the development of resistance.

In the present study, susceptible populations of A. fallacis and T. urticae were separately selected for resistance to azinphosmethyl, a common broad-spectrum organophosphate. In the first part of the study, a non-limiting food source was supplied to each population, eliminating the above hypothesized deterrant to resistance development. In the second part of the study, the selection was repeated with the addition of small numbers of resistant genotypes to each population. This experiment was conducted to reflect the importance of the initial gene frequency (of the major gene responsible for resistance) in the first selection experiment.

LITERATURE REVIEW

Biological, Physical and Behavioral Comparisons of *Tetranychus urticae* Koch and *Amblyseius fallacis* Garmen.

Basic Bionomics (see Table 1)

Both *T. urticae* and *A. fallacis* have four developmental stages (egg, six-legged larvae, protonymph and deutonymph), each of the last three being followed by a quiescent resting stage (nymphochrysalis, deutochrysalis, teliochrysalis) (McMurtry et al. 1970, van de Vrie et al. 1972). Males of *A. fallacis* have been reported to have no deutonymphal stage (Ballard 1954) whereas males of *T. urticae* do, although it is shorter in duration than that of the female (Boudreaux 1963).

Developmental and ovipositional periods and thus the intrinsic rate of increase for both species, vary greatly with temperature and to a lesser extent, relative humidity (McMurtry et al. 1970, van de Vrie et al. 1972). Developmental times (in days) for *A. fallacis* range from 5.0 (Ballard 1954) to 5.8 (McClanahan 1968) at 26°C to 11.6 (McClanahan 1968) at 20°C. Depending on temperature, values for *T. urticae* range from six to ten days (van de Vrie et al. 1972). Boudreaux (1954) listed an average preovipositional

TABLE 1

A Biological Comparison of *T. urticae* and *A. fallacis*

Parameter	*T. urticae*	*A. fallacis*	Source
Number of developmental stages	four	four	McMurtry et al. 1970; van de Vrie et al.1972
developmental period	6-10 days	5-11.6 days	Ballard 1954; McClanahan 1968; van de Vrie et al. 1972
preovipositional period	1 day (22-27°C)	1 day (26°C)	Boudreaux 1954; Bravenboer 1959
duration of oviposition	10.8-26.3 days	22 days	Caegle 1949; Ballard 1954
eggs/female/day	2.5-5.6	2.2	Caegle 1949; Ballard 1954
intrinsic rate of increase	.2585 (22°C)	.279 (25°C)	Croft unpubl. Wrensch and Young 1975
mode of reproduction	arrhenotokous parthenogenesis	arrhenotokous parthenogenesis	Helle and Bolland 1967; Hansell et al. 1964
# chromosomes			Helle and Bolland
male	3	4	1967; Hansell
female	6	8	et al. 1964
mating necessary for egg production	no	yes	Helle and Bolland 1967; Rock et al. 1976
sex ratio	varies average 2.5	varies 2.0-5.0	Overmeer and Harrison 1969; Burrel and McCormich 1964; Smith and Newsom 1970; Lee 1972; Croft unpubl., Rock et al. 1971; Ballard 1954
size (length by width)	450 by 300 (microns)	400 by 250 (microns)	-
diapause	fertilized adult female	fertilized adult female	McMurtry et al.1970; van de Vrie et al. 1972.

period for A. fallacis of one day at 78°F (26°C). Bravenboer (1959) reported values for T. urticae of .5 days (27-33°C), 1 day (22-27°C), 2 days (18-22°C) and 5 days (13.5°C). Ballard (1954) reported 22 days as the average duration of oviposition for A. fallacis at 26°C. Caegle (1949) reported ovipositional periods (depending on temperature) of 10.8-26.3 days for T. urticae. Values of eggs per female per day were 2.2 (Ballard 1954, 26°C) for A. fallacis and 2.5-5.6 (Caegle 1949) for T. urticae. Croft (unpubl.) obtained a value of .279 for the intrinsic rate of increase of A. fallacis at 25°C. Wrensch and Young (1975) determined a value of .2585 for T. urticae at 22°C.

Reproduction

In both T. urticae (Helle and Bolland 1967) and A. fallacis (Hansell et al. 1964) reproduction is based upon arrhenotokous parthenogenesis, the male having the haploid number of three (T. urticae) or four (A. fallacis) chromosomes. In T. urticae, virgin females produce male progeny (Helle and Bolland 1967). In A. fallacis, however, mating is necessary for egg production (Rock et al. 1976). This difference would be of significance only under low population densities in which male-female encounters were restricted.

Experiments by Overmeer and Harrison (1969) indicated that the sex ratio of T. urticae averages near 2.5:1.0 (female to male), fluctuating with the genotype of the

female parent. Mitchell (1972) further showed that the sex ratio was the result of several alleles inherited from the female which respond to natural selection. He pointed out that this was especially interesting in view of data indicating that the sex ratio is quite important in dispersal (Overmeer and Harrison 1969, Mitchell 1970).

The sex ratio of A. fallacis also seems to fluctuate with reported values falling in the 2.0-5.0 (female to male) range (2.0 by Burrell and McCormich 1964, Smith and Newsom 1970, Lee 1972; 3.0 by Croft unpubl.; 4.0 by Rock et al. 1971 and 5.0 by Ballard 1954).

Genetics and Mechanism of Resistance to Organophosphates

Mechanism of Resistance

The mode of action of organophosphorous insecticides involves the inhibition of an enzyme, cholinesterase, important in the transmission of nerve impulses (Brown 1969a). Cholinesterase normally acts by metabolizing acetylcholine, the chemical messenger linking nerve axons through their synapses. When cholinesterase is inhibited, acetylcholine accumulates at the synapse, resulting first in an increase and then in cessation of nervous conduction. The result is paralysis and death of the organism.

Two distinct mechanisms have been discovered for the resistance of T. urticae to organophosphates. The first was discovered by Smissaert (1964) and involves an altered

cholinesterase molecule which is both less active and less susceptible to organophosphorous inhibition. This mechanism has been reported in strains of T. urticae from the Netherlands (Smissaert 1964, Voss and Matsumura 1964), New Zealand (Ballantyne and Harrison 1967, Overmeer and Harrison 1969), Germany (Schulten 1968), Israel (Zahavi and Tahori 1970) and Great Britain (Cranham 1974).

A second mechanism was discovered for two US strains of T. urticae (Matsumura and Voss 1964, Herne and Brown 1969). In these strains, the cholinesterase activity of the resistant strain was shown to be identical with the susceptible strain and resistance was attributed to a higher detoxicative capacity of the resistant strain resulting from an increased carboxyesterase activity. Herne and Brown (1968) suggested that the difference between the European and the American strains may be due to the former having been selected with demeton and oxydemetonmethyl, whereas the latter were selected with parathion and malathion. According to this hypothesis, resistance of the strain of T. urticae used in the present study is assumed to be due to a higher detoxicative ability of the resistant strain over the susceptible strain.

Motoyama et al. (1972) discovered an identical mechanism for azinphosmethyl resistance in A. fallacis. Again, the resistant strain was found to degrade the organophosphate faster than the susceptible strain, resulting in less

inhibition of the cholinesterase activity. When bimolecular rate constants were compared, no difference was found between S and R strains, indicating that a modified cholinesterase was not associated with resistance. Further studies by Motoyama et al (1977) have indicated that the exact mechanism of azinphosmethyl resistance in A. fallacis involves glutathione S-transferases.

Genetics of Resistance

Most cases of strong resistance to an insecticide depend upon allelism in a single major gene (Brown 1969b). During the development of resistance, however, several modifying genes may be important.

Independent of the mechanism involved, the resistance of T. urticae to both malathion (Taylor and Smith 1956) and parathion (Schulten 1966, Ballantyne and Harrison 1967, Herne and Brown 1969) has been shown to be due to a single major dominant gene, with or without modifiers.

Croft et al. (1976) determined that the resistance of A. fallacis to azinphosmethyl is due principally to a single allele which exhibits partial dominance. They hypothesized that partial dominance may be a characteristic of resistance to azinphosmethyl as suggested by the study of Dittrich (1972). Dittrich (1972) demonstrated complete dominance for parathion, incomplete dominance for paraoxon, and no dominance for oxydemetonmethyl in a demeton-parathion selected strain of T. urticae.

Previous Selection Experiments with T. urticae and A. fallacis

Two-spotted spider mites, Tetranychus urticae, are infamous for their ability to develop resistance to a wide variety of pesticides. Resistance is especially prevalent in greenhouses where more intensive control programs and greater numbers of spider mite generations are present (Helle 1965). Numerous laboratory selection experiments with organophosphorous compounds and T. urticae have been carried out (Watson 1956, Hanson 1958, Helle 1959, Saba 1960, Watson and Naegele 1960, Abul-Hab and Stafford 1961, Dittrich 1963, Helle 1965, Overmeer 1966) with common results; most often a resistant strain is produced. In highly inbred strains, however, slower resistance development has been encountered. McEnroe and Harrison (1968) hypothesized a slow response to selection in an "internally balanced" strain (highly inbred) and a "rapid response to selection when strains are outcrossed". This hypothesis was experimentally confirmed by McEnroe and Naegele (1968) and McEnroe and Kot (1968).

The first reported case of OP-resistance in A. fallacis was by Motoyama et al. (1970). Since then, numerous reports have indicated both a high magnitude and widespread distribution of resistance throughout the mid-western and eastern deciduous fruit-growing regions of North America (Croft and Brown 1975, Croft et al. 1976, Croft and Nelson 1972). One of the few successful selection

experiments with A. fallacis has been performed by Croft and Meyer (1973). They produced a carbaryl-azinphosmethyl resistant strain through the selection and hybridization of two strains; one carbaryl resistant and one azinphosmethyl resistant. Croft (1972) and Croft and Meyer (1973) commented on the lack of success in laboratory selection attempts and suggested that "greater success might be realized by periodically introducing genetic variability (wild genotypes) during laboratory selection experiments".

MATERIALS AND METHODS

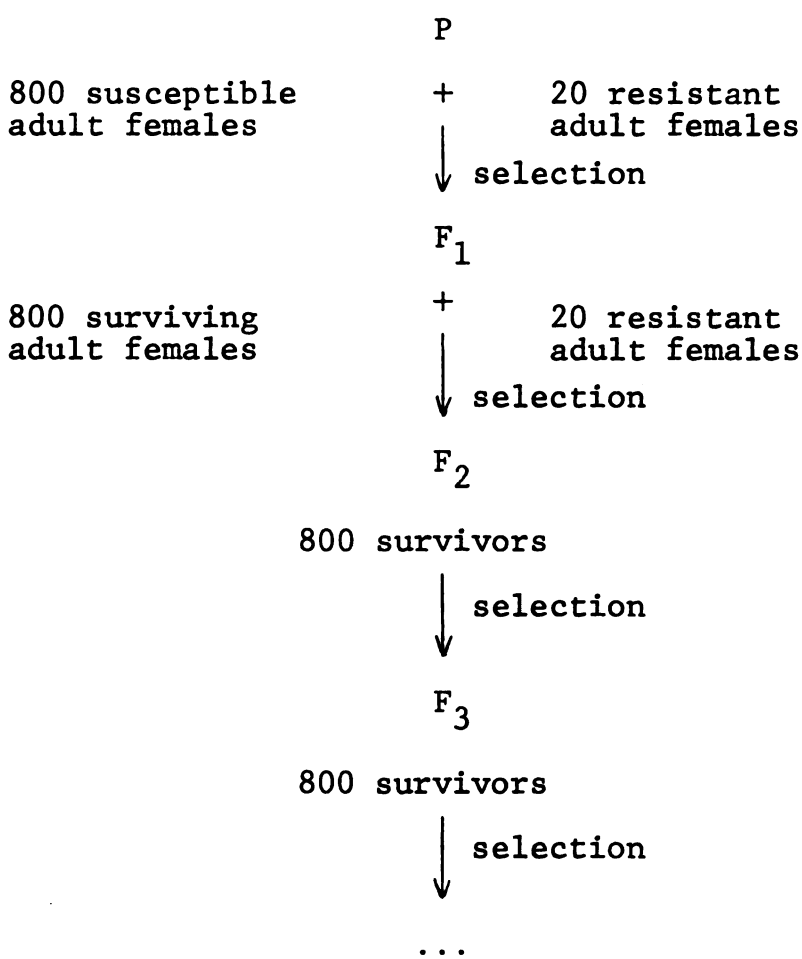
The alternative experiments of the present study are outlined in Figure 1. In Experiments 1 and 3 (the form of Experiment 1 was repeated in Experiment 2 with a different strain of susceptible A. fallacis) susceptible populations of Tetranychus urticae and Amblyseius fallacis were separately selected for resistance to azinphosmethyl under similar greenhouse conditions. Azinphosmethyl was chosen as the selecting chemical for two reasons: (1) azinphosmethyl is one of the primary broadspectrum organophosphorous insecticides used in tree fruit pest management and (2) the genetics of resistance to azinphosmethyl has been determined for A. fallacis (Motoyama et al. 1972, Croft et al. 1976). Crucial to these experiments is the provision of a non-limiting food supply to each species, thus eliminating one of the hypothetical deterrants to the development of resistance. Resistant T. urticae were supplied to the A. fallacis as prey, thus allowing survival through chemical selections.

