ACARICIDE RESISTANCE AND GENETIC AFFINITIES OF SOME SELECTED POPULATIONS OF *TETRANYCHUS URTICAE* KOCH IN NEW ZEALAND.

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ABSTRACT

A study of resistance to acaricides in a number of populations of the two-spotted spider mite, Tetranychus urticae, in New Zealand had been carried out. Natural genetic and cytoplasmic incompatibilities between populations were also investigated with a view to possible biological control of the pest. Facets of acaricide resistance that were studied included multi-resistance, cross-resistance, negatively correlated resistance and the inheritance of resistance. Chemicals used included an organophosphate representative (parathion-methyl), a carbamate (formetanate), an ungrouped compound (tricyclohexyltin hydroxide) and an organochlorine (dicofol). Cross-resistance was demonstrated between parathion-methyl and formetanate in five populations obtained from widely separate areas of New Zealand. The resistance to parathion of three strains was found to be inherited as a single dominant character and transmissible by both sexes. Cytoplasmic factors (or nucleo-cytoplasmic interactions) and minor genes were found to contribute slightly to the expression of total resistance. No resistance to tricyclohexyltin hydroxide (Plictran) and dicofol (Kelthane) was detected.

High degrees of incompatibility (haploid egg lethality) were observed in the hybrids of crosses between the various populations. Chromosomal rearrangements in balanced heterozygous conditions, in conjunction with the cytoplasm, were considered to be important factors determining the interpopulational sterilities. The interpopulational incompatibility phenomenon was found to be multifactorial and not associated with the resistance factor. The egg

mortalities of some backcross series which remained constantly high in spite of several crossings, implicated that the introduction of normal males to a resistant mite population in an enclosed area (e.g. in a glasshouse) might be a worthwhile proposition in the integrated control of spider mites. Backcross hybrids, on allowing to multiply randomly, were capable of forming new gene combinations, leading consequently to the formation of new strains which were genetically different from the original parents used in the backcross series.

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CHAPTER 1

INTRODUCTION

'Can insects become resistant to sprays?. That now historical question was asked by Melander (1914) in 1914. Following the introduction of DDT in the early 1940s, the answer was clearly, yes, and the problem of resistance has had a profound influence ever since on the orientation of entomological research. Such influence is obvious since the development of resistant populations of insects will threaten man's hopes for improvement of his health standards and protection of his food reserves. While only 8 insect species were known to have developed resistance prior to 1940 (Brown, 1961a), the number of resistant strains began a sharp upward trend soon after the introduction and use of DDT and other synthetic organic insecticides. The history of insecticide resistance since then virtually parallels the history of insecticide development. Up to 1967, resistance had developed in 224 species of insects and acarines. Of these 97 are of public health or veterinary importance and 127 attack field or forest crops or stored products (Brown, 1972).

In similar vein, the control of spider mites (Acarina: Tetranychidae) did not constitute a problem until about two decades ago when resistance to agricultural insecticides became widespread.

The first case of resistance in mites probably occurred in 1937, when Compton and Kearns (1937) found inadequate control by Selocide sprays of a two-spotted spider mite population. Selocide resistance was a prelude to the unlimited resistance development which set in after the war, following the use of synthetic insecticides. In many situations, the Tetranychids were promoted from the

role of a minor pest to that of a major one as a result of the use of DDT (Helle, 1965a).

An extraordinary genetic potential to adapt to various environments, plus subjection to the high selection pressure encountered in the commercial growing areas, are factors that make the frequency of the resistance phenomenon high in the family Tetranychidae. For these very reasons, the two-spotted spider mite, Tetranychus urticae, has recently been the most difficult to control among all the pests that confront horticulturists and agriculturists (Naegele and Jefferson, 1964). In spite of control measures, many orchardists and ornamentalists suffer economic loss due to defoliation, reduced tree vigour, poor fruit colour, or small fruit brought on as a result of mite feeding. The major problem in the chemical control of spider mites throughout the world is the continued development of strains resistant to the common chemical compounds. Each year, the problem becomes more severe with the number of non-effective types ever increasing, especially the organophosphorus compounds.

The resistance of insects of medical interest, such as Anopheles, Aedes and Musca, had been extensively studied for many years (Brown, 1960). Research on the resistance of agricultural pests had been done but on a smaller scale. As the chemical control of spider mites threatens to develop into a neck and neck race between the chemical industry and the resistance response of the mites, the desirability of an exhaustive investigation into the biological background of the organism, and the physiological and genetical base of the resistance become evident.