In Experiments 4 and 5, the same two susceptible populations were again selected for resistance to azinphosmethyl. Here, however, small numbers of resistant adult females of each species were added to the first (P) and

Figure 1. Outline of the experimental cells of Experiments 1-5.

Figure 2. Diagram of Experiments 4 and 5.

Experiment 1,2 susceptible *A. fallacis* with non-limiting food supply	Experiment 3 susceptible *T. urticae* with non-limiting food supply
Experiment 4 *A. fallacis* plus small numbers of resistant *A. fallacis*	Experiment 5 *T. urticae* plus small numbers of resistant *T. urticae*



second (F_1) generations (see Figure 2). Experiments 4 and 5 provide a comparison of the "innate" ability of the two populations to develop resistance under similar conditions, since any differences in the initial gene frequence (of the major gene responsible for resistance) will be negated by flooding the system with resistant genotypes. A comparison of Experiments 2 and 3 vs. 4 and 5 should thus indicate the importance of the initial gene frequencies in the development of resistance.

The origins of both T. urticae and A. fallacis strains used in the experiments are listed in Table 2. The Rose Lake susceptible (S) strain was collected in 1974 from a weed-groundcover habitat which had no known history of pesticide application (Croft et al. 1976). Mites were maintained on units similar to those described by McMurtry and Scriven (1964) and Hoying (1976) until their use in the experiments. The Monroe S and Garden S strains were also collected from areas (Monroe Co., MI on soybean and Lansing, MI on beans and cucumber respectively) which had no known history of pesticide application.

The resistant (R) A. fallacis strain was collected in September 1976 from apple leaves and groundcover obtained from a commercial apple orchard near Belding, MI. For the previous ten years, azinphosmethyl supplemented occasionally with diazinon had been the principal broadspectrum OP compound applied for pest control (see Croft et al. 1976 for method of collection).

TABLE 2

Origins of Mite Strains Used in the Experiments

Strain	Origin and History
Susceptible *A. fallacis*	
Rose Lake S	Rose Lake State Game Area (near Lansing, MI) collected from weed-ground cover habitat (Croft et al. 1976)
Monroe S	Monroe Co., MI collected from soybean August 1976
Garden S	Lansing, MI collected from beans and cucumber August 1976
Resistant *A. fallacis*	
Belding R	commercial apple orchard near Belding, MI Sept.1976
Susceptible *T. urticae*	
New Zealand S	derived from the susceptible LN4 strain, Lincoln College, Canterbury, New Zealand (courtesy R.A. Harrison)
Resistant *T. urticae*	
Greenhouse R	greenhouse *T. urticae* from Michigan State University, E. Lansing, MI

The susceptible T. urticae strain was obtained from a susceptible New Zealand colony of T. urticae (derived from the LN4 strain) courtesy of Dr. R.A. Harrison (Lincoln College, Canterbury, New Zealand).

The resistant T. urticae strain was collected from a Michigan State University greenhouse (E. Lansing, MI) in which numerous insecticidal treatments (organophosphates and others) had been made for the previous ten years.

Both T. urticae and A. fallacis populations were reared and selected on 16-day (an 18-day cycle was used, with two days added to the beginning of each cycle, during the winter months, Nov.-March) generation cycles as shown in Table 3. Each cycle was initiated with the hand transfer (using a fine camel hair brush) of 200 adult female mites to each of four fresh lima bean plants (Phaseolus limensis). The lima bean plants used for the A. fallacis populations were infested with an abundant prey population (resistant T. urticae) one day prior to each transfer. On day eight the leaves from the initial four plants were transferred to eight fresh plants in order to insure good plant condition (plants deteriorate under continuous T. urticae feeding in about ten days) and an adequate food supply. On day twelve the dried leaves from the four initial plants were removed (active life stages crawled onto the fresh plants), the resistance level of the populations were determined, and the plants were surveyed to

TABLE 3

Events in the 16-day Generation Cycle

Day #	Event
0	transfer 200 mated adult females to each of four fresh lima bean plants (A. fallacis plants were previously infested with T. urticae)
8	transfer leaves from the initial four plants to eight fresh plants to insure good plant condition and adequate food supply.
12	remove dried dead leaves of four initial plants; slide-dip to determine present resistance levels; survey plants to determine pre-spray population levels.
14	spray seven plants to run-off at levels predicted to give approximately 75% mortality; leave eighth plant in case of excessive mortality.
16	assess post-spray population levels; initiate next generation by transferring 200 adult female survivors to each of four new plants.

determine approximate pre-spray population levels (a description of the survey technique follows). On day fourteen the plants were sprayed to run-off (using a compressed air knapsack sprayer) at a concentration calculated to give approximately 75% mortality. Wettable-powder insecticide (Guthion^{R} 50-WP) was used for both resistance level determinations and spraying operations. In order for any residual action to occur, the plants were left for 48 hours (day 16) before post-spray population levels were determined. Surviving adult females were then removed and used to initiate the next generation cycle.

The slide-dip method of Anon. (1968) as modified by Croft et al. (1976) was used to determine resistance levels for both T. urticae and A. fallacis populations. Twenty adult females were placed on their backs on Permacel Brand^{R} filament tape which was affixed by Scotch Brand^{R} double-stick tape to a microscope slide. Adult females were used once distension of their abdomen indicated that oviposition had begun.

Six-hundred mites (one-hundred at each of five concentrations and a control) were dipped in toxicant solutions of 50-WP insecticide dissolved in distilled water. Slides were dipped for five seconds, blotted on paper, and allowed to dry for fifteen minutes. Slides were then held for 48 hours at 25^{o}C and 95% RH before mortality was determined by failure to exhibit leg or mouthpart movement when

mites were lightly prodded with a camel hair brush. Results were plotted on logarithmetic-probability paper; LC_{50} and slope were calculated using a computer. Spray dosages predicted to give approximately 75% mortality were determined from the plotted dosage-mortality curves.

Each lima bean plant contained approximately 100 basal leaves (trifoliets were removed to keep the plants under vegetative control). A random survey technique was used in order to determine pre-spray population levels. Ten uniformly distributed basal leaves were sampled from each plant (chosen in an X pattern) and appropriate conversions were made to determine the total number of mites present. 48 hours after spraying (day 16), the total plant was surveyed to determine post-spray survival.

Populations of A. fallacis and T. urticae were reared and selected in adjacent greenhouse rooms in order to maintain nearly equal environmental conditions. Temperature records were maintained to check that similar temperature ranges (65-85^{o}F) were present. Mites were reared on plants placed in large water trays in order to minimize dispersion.

RESULTS AND DISCUSSION

Experiment 1

In Experiment 1, the Rose Lake susceptible strain of A. fallacis was selected with azinphosmethyl through seven generations (see Table A-1). The change in the dosage-mortality lines with selection is shown in Figure 3, with the change in LC_{50} (50% lethal concentration) plotted in Figure 4. As seen in Figure 4, the LC_{50} did not increase with selection, but in fact, decreased somewhat. The Rose Lake susceptible strain was a highly inbred strain derived originally from a limited number of individuals. The high slope of 4.391 (of the dosage-mortality line per decade) indicates a narrow genetic base with respect to azinphosmethyl tolerance. Limited genetic variability was hypothesized to explain the inability of the Rose Lake strain to respond to azinphosmethyl selection. This hypothesis is tested in Experiment 2.

Experiments 2 and 3

The same selection format was followed in Experiments 2 (A. fallacis) and 3 (T. urticae). In Experiment 2, however, the A. fallacis parental population (generation number 0) was derived from equal numbers of three susceptible populations (300 mites each from the Rose Lake S,

Monroe S and Garden S strains). Dosage-mortality lines for the composite strain as well as for the three susceptible strains from which it was derived are shown in Figure 5. The increased genetic variability available to the composite strain decreased the slope of the parental population (of Experiment 2 versus 1) from 4.391 to 1.780. Figure 6 shows the change in the dosage-mortality lines with selection for Experiment 2. As shown in Table A-2, the LC_{50} increased by a factor of 23.87 (.00107 to .02549 % A.I.) in 14 selections.

The susceptible T. urticae strain used in Experiment 3 was obtained from New Zealand (derived from the LN4 strain, courtesy of Dr. R.A. Harrison, Lincoln College, New Zealand) and was found to have an LC_{50} of .03039 (% A.I.) with a slope of 2.200. The slopes of the two susceptible populations used in starting Experiments 2 and 3 compare quite favorably (1.739 for A. fallacis and 2.200 for T. urticae). The parental (generation 0) LC_{50} levels for the two species, however, differ by a factor of approximately 30 (.00107 for A. fallacis and .03029 for T. urticae, % A.I.). Questions as to whether (1) this reflects the true "intrinsic" susceptibility of the two species and (2) whether this difference in toxicant ranges used in the two experiments will differentially affect the ability of the two species to acquire resistance, remain as yet unanswered.

Figure 7 shows the change in dosage-mortality lines for Experiment 3. The LC_{50} of the T. urticae population increased by a factor of 20.41 (.03029 to .61080 % A.I.) in 22 selections. Figure 8 shows the relative change in LC_{50} (with the LC_{50} of the parental strain used as a base) with selection for the two species in Experiments 2 and 3 (data in Table A-6).

In Figures 9 and 10, the population sizes prior to selection and the percent mortality after selection, respectively, are compared for Experiments 2 and 3 (data in Tables A-2 and A-3). The figures demonstrate that population sizes prior to selection (average of 4658 for A. fallacis and 4779 for T. urticae) and percent mortality after selection (average of 71.0% for A. fallacis and 76.1% for T. urticae) were similar for the two experiments.

Experiments 4 and 5

Experiments 4 (A. fallacis) and 5 (T. urticae) test the importance of the initial gene frequencies (of the genes responsible for resistance development) in Experiments 2 and 3. The addition of resistant individuals to each population prior to selection should negate any differences in initial gene frequency. Thus, any differences in the two species, important in the development of resistance (other than the initial gene frequency) should result in differential rates at which resistance is acquired.

Figures 11 and 12 show the change in dosage-mortality lines for the two populations. Again the LC_{50}'s of the parental populations (.0153 for A. fallacis and .5048 for T. urticae) differ by a factor of approximately 30. In addition, the distance between the LC_{50}'s of the parental (line 00) and the resistant (line R) populations differ for the two species (.46512/.00092=506.67 for A. fallacis; .61847/.03029=20.42 for T. urticae).

Two methods of comparing the change in LC_{50} for the two species are contrasted in Figures 13 and 14. In Figure 13, the "X"-fold change in LC_{50} (using the parental LC_{50} as a base) is plotted. In this figure, resistance development of the A. fallacis population appears to have surpassed the T. urticae population. In Figure 14, the fractional change in LC_{50} with respect to the LC_{50} of the resistant strain ($LC_{50(parental)}$=0.00; $LC_{50(R)}$=1.00) is plotted as a function of selection. Here it is seen that the T. urticae population approaches the LC_{50} of the resistant strain much more quickly than the A. fallacis population.

Great care must be taken in interpreting these results. The two resistant populations used in adding resistant genotypes into Experiments 4 and 5 were obtained from quite different sources (see Table 2) than the susceptible populations used in all five experiments. Incompatibilities between strains of both T. urticae (Helle and

Pieterse 1965) and A. fallacis collected from different areas is quite common. In addition, the differences in the resistant/susceptible LC_{50} ratios for the two species further complicates comparisons. Further studies are presently under way in our laboratory to resolve these problems.

Figures 15 and 16 show population sizes prior to selection and percent mortality after selection, respectively, for the two experiments. Again, comparable ranges are present for the two species.

Table 4 shows a summary of Experiments 1-5. In Experiment 1, the Rose Lake susceptible strain failed to acquire resistance through 7 selections. Experiments 2 and 3 demonstrate that under the present selection regime (similar population sizes, mortality levels, environmental conditions, unlimited food supplies, etc.) both species acquired an appreciable level of resistance, although the A. fallacis population did so somewhat faster. Experiments 4 and 5 fail to resolve the question of whether the two species differ in their abilities to acquire resistance when differences in initial gene frequency (of the major gene responsible for resistance) are negated.

Predicted Versus Actual Selection Mortalities

Dosage-mortality lines obtained using the slide-dip method were used to calculate spray concentration levels to give approximately 75% mortality during selections. Using

TABLE 4

A Summary of Experiments 1-5

Experiment	Species	Strain(s) Used	Initial LC_{50} (% A.I.)	Final LC_{50}	X-fold Change in LC_{50}	Number of Selections	Average Population Size/ Selection	Average Mortality/ Selection
1	A. fallacis	Rose Lake S	.00146	.00060	.41	7	3971	79.4
2	A. fallacis	Rose Lake S Monroe S Garden S	.00107	.02549	23.87	14	4658	71.0
3	T. urticae	New Zealand S	.03029	.61808	20.41	22	4779	76.1
4	A. fallacis	Rose Lake S Garden S Monroe S Belding R	.00092	.04740	51.63	5	4625	67.5
5	T. urticae	New Zealand S Greenhouse R	.03039	.63335	20.91	6	5560	73.2

the actual spray concentrations on the x-axis of the dosage-mortality line and reading across to the y-axis (see Figure 17) yielded a predicted mortality level. Actual mortality levels 48 hours after spraying were calculated as previously described (see Materials and Methods).

Actual/predicted probit mortality ratios (probit ratios were used in order to obtain a linear relationship between dosage and mortality) were calculated for each selection in which the appropriate data were available (see Table A-8). Probit ratios were pooled for each species (Experiments 1,2,4 for A. fallacis and 3,5 for T. urticae) with the results summarized in Table 5. Figure 16 graphs the probit mortality ratio for each species broken down by experiment number. An F-test of probit ratios showed probit mortality means were significantly different for the two species using a 99.5% confidence level ($1.091 \pm .105$ for A. fallacis and $1.219 \pm .134$ for T. urticae). These results should be of use in more accurately determining spray concentrations to give desired spray mortality (using the same spray methods).

TABLE 5

A Comparison of Predicted and Actual Mortalities Following Spray Application for T. urticae and A. fallacis (see Appendix, Table A-8)

		A. fallacis	T. urticae
number of samples		21	21
average predicted mortality (%)		56.9	42.0
range	high	81.9	70.9
	low	25.0	15.3
average predicted mortality (probits)		5.1942	4.7989
average actual mortality (%)		72.6	76.1
range	high	88.7	94.6
	low	55.8	43.6
average actual mortality (probits)		5.5584	5.7534
average ratio (probits)		1.091*	1.206*
range	high	.957	.981
	low	1.337	1.504
standard deviation		.10530	.13431
95% confidence intervals		(1.04164, 1.13988)	(1.15826, 1.28019)

*significantly different at .005 level.

CONCLUSIONS

Numerous references (McEnroe and Harrison 1968, McEnroe and Kot 1968, McEnroe and Naegele 1968, Croft 1972, Croft and Meyer 1973) have been made to the importance of genetic variability (i.e. a wide genetic pool) in selection experiments. This principle is demonstrated in a comparison of Experiments 1 and 2 (A. fallacis). The Rose Lake susceptible strain failed to increase its tolerance to azinphosmethyl through seven selections in Experiment 1. When the Monroe and Garden susceptible strains were combined with the same Rose Lake strain (decreasing the slope from 4.391 to 1.780), however, a resistance factor of 23.87 was achieved in 14 generations.

Great difficulty was encountered in obtaining a susceptible T. urticae strain for use in Experiments 3 and 5. The strain finally used in these experiments (New Zealand S) had a higher LC_{50} of .03029 as compared to a value of .00107 (% A.I.) for the susceptible A. fallacis population with which it was compared in Experiments 2 and 4. As mentioned previously, it is unclear whether the different toxicant ranges present in Experiments 2 (A. fallacis) and 3 (T. urticae) differentially favored

either population in the development of resistance.

Biological, physiological and behavioral characteristics of A. fallacis and T. urticae are almost identical. The two major exceptions are that (1) fertilization is necessary for egg production in A. fallacis (unfertilized T. urticae produce male progeny) and (2) A. fallacis and T. urticae differ in their mode of food uptake (predator versus herbivore, respectively). The first of these two differences (fertilization and egg production) would be significant only under low population densities which were not present in these experiments. Two effects may arise from the second major difference between the two species - (1) the possibility of secondary poisoning exists for A. fallacis and (2) A. fallacis might be subject to greater toxicant exposure due to more extensive searching for its prey.

Azinphosmethyl is a contact insecticide. Since the mites were exposed to the chemical for only two days, direct contact with the toxicant would seem to be more significant to the predator than the possibility of secondary poisoning.

The claim that A. fallacis would encounter more toxicant in searching for its prey deserves consideration. Although prey populations (T. urticae) were maintained at high levels throughout the experiments, it was observed that the mobility of A. fallacis was greater than that of the more sedentary T. urticae.

The results of the actual versus predicted mortality comparison are quite interesting in this context. The slide-dip technique does not allow for differences in mobility since all mites are held stationary throughout testing. One might then expect that the actual/predicted mortality ratio would be higher for A. fallacis since mites were free to move about in the actual selection experiments. In fact, the ratio was somewhat higher for T. urticae. Although other factors may be at work here, this is an indication that mobility is probably not a significant factor in the present study.

An interpretation of Experiments 4 and 5 is quite difficult in view of the many factors involved in these experiments. Parental LC_{50}'s for the two species differ by a factor of 30 as in Experiments 2 and 3. Additionally, the distance between the dosage-mortality of the parental and resistant strains (from which the small numbers of resistant mites were added) is quite different for the two species (see Table A-7). Ideally, comparisons should be made between the two species over identical toxicant ranges.

Although Experiment 4 is at present incomplete, it can be seen that the two species did not respond identically in Experiments 4 and 5. The susceptible T. urticae strain was able to attain the LC_{50} of the resistant T. urticae strain in 6 generations. After 6 generations, the susceptible A. fallacis strain had failed to do so,

although it had made significant progress towards the LC_{50} of the resistant A. fallacis strain (a 51.63-fold increase in LC_{50}) and in fact had shown a greater absolute increase in LC_{50} level than did the T. urticae strain. The Belding R strain (A. fallacis) used in Experiment 4 had been intensively selected with organophosphates for approximately 10 years prior to use in the experiment. Possibly the inability of the susceptible A. fallacis strain to attain the LC_{50} of the resistant strain was due to genetic incompatibilities between the two strains. A future study mentioned previously will circumvent this problem by repeating Experiments 4 and 5 using the resistant strains developed in Experiments 2 and 3 for introducing resistant genotypes into the two susceptible populations.

The main purpose of Experiments 1-5 was to test the hypothesis that the major obstacle in resistance development in a specific natural enemy (A. fallacis) is a food limitation following pesticide selection. This added stress (in addition to the direct chemical stress) upon the natural enemy, hypothetically limits natural enemy resistance development as compared to that achieved in pest species. Although Experiments 4 and 5 have failed to concretely eliminate the question of the initial gene frequency, the results of Experiments 2 and 3 do indicate that a natural enemy can develop resistance as quickly as a similar pest species (23.87-fold resistance in the natural enemy in 14

generations versus 20.21-fold resistance in the pest in 22 generations). These results may have practical significance in the applied aspect of pest-management. If economic thresholds for pest species were raised to maximum levels, the additional prey available for natural enemies (following pesticide selection) would allow maximum resistance development in the natural enemy.

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APPENDIX A

FURTHER FIGURES

FIGURE 3

Dosage-Mortality Lines for Experiment 1: Selection of an A. fallacis population through 7 generations.