Genetic principles and methodology have been invaluable both from the point of view of understanding the development, spread and regression of resistance, and in providing pure strains for funda-

mental investigations on the interrelationships of genes, enzymes and toxicological responses to insecticides. From the practical standpoint, knowledge of the genetic identity of phenotypes has made possible the detection of genes for resistance in field strains prior to the use of insecticides or during the course of control operations, thus indicating the advisability of change to another insecticide. Additionally, information from hybridization, indicating reproductive barriers or genetic isolation, obtained during the course of studies on the genetics of resistance, has generated considerable interest in the feasibility of genetic control of insect populations.

With the various problems in mind, the aims of the present research are:

1) to determine the distribution of acaricide resistance among the two-spotted spider mite populations in certain selected areas of New Zealand.
2) to determine the effectiveness of particular groups of acaricides under laboratory conditions.
3) to study cross-resistance, multiple-resistance and negatively-correlated resistance patterns in the resistant populations chosen.
4) to study the mechanism of inheritance of the resistances in these populations.
5) to study the patterns of genetic and cytoplasmic incompatibility that occurs among the chosen populations of spider mites.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 INTRODUCTION

The survey of the literature is intended to consist of four portions. The first part, which consists of a summary of general concepts regarding resistance in insects as a whole, provides a better understanding of the second part, which deals with the specific problem of resistance in spider mites. The third part of the literature review attempts to gather available information regarding incompatibility, sex-ratio and sex determination of T.urticae. The last section deals with the current genetical concepts in insect control in general and their possible application in control of two-spotted spider mites.

2.2 DEFINITIONS

Throughout the thesis, there are several terms which are repeatedly used and it is essential to define them. Some terms, such as "incompatibility", "sterility", "incompatible genes", "sex-ratio", "chromosomal races", "strain hybrids", etc. are counted as being self-explanatory, but on the other hand there are terms like "resistance" and "tolerance" which are confusing and have in the past been misinterpreted and misused. Thus, the latter terms are defined in detail

Tolerance

a) Natural tolerance — All living organisms can carry

on their life processes with little or no impairment resulting from the presence of a chemical up to some level of concentration. Obviously, this concentration depends upon the species, the chemical, the method of exposure and the criterion of effect. If these factors were to remain constant, the level will give an indication of the measurement of natural resistance or tolerance (Hoskins and Gordon, 1956). When a measurement of tolerance is used, it is usually necessary to specify information such as the stage of the life cycle, age within a stage, sex and sometimes race or strain. Although tolerance can be specified only within a range which may be rather wide, it provides a basic measure of the ability of a population to withstand a toxicant and which can be used for comparative purposes.

A number of factors such as the permeability of the integument (a function of its anatomical structure and thickness), ease of absorption from the digestive tract, patterns of behaviour which affect the degree of contact with a toxicant, and biochemical reactions into which absorbed toxicants enter, affect tolerance.

The tolerance of various species may differ greatly but representatives of a species living under natural conditions in different regions do not vary as a rule. There are some exceptions however; for example, citrus thrips, Scirtothrips aurantii, from different regions of S.Africa differs markedly in tolerance to tartaremetic (Smith, 1946).

b) Vigour tolerance Hoskins and Gordon (1956) refers this as the added ability to withstand a toxicant which appears to stem from improved nutrition, extra weight, or any other factor associated with what may be called extra vigour. A strain developed by breeding only from those individuals which survive

exposure to such diverse stresses as extremes of temperature, lack of moisture, abnormal food, or an injurious chemical will have an altered ability to withstand many kinds of stresses including exposure to chemicals. This change from the normal tolerance may either augment or decrease the normal tolerance. To illustrate this, two examples can be cited in which the specific properties contributing to vigour tolerance have been identified. The increased arsenic tolerance of codling moth is correlated with their larger size and greater resistance to desiccation, which enables them to survive longer and reach safety beside an untreated point of entry (Ascher and Bergmann, 1962). A pyrethrum resistant strain of granary weevil, Sitophilus granarius, (52x), was found also to be more tolerant to environmental stresses, such as starvation (2x), heat (1.2x), cold (1.1x) and desiccation (1.8x, 1.7x)(Lloyd and Parkin, 1963).

Resistance

The World Health Organisation defines resistance as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (WHO Tech. Rept. Ser., 1957).