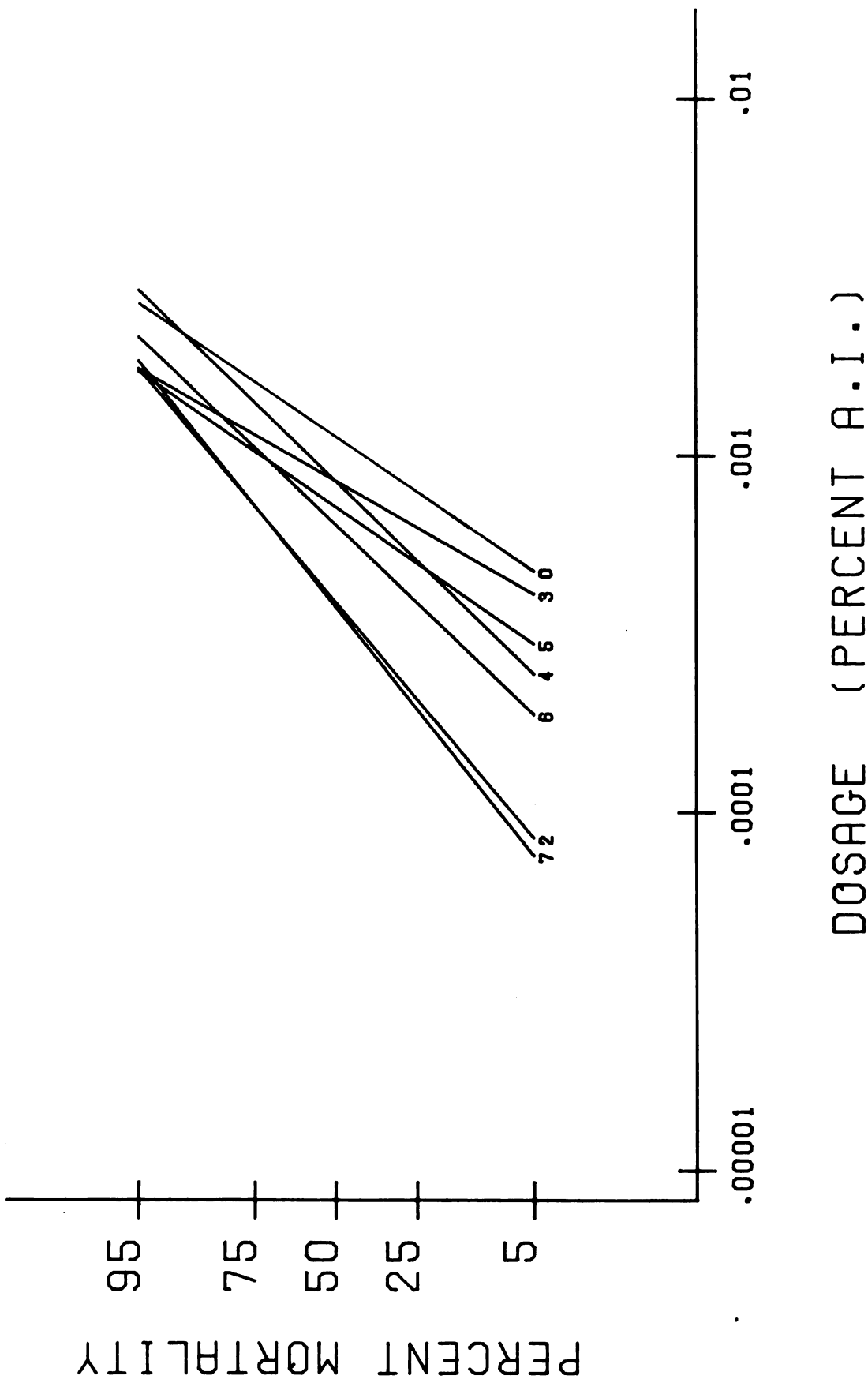

PERCENT MORTALITY
95
75
50
25
5
.00001
.0001
.001
.01
DOSAGE (PERCENT A.I.)
72
8
4
5
3
0

FIGURE 4

Change in LC_{50} with Time for Experiment 1

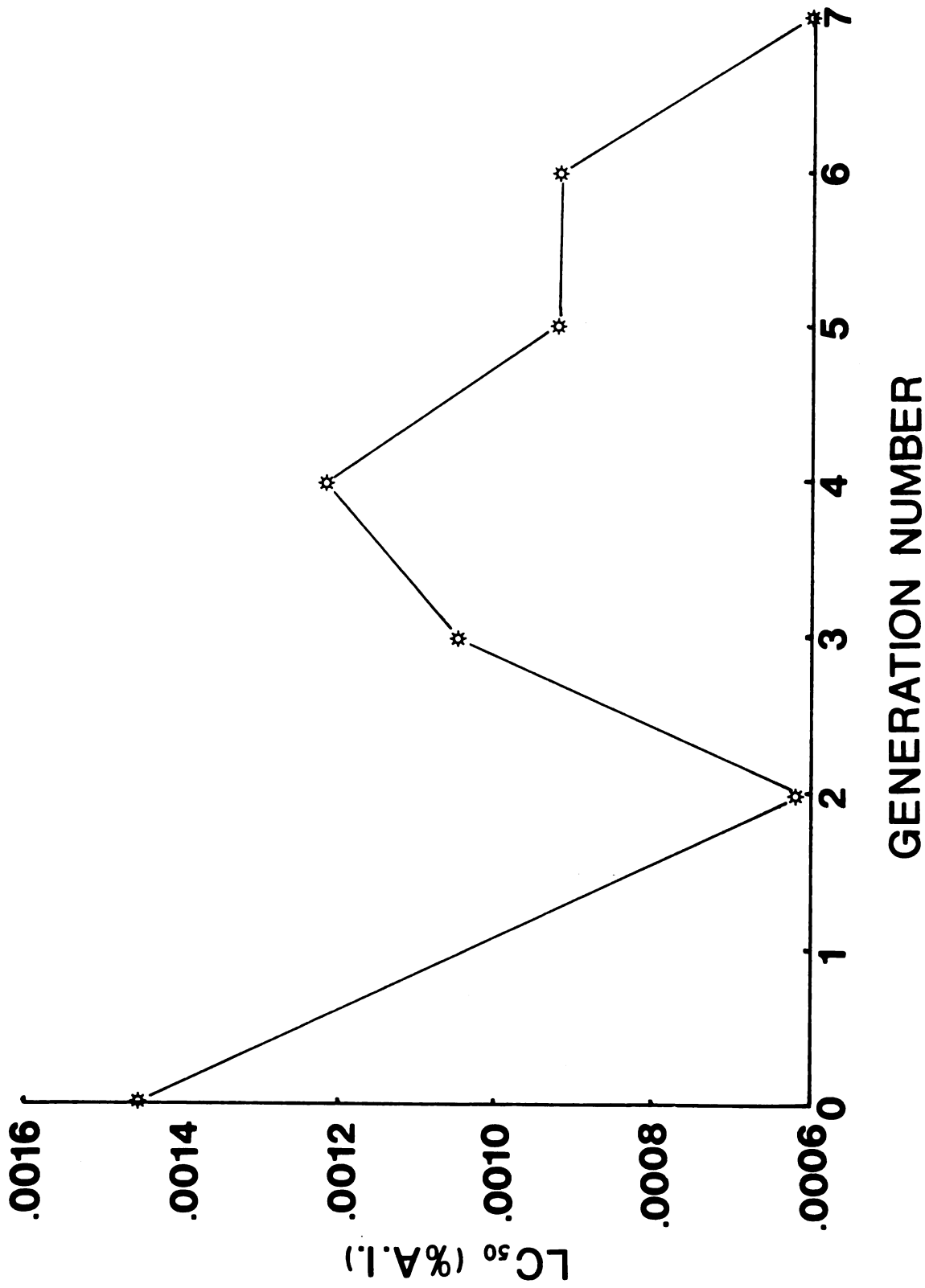

.0016
.0014
.0012
.0010
.0008
.0006
LC50 (%A.I.)
0
1
2
3
4
5
6
7
GENERATION NUMBER

FIGURE 5

Dosage-Mortality Lines for the Three Susceptible Strains (R = Rose Lake S, M = Monroe S, G = Garden S) and the Resulting Composite Strain (CC = Composite) Used in Experiment 2.

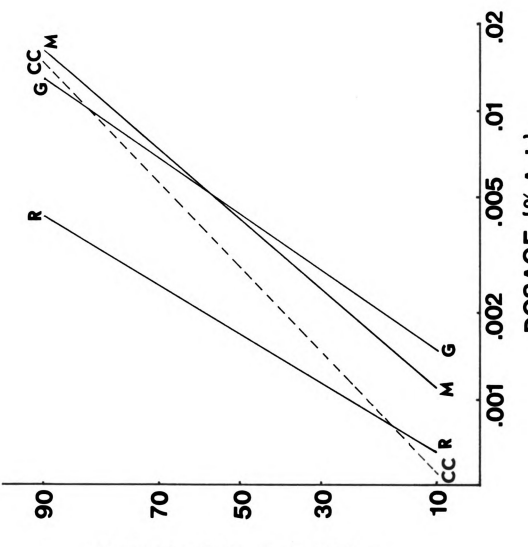

PERCENT MORTALITY
90
70
50
30
10
.001
.002
.005
.01
.02
DOSAGE (% A.I.)
R
G, CC
M
CC
R
M
G

FIGURE 6

Dosage-Mortality Lines for Experiment 2: Selection of an A. fallacis Population Through 14 Generations.

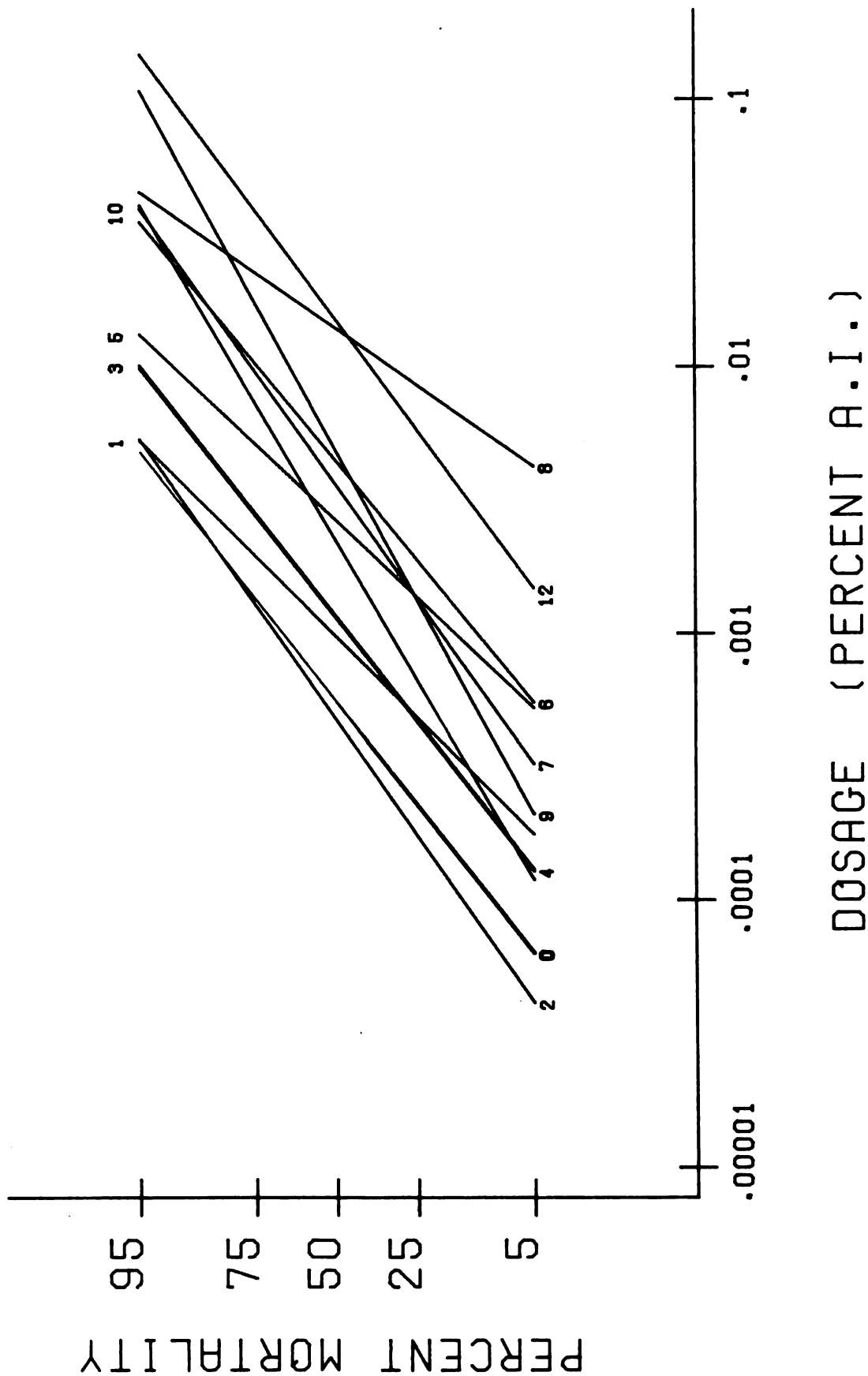

PERCENT MORTALITY
95
75
50
25
5
.00001
.0001
.001
.01
.1
DOSAGE (PERCENT A.I.)
1
3
5
10
2
0
4
9
7
6
12
8

FIGURE 7

Dosage-Mortality Lines for Experiment 3: Selection of a T. urticae Population Through 22 Generations.

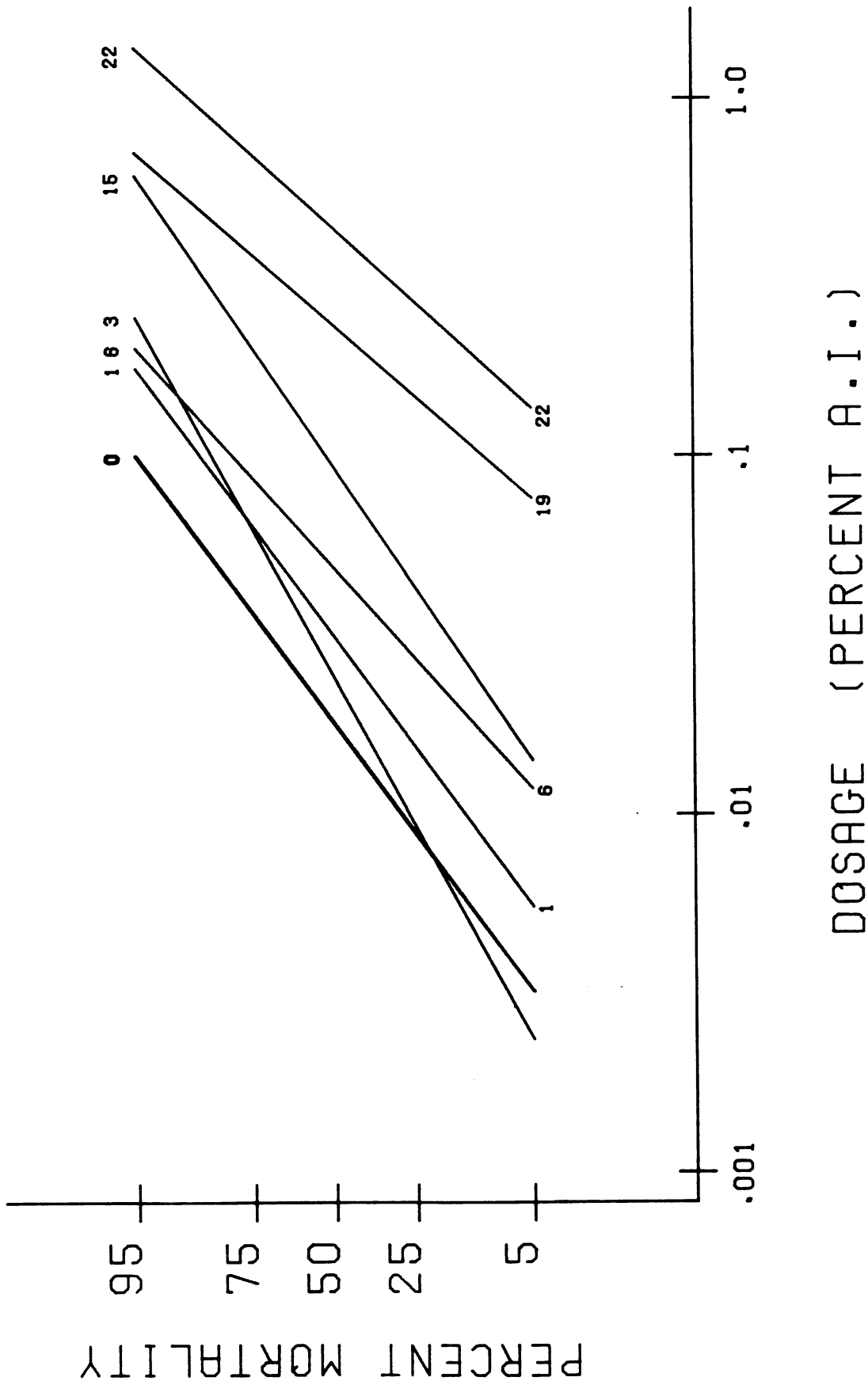

PERCENT MORTALITY
95
75
50
25
5
.001
.01
.1
1.0
DOSAGE (PERCENT A.I.)
0
1
3
6
15
19
22
22

FIGURE 8

Relative Change in LC_{50} for Experiments 2 and 3

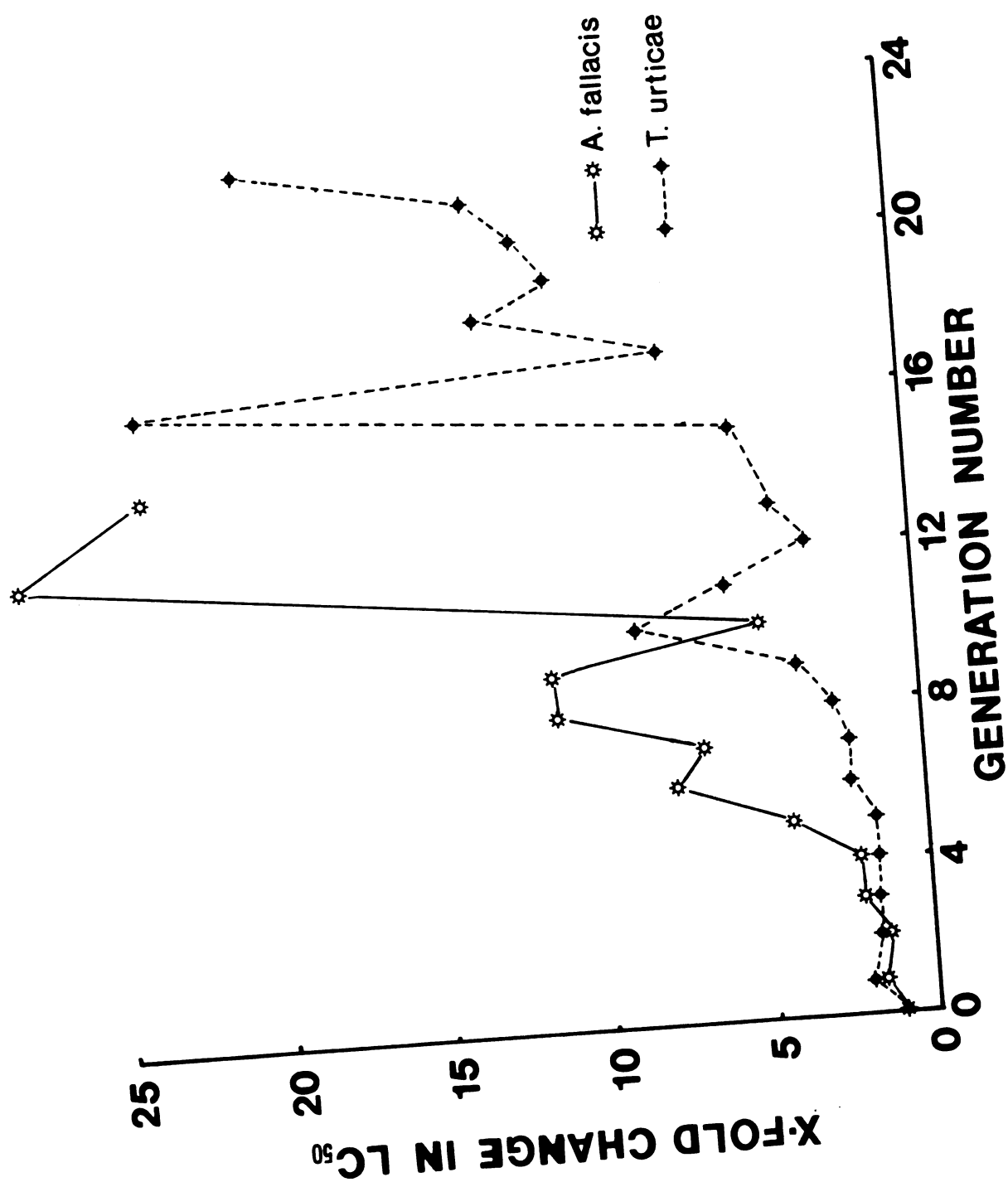

A. fallacis
T. urticae
X-FOLD CHANGE IN LC50
25
20
15
10
5
0
0
4
8
12
16
20
24
GENERATION NUMBER

FIGURE 9

Comparison of Population Sizes Prior to Selection in Experiments 2 and 3

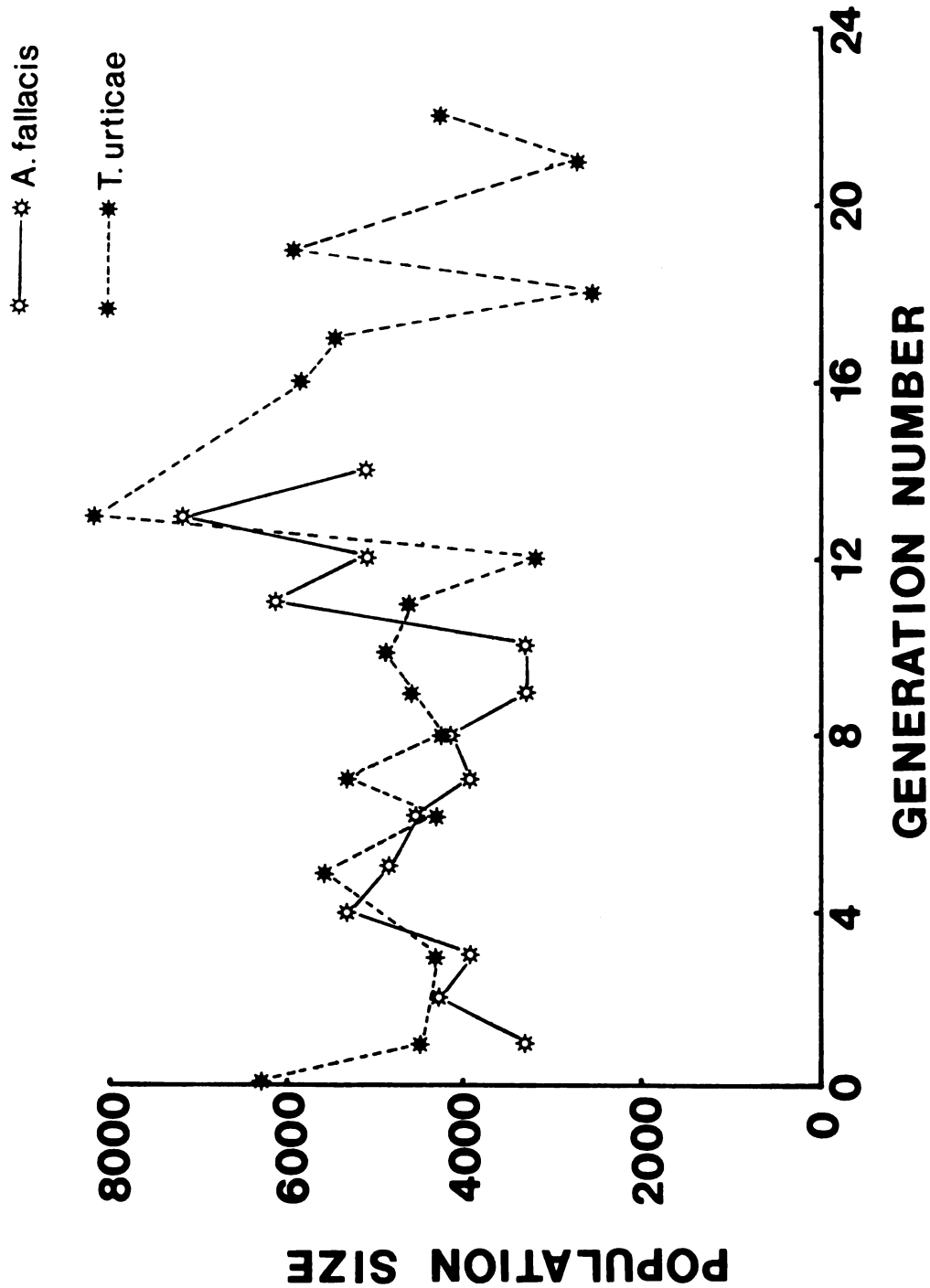

A. fallacis
T. urticae
8000
6000
4000
2000
0
0
4
8
12
16
20
24
POPULATION SIZE
GENERATION NUMBER

FIGURE 10

A Comparison of Percent Mortality After Selection in Experiment 2 and 3

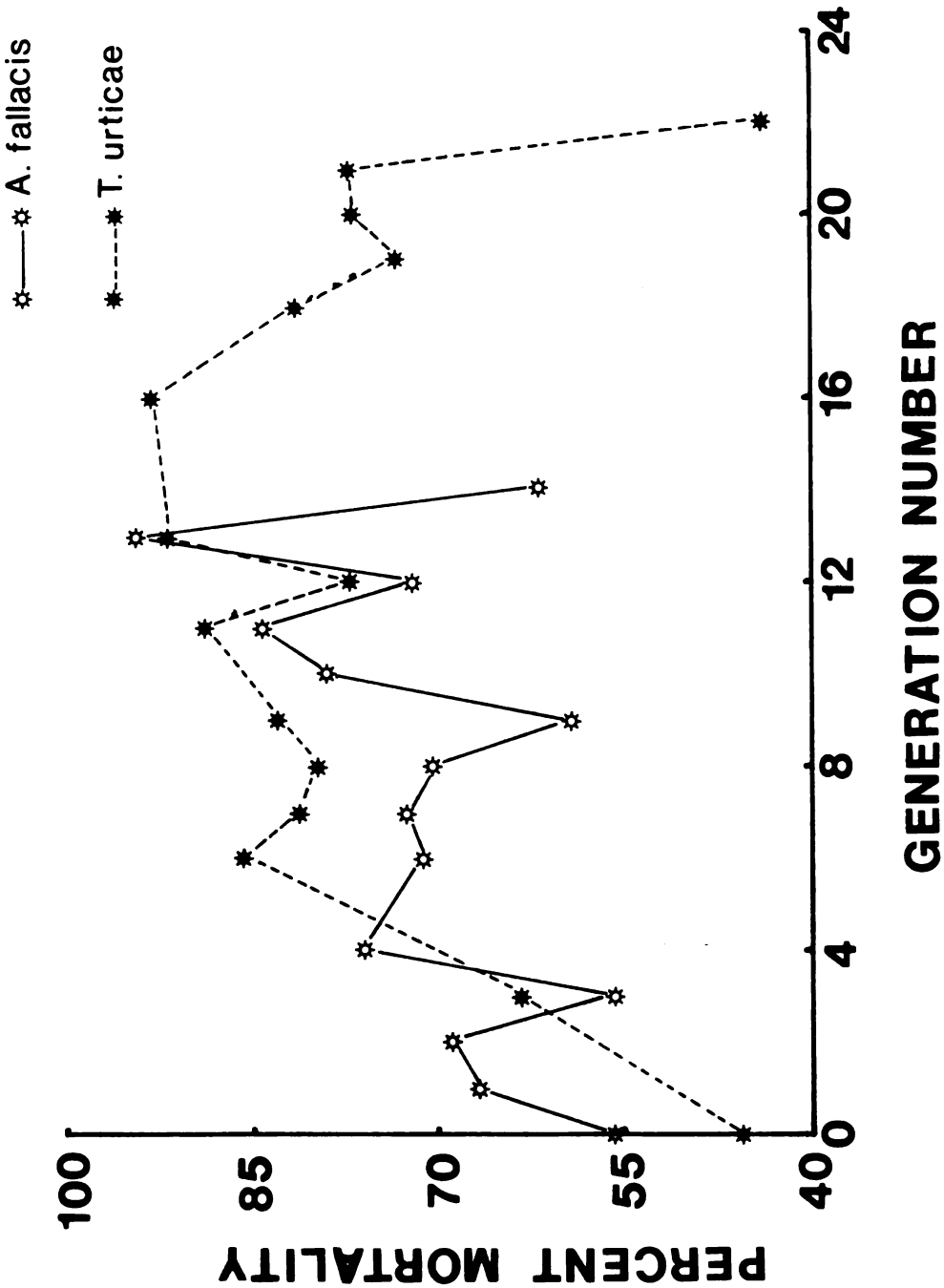

A. fallacis
T. urticae
PERCENT MORTALITY
100
85
70
55
40
0
4
8
12
16
20
24
GENERATION NUMBER

FIGURE 11

Dosage-Mortality Lines for Experiment 4: Selection of an A. fallacis Population Through 6 Generations

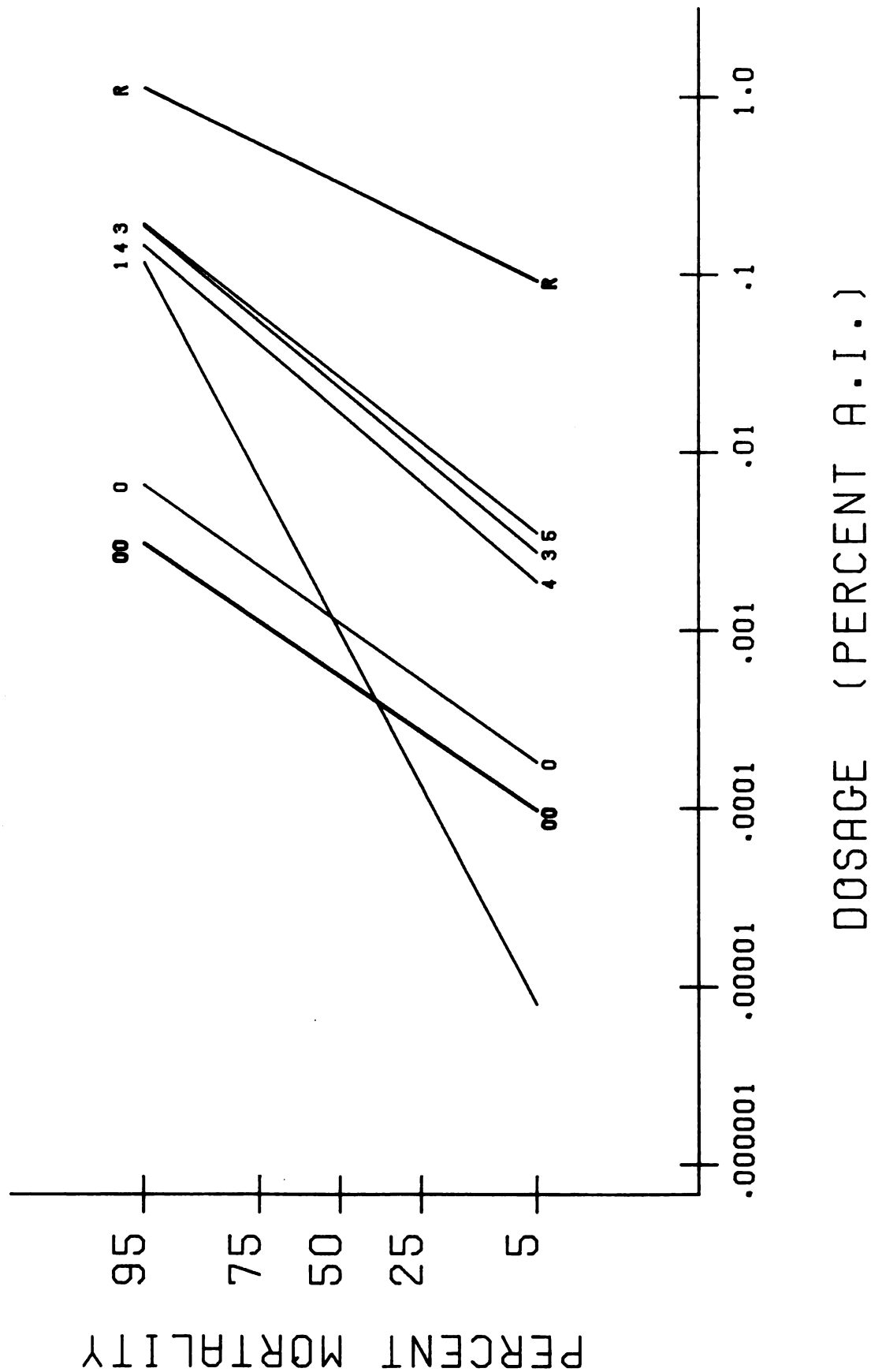

PERCENT MORTALITY
95
75
50
25
5
.000001
.00001
.0001
.001
.01
.1
1.0
DOSAGE (PERCENT A.I.)
R
143
0
00
436
0
00
R

FIGURE 12

Dosage-Mortality Lines for Experiment 5: Selection of a *T. urticae* Population Through 6 Generations