Precise laboratory methods of bioassay of response of insects to chemicals are now available for the quantitative evaluation of changes in the susceptibility of populations, but the phenotypic responses of populations is influenced by variation caused by such factors as dosage and intrinsic toxicity of the chemical, type and volume of solvent used per insect, length of exposure and temperature. A change in any of these factors can alter the quantitative estimations of the degree of apparent resistance. The elucidation of the

physiological and biochemical mechanisms involved in the phenomenon of resistance by using strains genetically pure for the mechanisms have placed the phenomenon of resistance in proper perspective. Although phenotypic results are good evidence of the existence of resistance, the phenomenon is most convincingly evident when the specific mechanisms responsible for the observed resistance are traced (Georghiou, 1965).

2.3 ORIGIN OF RESISTANCE

Since resistance generally arises following the exposure of a population to a toxicant, it is recognised that resistance is an example of evolutionary change (Crow, 1957). In the past there had been a great deal of controversy as to whether resistance is post-adaptive (change to resistance is physiological and does not depend on the genetic constitution, or if the change is genetic, it is induced directly by the poison) or preadaptive (genetic differences already are present in the population and the poison acts as a selective agent favouring the resistant genotypes) in origin.

Considerable evidences seem to show that the postadaptive explanation is very unlikely, as illustrated by the following examples: Drosophila larvae exposed to sublethal doses of DDT for 50 generations develop no increase in tolerance level (Crow, 1957; Brown, 1958); sublethal treatments of houseflies with DDT lead to a decrease in tolerance (Hadaway, 1956); a strain of Aedes aegypti under sublethal doses of DDT produce an insignificant two-fold increase in LD50 level after 3 generations (Brown, 1961b). These experiments lead to the conclusion that a physiological immunity can-

not be acquired during the life time of an insect. Futhermore, tests with DDT on Drosophila seem to indicate that it does not induce mutations conferring immunity or resistance, nor does it increase the normal mutation rate (Crow, 1957).

On the other hand, the evidence for the pre-adaptive nature of resistance is considerable. If sublethal doses do not produce resistance and lethal doses do, then this must suggest that the insecticide is acting solely as a selective agent. That resistant mutants are already present and available for selection has already been demonstrated. Populations of some African strains of the Anopheles mosquito in the absence of Dieldrin treatment contains 0.04% to 12% of individuals heterozygous for the Dieldrin-resistance gene (Crow, 1957; Brown, 1958). In unsprayed villages of Taiwan (Brown, 1958), 1 in 200 bedbugs (Cimex hemipterus) are resistant to DDT. In the selection for resistant Drosophila within inbred lines, there is no indication of appreciable change in resistance (Merrell and Underhill, 1956). These experiments offer strong support for preadaptation, since there is no reason to think that direct effects of the insecticide will be any less effective in isogenic than in heterogeneous strains. Further, this shows that selection must act mainly on the supply of genetic variants already in the population at the time the selection programme begins, not on mutations that occur during the process of selection. To support this, it can be shown that there is considerable natural variation in resistance in different Drosophila cultures not previously exposed to insecticides (Beard, 1952).

Such cases as these, while perhaps constituting instances of balanced polymorphism in which the resistance factor is advanta-

geous to the insect, may be out of the ordinary, but they do serve to show that the resistance gene can be present at lower or higher frequencies, so permitting the progressive development of resistance by selection. It seems safe to assume, therefore, that the sole effect of the insecticide is as a selective agent, although the postadaptive concept should not be completely rejected, as the effects, especially mutagenic effects, of other compounds besides DDT had not been studied. Recent studies have shown that DDE, a metabolic product of DDT, is capable of inducing mutations in a Chinese Hamster cell line (Kelly-Garvert and Legator, 1973). In a mammalian cell line, DDT and DDE have been shown to produce chromosome aberrations (Palmer et al., 1972).

So far, the resistance factors have been regarded as pre-existing as part of the genetic variability of the species. To view the situation in another way, it can be assumed that resistance factors may also arise as new mutations shortly before or during the selective process. Assuming that a mutation occurs in the germ line of a normal individual, this occurrence will not confer any immediate advantage on the organism. Only individuals of the succeeding generations which carry the mutation in the somatic as well as the germ line are potentially capable of benefiting. The chance of passing the mutation through to the succeeding generation depends on the probability of the susceptible parent which carries the mutation being killed (Milani, 1958a). The mutation that has been passed to succeeding generations has to be non-deleterious to the organisms. If the mutation is harmful as most spontaneously occurring mutations are (Auerbach, 1956), the situation will be akin to the opposite of balanced polymorphism, under normal condition (no insecticide). This situation has often been shown to be the case; that is, the resistance

factors tend to be associated with reduced vitality and fitness (Maelzer and Kirk, 1953; Dittrich, 1961).