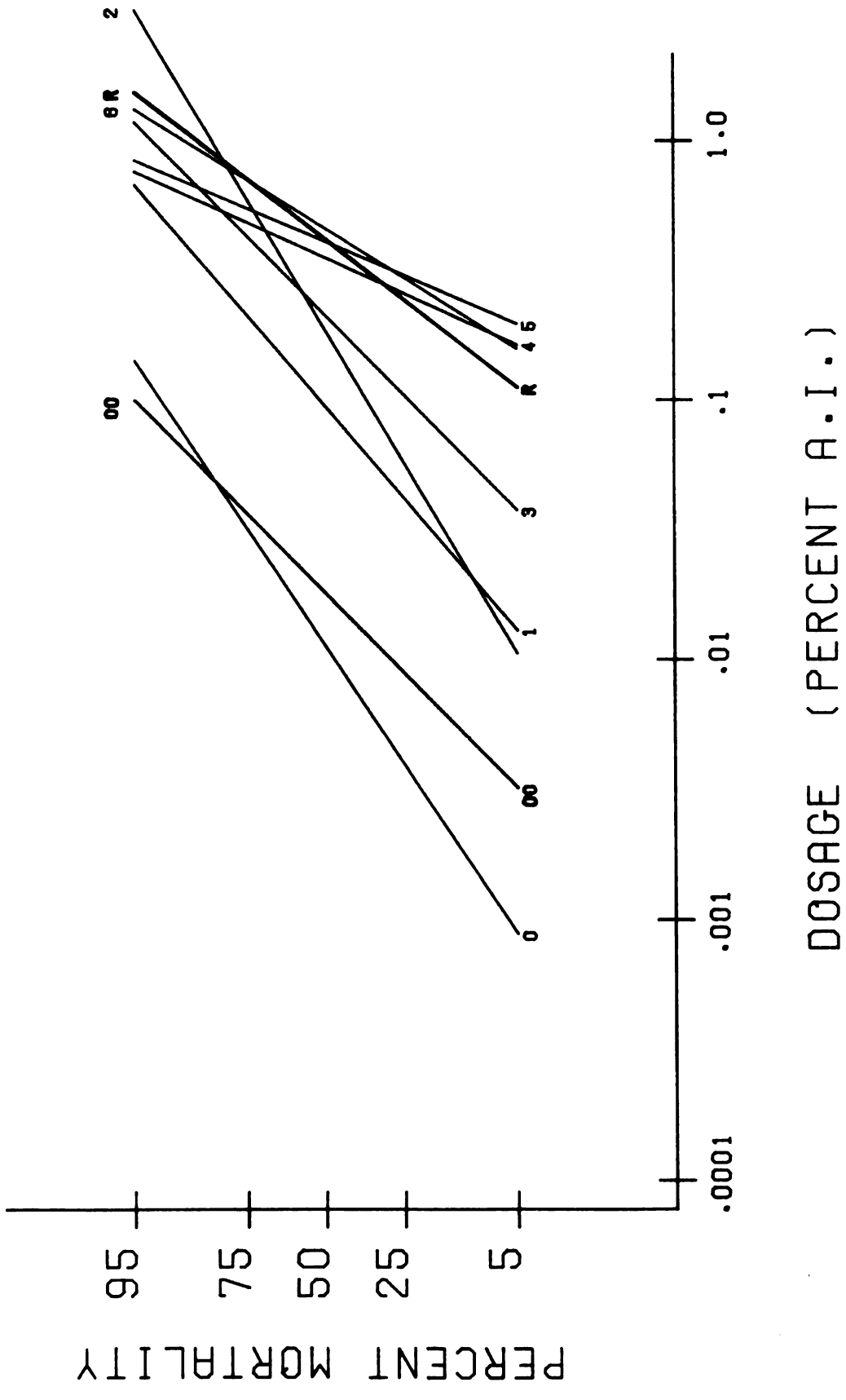

PERCENT MORTALITY
95
75
50
25
5
.0001
.001
.01
.1
1.0
DOSAGE (PERCENT A.I.)
0
00
000
1
3
4 5
R
6R
2

FIGURE 13

X-Fold Change in LC_{50} for Experiments 4 and 5

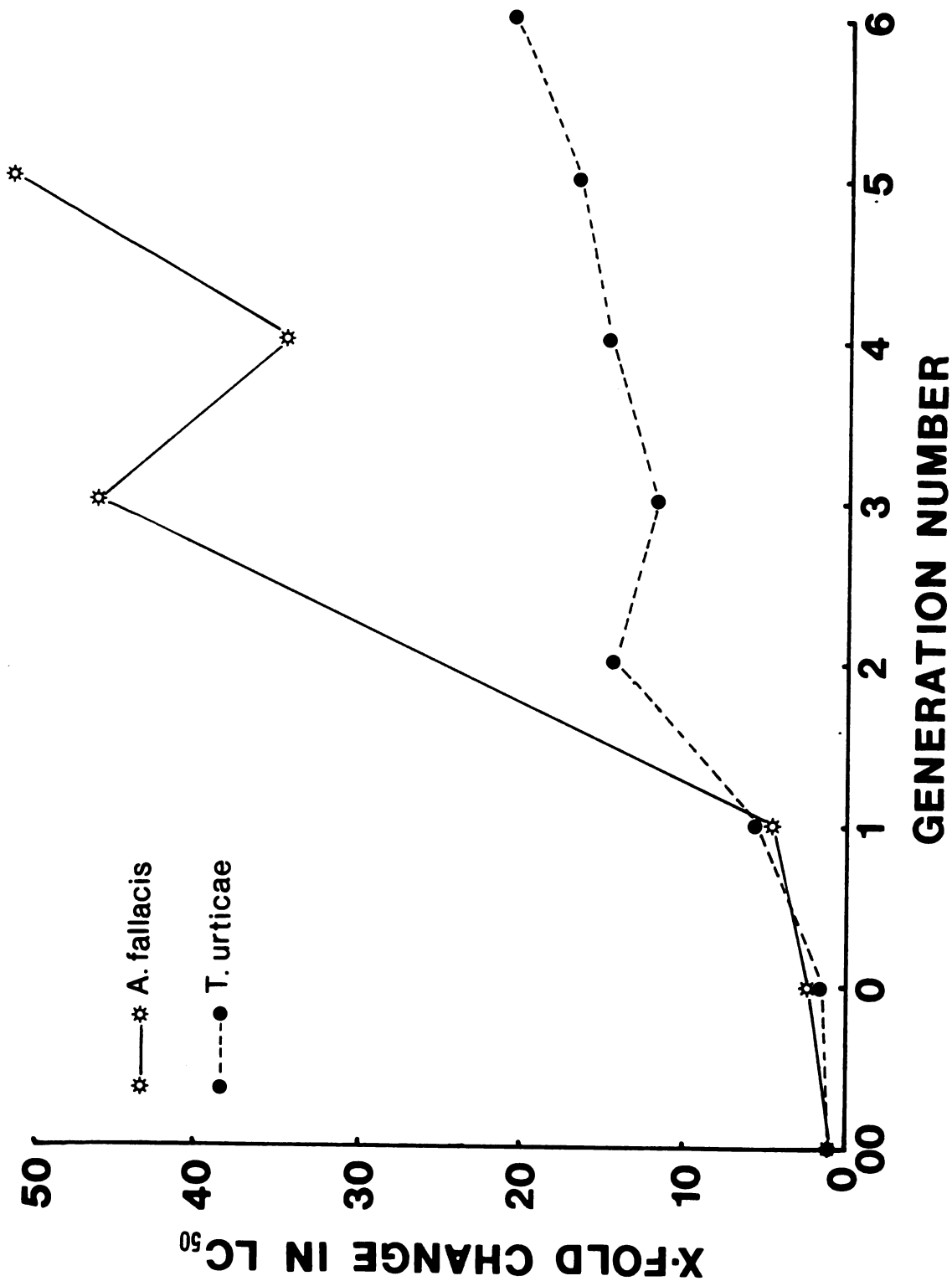

X-FOLD CHANGE IN LC50
GENERATION NUMBER
A. fallacis
T. urticae
0
10
20
30
40
50
00
0
1
2
3
4
5
6

FIGURE 14

Y-Fold Change LC_{50} for Experiments 4 and 5

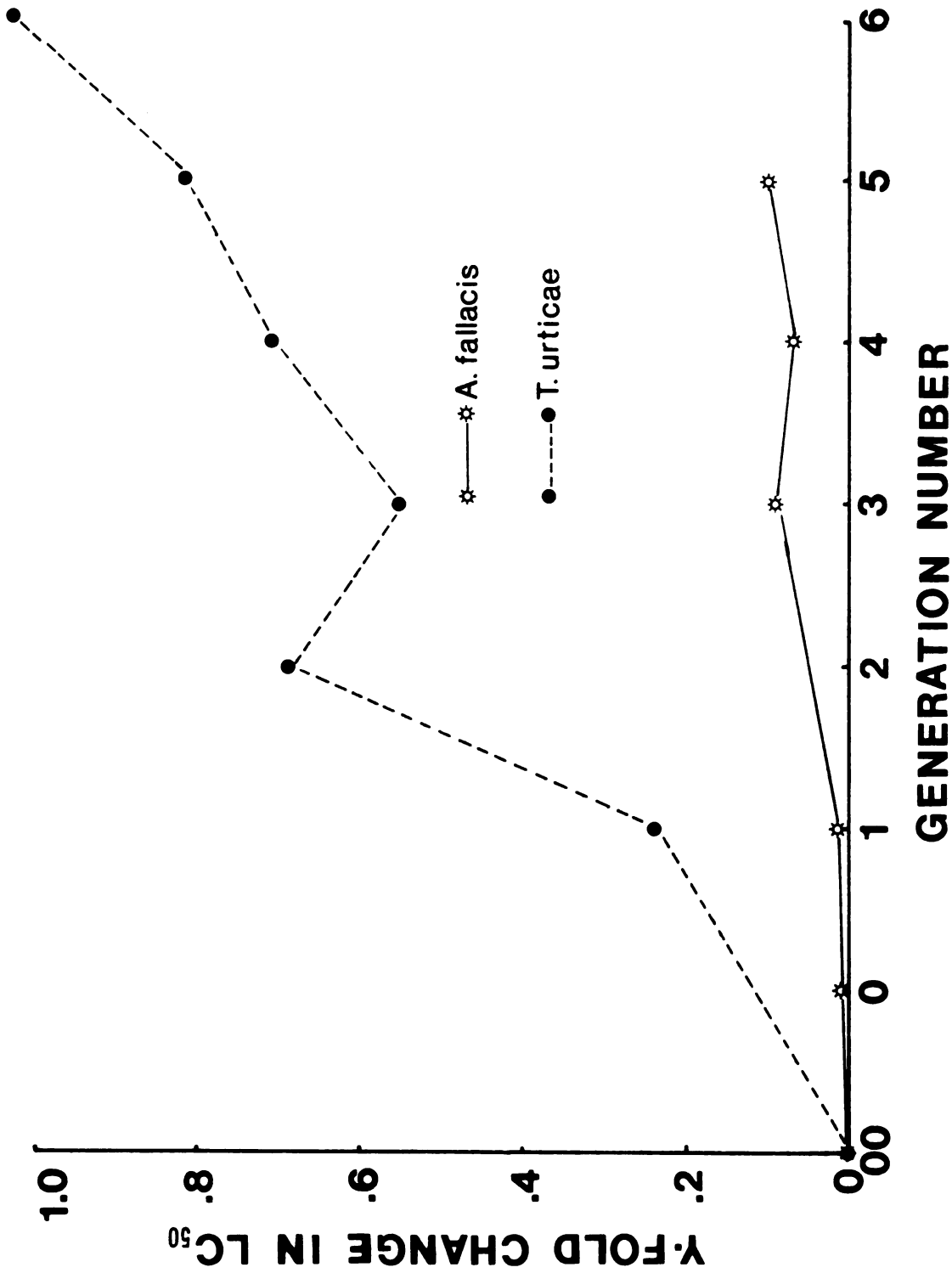

Y-FOLD CHANGE IN LC50
1.0
.8
.6
.4
.2
0
00
0
1
2
3
4
5
6
GENERATION NUMBER
A. fallacis
T. urticae

FIGURE 15

A Comparison of Population Sizes Prior to Selection in Experiments 4 and 5

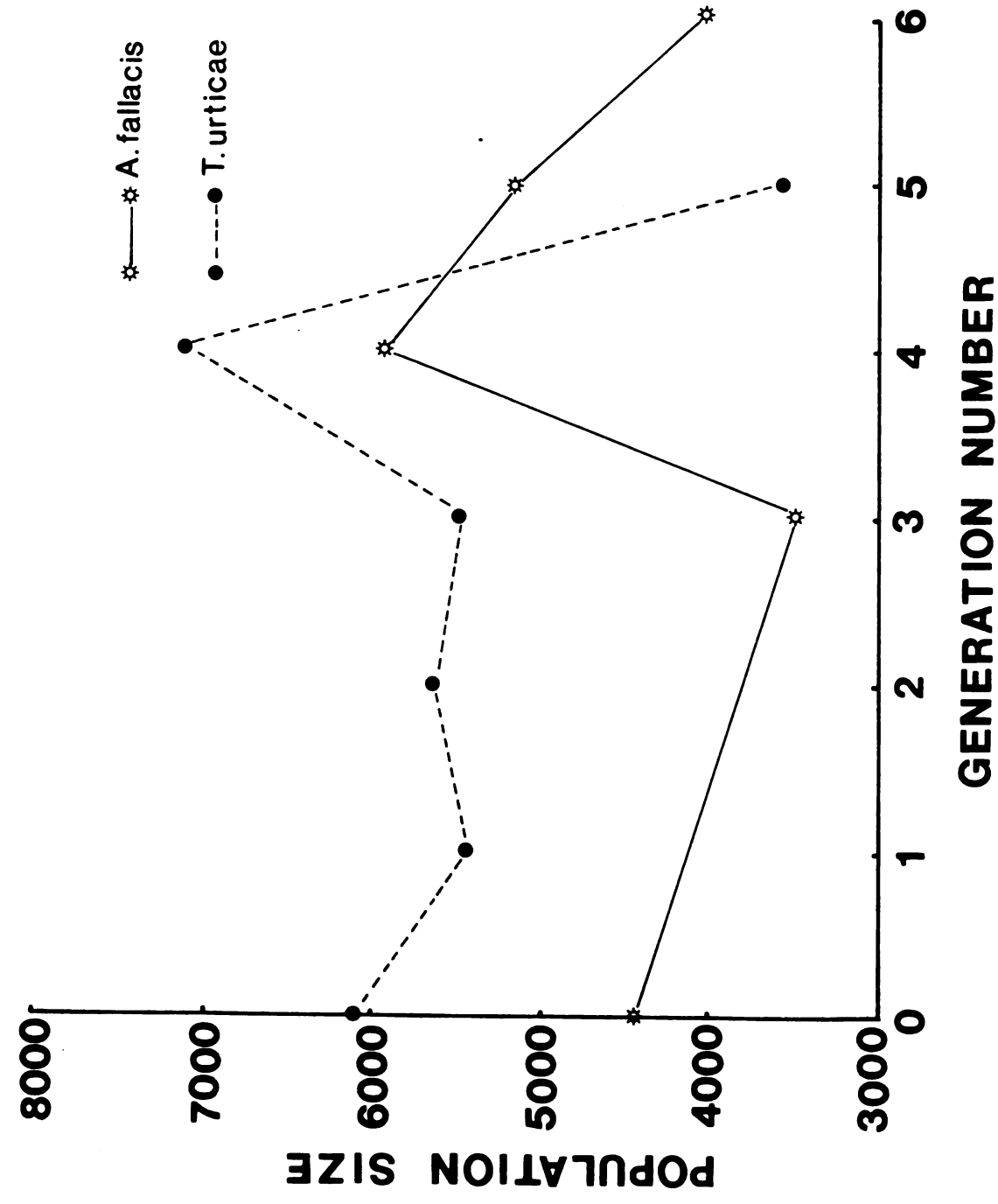

A. fallacis
T. urticae
POPULATION SIZE
8000
7000
6000
5000
4000
3000
0
1
2
3
4
5
6
GENERATION NUMBER

FIGURE 16

A Comparison of Percent Mortality After Selection in Experiments 4 and 5

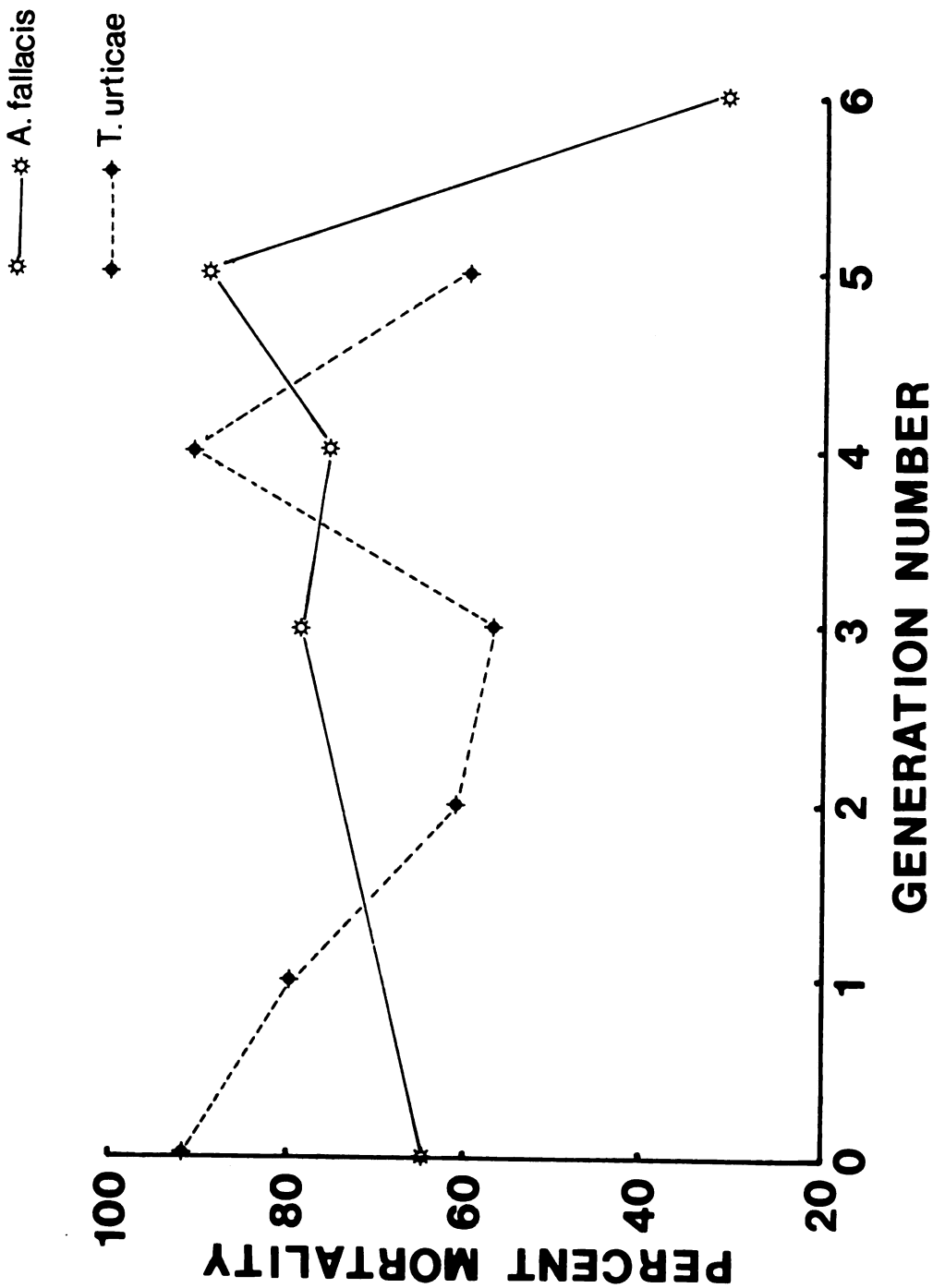

A. fallacis
T. urticae
PERCENT MORTALITY
100
80
60
40
20
0
1
2
3
4
5
6
GENERATION NUMBER

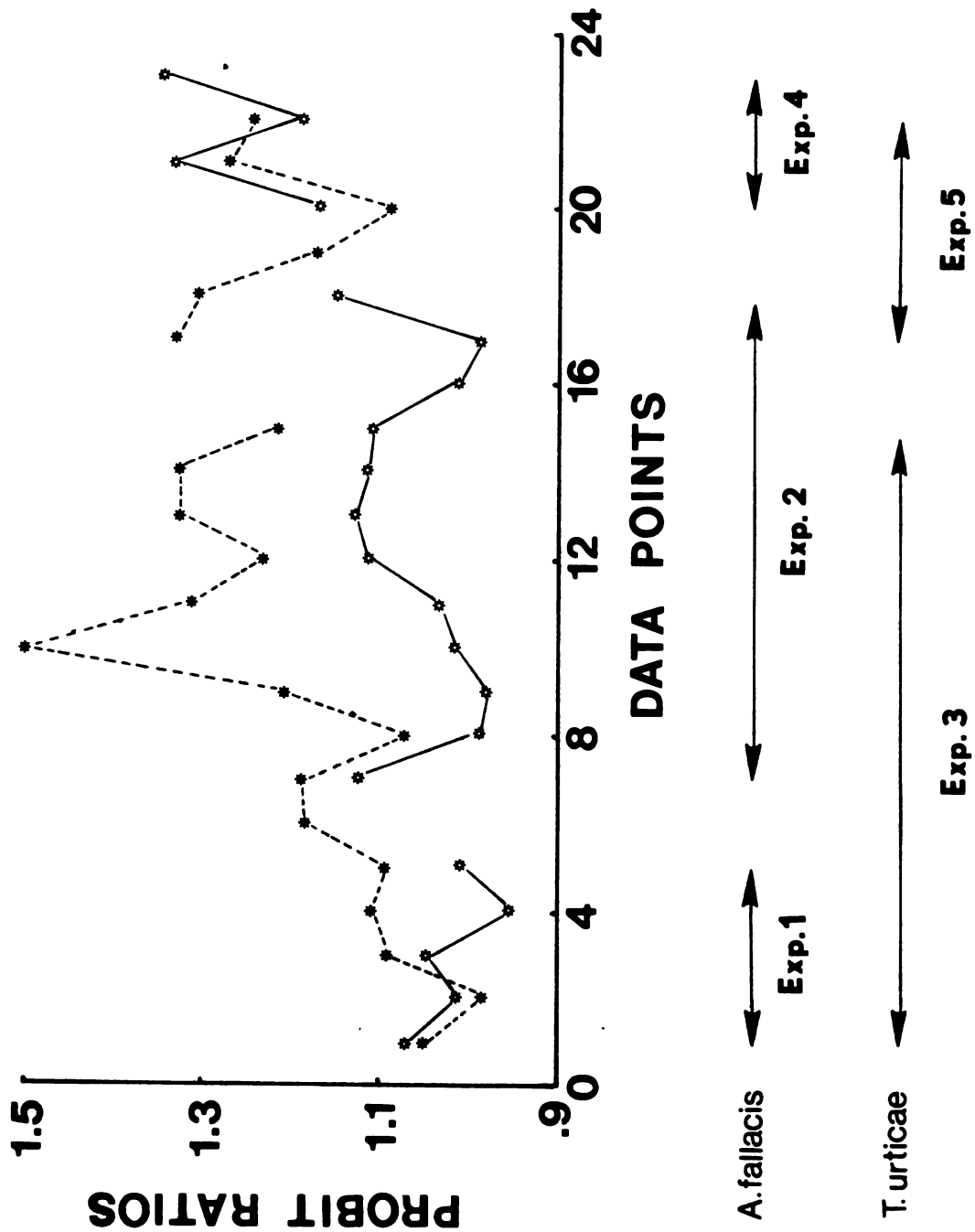
FIGURE 17

Relationship between predicted probit mortality (P), actual spray concentration (S) and the dosage-mortality line (D-M line)

S
D.M LINE
P
LOG10 DOSAGE
PROBIT MORTALITY

FIGURE 18

A Comparison of Mortality Probit Ratios (Actual/Predicted Mortality) Following Selection for A. fallacis and T. urticae


PROBIT RATIOS
1.5
1.3
1.1
.9
0
4
8
12
16
20
24
DATA POINTS
A.fallacis
T. urticae
Exp.1
Exp. 2
Exp. 3
Exp.4
Exp.5

APPENDIX B

EXPERIMENTAL DATA

TABLE A-1

Selection Data for Experiment 1 (A. fallacis)

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-square**
0 (P)	-	.0243	.00146	4.391	.034	73.8	-	1.38
1 (F_1)	4813	-	-	-	.034	-	82.1	-
2	4003	.0101	.00061	2.507	.020	77.0	87.0	5.31*
3	3152	.0175	.00105	5.224	.025	79.0	80.7	4.64
4	3606	.0204	.00122	3.065	.025	60.6	69.5	3.24
5	3284	.0155	.00093	4.321	.025	81.5	74.0	5.07
6	4968	.0153	.00092	3.114	.030	81.9	83.2	2.42
7	-	.0100	.00060	2.376	-	-	-	6.44
Average	3971					75.6	79.4	

*Significant at .05 level

**Applies to LC_{50}, Slope and Predicted Mortality

TABLE A-2

Selection Data for Experiment 2 (A. fallacis)

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-** square
0 (P)	-	.0178	.00107	1.739	.010	33.1	56.1	8.41*
1 (F_1)	3350	.0269	.00161	2.231	.045	69.1	66.9	.34
2	4303	.0164	.00098	1.559	.040	72.8	68.9	5.60
3	3944	.0370	.00222	1.743	.040	52.3	55.8	9.89*
4	5353	.0362	.00217	1.747	.070	69.2	76.0	3.27
5	4859	.0715	.00429	2.351	.075	52.0	74.1	.33
6	5489	.1364	.00818	1.832	.125	47.2	71.4	6.19
7	3928	.1220	.00732	1.585	.125	50.7	72.9	4.92
8	4101	.2024	.01214	1.608	.200	49.7	70.4	.01
9	3237	.2031	.01208	1.217	.300	58.2	59.3	6.87*
10	3253	.0879	.00527	1.303	.400	80.4	79.0	3.32
11	6155	-	-	-	.400	-	84.2	-
12	5041	.4914	.02948	1.652	.400	44.1	72.6	3.03
13	7159	-	-	-	.500	-	94.4	-
14	5035	.4249	.02549	1.842	.400	48.1	62.5	22.38*
Average	4658					55.9	71.0	

*Significant at .05 level

**Applies to LC_{50}, Slope and Predicted Mortality

TABLE A-3

Selection Data for Experiment 3

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-** square
0 (P)	6292	.5048	.03029	2.200	.34	35.3	45.3	8.57*
1	4424	.8800	.05280	2.189	1.50	69.2	-	2.78
2	-	.5345	.03207	1.790	1.00	68.7	-	23.05*
3	4293	.8090	.04854	1.632	1.50	66.9	63.1	3.49
4	-	.7699	.04619	5.165	1.50	66.3	-	4.16
5	5530	.7852	.04711	1.652	2.00	74.9	-	22.00*
6	4357	1.2458	.07475	2.673	2.00	70.9	85.7	.00
7	5383	1.1569	.06941	1.528	1.75	60.8	80.9	14.80*
8	4127	1.3678	.08207	1.939	2.00	62.5	79.8	5.45
9	4538	1.8786	.11272	1.553	2.00	51.7	83.2	22.97*
10	4854	4.3380	.26029	1.218	4.00	48.3	-	7.03*
11	4613	2.9870	.17922	1.775	4.00	58.9	88.4	28.47*
12	3158	1.6391	.09835	1.363	3.00	64.1	77.1	10.43*
13	8144	2.171	.13030	1.063	3.00	61.4	91.9	1.18

TABLE A-3 (Continued)

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-** square
14	-	-	-	-	2.00	-	-	-
15	-	2.7378	.16427	2.013	2.50	46.8	-	41.83*
16	5857	12.1010	.72606	1.420	4.00	24.7	93.0	36.85*
17	5429	3.7515	.22509	2.505	3.25	43.8	-	5.83
18	2521	6.5702	.39421	3.072	4.50	30.7	81.7	44.48*
19	5955	5.4267	.32560	3.398	4.00	32.6	73.0	6.76
20	4331	5.9590	.35751	3.872	4.00	25.1	76.7	6.71
21	2751	6.7056	.40234	2.959	4.00	25.3	77.3	20.34*
22	4235	10.3013	.61808	3.262	5.00	15.3	43.6	22.55*
Average	4779					50.2	76.1	

*Significant at .05 level

**Applies to LC_{50}, Slope and Predicted Mortality

TABLE A-4

Selection Data for Experiment 4

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-** square
00 (P)	-	.0153	.00092	2.195	-	-	-	.72
0 (F_1)	4446	.0312	.00187	2.113	.02	34.1	64.8	5.32
1 (F_2)	-	.0686	.00412	.789	.04	-	-	.01
2	-	-	-	-	.10	-	-	-
3	3490	.7123	.04274	1.796	.30	25.0	78.3	4.29
4	5983	.5287	.03172	1.744	.40	41.7	75.2	.86
5	5163	.7900	.04740	1.896	.50	35.3	88.7	7.89*
6	4043	-	-	-	.60	-	30.7	-
Average	4625					34.0	67.5	
R***	-	7.7520	.46512	3.032	-	-	-	9.04*

* Significant at .05 level

** Applies to LC_{50}, Slope and Predicted Mortality

*** Dosage-Mortality line for Resistant A. fallacis added into P and F_1 generations

TABLE A-5

Selection Data for Experiment 5

Generation Number	Population Size	LC_{50} (lb/100g)	LC_{50} (% A.I.)	Slope	Spray Level (lb/100g)	Predict Mortality(%)	Actual Mortality(%)	Chi-** square
00 (P)	-	.5048	.03029	2.200	-	-	-	8.58*
0 (F_1)	6101	.4043	.02426	1.492	.30	42.4	91.9	3.53
1 (F_2)	5422	2.8563	.17138	1.917	1.50	29.6	79.9	.15
2	5623	7.2737	.43642	1.327	3.00	30.5	60.8	13.82*
3	5488	5.9433	.35660	2.198	4.50	39.5	56.3	35.72*
4	7153	7.4462	.44677	4.911	6.00	32.3	90.4	4.08
5	3575	8.4906	.50944	5.197	6.00	21.7	59.7	17.59*
6	-	10.5558	.63335	3.554	-	-	-	11.56*
Average	5560					32.7	73.2	
R***	-	10.3079	.61847	2.896	-	-	-	11.75*

* Significant at .05 level

** Applies to LC_{50}, Slope and Predicted Mortality

*** Dosage-Mortality line for Resistant T. urticae added into P and F_1 generations

TABLE A-6

Relative Changes in LC_{50} for Experiments 2 and 3

Generation Number	*A. fallacis*		*T. urticae*	
	LC_{50} (lbs/100g)	X-fold Change*	LC_{50} (lbs/100g)	X-fold Change*
0	.0178	1.00	.5048	1.00
1	.0269	1.51	.8800	1.74
2	.0164	.92	.5345	1.06
3	.0370	2.08	.8090	1.60
4	.0362	2.03	.7699	1.53
5	.0715	4.02	.7852	1.56
6	.1364	7.66	1.2458	2.47
7	.1220	6.85	1.1569	2.29
8	.2024	11.37	1.3678	2.71
9	.2031	11.41	1.8786	3.72
10	.0879	4.94	4.3380	8.59
11	-	-	2.9870	5.92
12	.4914	27.61	1.6391	3.25
13	-	-	2.1717	4.30
14	.4249	23.87	-	-
15			2.7378	5.42
16			12.1010	23.97
17			3.7515	7.43
18			6.5702	13.02
19			5.4267	10.75
20			5.9590	11.80
21			6.7056	13.28
22			10.3013	20.41

*LC_{50} of gen. 0 used as base (LC_{50}/LC_{50} (gen.00)

TABLE A-7

Relative Changes in LC_{50} for Experiments 4 and 5

Generation Number	*A. fallacis*			*T. urticae*		
	LC_{50} (lb/100g)	X-fold Change	Y-fold Change	LC_{50} (lb/100g)	X-fold* Change	Y-fold** Change
00	.0153	1.00	.000	.5048	1.00	.000
0	.0312	2.04	.002	.4043	.80	-
1	.0686	4.48	.007	2.8563	5.66	.240
2	-	-	-	7.2737	14.41	.690
3	.7123	46.56	.090	5.9433	11.77	.555
4	.5287	34.56	.066	7.4462	14.75	.708
5	.7900	51.63	.100	8.4906	16.82	.815
6	-	-	-	10.5558	20.91	1.025
R	7.7520	506.67	1.000	10.3079	20.42	1.000

---

* $LC_{50}/LC_{50(gen.00)}$

** expressed as fraction of way to LC_{50} of R strain

i.e. $$\frac{LC_{50}-LC_{50(gen.00)}}{LC_{50(R)}-LC_{50(gen.00)}}$$

TABLE A-8

A Comparison of Predicted vs. Actual Mortalities for Pooled A. fallacis and T. urticae Populations

Source and Generation Number	Predicted Mortality (%)	Predicted Mortality Probits	Actual Mortality (%)	Actual Mortality Probits	Ratio of Actual/ Predicted Probits
Experiment 1					
2	77.0	5.7388	87.1	6.1311	1.068
3	79.0	5.8056	80.7	5.8669	1.011
4	60.6	5.2695	69.5	5.5101	1.046
5	81.5	5.8962	74.0	5.6433	.957
6	81.9	5.9105	83.2	5.9621	1.009
Average	76.0	5.7241	78.9	5.8227	1.018
Experiment 2					
0	33.1	4.5627	56.1	5.1535	1.129
1	69.1	5.4974	66.9	5.4372	.989
2	72.8	5.6055	68.9	5.4930	.980
3	52.3	5.0583	55.8	5.1459	1.017
4	69.2	5.5003	76.0	5.7063	1.037
5	52.0	5.0494	74.1	5.6464	1.118
6	47.2	4.9306	71.4	5.5651	1.129
7	50.7	5.0170	72.9	5.6098	1.118
8	49.7	4.9918	70.4	5.5359	1.109
9	58.2	5.2062	59.3	5.2353	1.006
10	80.4	5.8576	79.0	5.8064	.991
12	44.1	4.8524	72.6	5.6008	1.154
Average	56.6	5.1774	68.6	5.4946	1.065

TABLE A-8 (Continued)

Source and Generation Number	Predicted Mortality (%)	Predicted Mortality Probits	Actual Mortality (%)	Actual Mortality Probits	Ratio of Actual/ Predicted Probits
Experiment 4					
0	34.1	4.5910	64.8	5.3799	1.172
3	25.0	4.3256	78.3	5.7824	1.337
4	41.7	4.7887	75.2	5.6808	1.186
5	35.3	4.6235	88.7	6.2107	1.343
Average	34.0	4.5822	76.8	5.7635	1.260
Average overall *A. fallacis*	56.9	5.1942	72.6	5.5584	1.091
Experiment 3					
0	35.3	4.6224	45.3	4.8819	1.056
4	66.9	5.4375	63.1	5.3345	.981
7	70.9	5.5495	85.7	6.0669	1.093
8	60.8	5.2746	80.9	5.8742	1.114
9	62.5	5.3198	79.8	5.8345	1.097
10	51.7	5.0422	83.2	5.9621	1.182
12	58.9	5.2251	88.4	6.1952	1.186
13	64.1	5.3601	77.1	5.7421	1.071
14	61.4	5.2895	91.9	6.3984	1.210
17	24.7	4.3172	93.0	6.4758	1.500
19	30.7	4.4951	81.7	5.9040	1.313
20	32.6	4.5498	73.0	5.6128	1.234
21	25.1	4.3297	76.7	5.7290	1.323
22	25.3	4.3361	77.3	5.7488	1.326
23	15.3	3.9761	43.6	4.8389	1.217
Average	45.8	4.8750	76.0	5.7733	1.194

TABLE A-8 (Continued)

Source and Generation Number	Predicted Mortality (%)	Predicted Mortality Probits	Actual Mortality (%)	Actual Mortality Probits	Ratio of Actual/ Predicted Probits
Experiment 5					
0	42.4	4.8088	91.9	6.3984	1.331
1	29.6	4.4638	79.9	5.8381	1.308
2	30.5	4.4894	60.9	5.2767	1.175
3	39.5	4.7344	56.3	5.1586	1.090
4	32.3	4.9394	90.4	6.3047	1.276
5	21.7	4.2164	59.7	5.2456	1.244
Average	32.7	4.6087	76.1	5.7037	1.237
Average overall *T. urticae*	42.0	4.7989	76.1	5.7534	1.206
Average* overall mites	49.5	4.9966	74.3	5.7584	1.149

*21 observations for both *A. fallacis* and *T. urticae*