In summary, two conditions must be fulfilled before resistance to insecticides can develop. Firstly, resistant mutants must occur in the original populations. Secondly, the insecticide must act as a powerful selective agent favouring these mutants. The net effect is the concentration of resistant mutants that are present in low frequencies in the original population.

2.4 DEVELOPMENT OF RESISTANCE

2.4.1 Pattern of resistance development

. Data from both laboratory and field studies on the development of resistance seem to indicate a common pattern of geometric or exponential response. By using the tolerance values of LD50 there appears to be an initial period of slow response extending over a few or many generations of selections, which is followed by a rapidly accelerating period of response often occurring within one or two generations (Crow, 1957; Milani, 1958; Brown, 1958; Unsterstenhofer, 1960). This latter period generally carries the resistance level to a maximum or limit characteristic of the insecticide and the populations (Hoskins and Gordon, 1956).

Various authors explain the geometric or exponential response in different ways and the two most popular interpretations are that of a kinetic or mathematical relationship between gene frequencies and selection (Hoskins and Gordon, 1956; Crow, 1957), and that of gradual perfection (Milani, 1958).

2.4.2 Factors affecting the development of resistance

The rate of resistance development by selection depends on the amount of heritable variance in the population and the intensity of selection.

a) Amount of heritable variance With other factors constant, the bigger the populations, the greater will be the amount of heritable variance. In the spider mites, many generations are produced during each season and very high populations can develop rapidly in midsummer. If coupled with high selection pressure, populations with high acaricidal resistance can develop. On the other hand, in small populations, there is obviously a lack of genetic variability and the resistance gene may not be present or may be lost due to chance (Crow, 1957). As an illustration of this point, the experiments done by King (1954; 1955) serve the purpose. He found that more rapid development of DDT resistance in Drosophila with 50% mortality treatment than the one which gives 95% mortality. Crow (1957) explained that the higher mortality appears to leave behind very few survivors and as a consequence the population loses much of its genetic variability. In general, rapidity of progress will increase with intensity of selection, but only if the number of survivors is large enough to maintain the genetic variability.

Further evidence that the amount of heritable variance is an important factor in the development of resistance come from the observation that certain T.urticae strains in the fields and glasshouses are not capable of developing resistance to Pentac selection (McEnroe and Lakocy, 1969). Probably the low heritable variance in these two situations is due to the low population density maintained by Pentac, leading to a high degree of inbreeding (McEnroe and Lakocy,

1969). However they do possess the ability to develop resistance if they outcross repeatedly with strains from elsewhere, a process which leads to the formation of balanced linkage combinations (Thoday et al., 1964), a pre-requisite to the release of variability for the response to selection. Outcrossing between populations serves to release cryptic variability held in balanced combinations by inducing crossing-over between homologous chromosomes (Mather, 1943). This type of radical change gives rise to an accelerated response. According to McEnroe and Harrison (1968), linked polygenic complexes probably play a more important role than free genetic diversity in response to selection as regard arrhenotokous species, which are commonly inbred. The mating behaviour of the arrhenotokous T.urticae, provides a built-in system for selection of inbred populations followed by periods of outcrossing. Under low population density this mite forms highly inbred populations. When the population pressure rises, the mites enter a migration phase and outcrossing can occur (McEnroe, 1969).

b) Selection pressure Generally, the greater the proportion killed each generation, the more rapid the increase in resistance. This statement is based on the assumption that there is adequate heritable variance in the surviving population; otherwise it would contradict Crow's explanation on King's experiment mentioned above (Crow, 1957; King, 1954, 1955).

Selection pressure can be adjusted by repeating applications, altering the concentration of toxicant and varying the period of toxicological exposure. With the genetic constitution remaining constant, the following generalizations regarding selection apply (Watson and Naegele, 1960):

a) Resistance develops more rapidly under high pressure.

b) Under regular periodic selection the same level of resistance is reached in time regardless of selection pressure.

c) Low selection pressure appears to produce more homogeneity than high selection pressure.

d) Higher levels of resistance can be demonstrated after one severe selection.

The quantitative relation between selective intensity and rate of progress do not follow any fast rules. The relationship is complex and depends on the number of genes involved, dominance and epistasis, amount of environmental effect, counteracting effects of natural selection, etc.. It is generally recognized that the fewer the factors involved, the more quickly they will become widespread through the population and the faster resistance will develop (Hoskins and Gordon, 1956). If selection intensity is assumed to be high, such that susceptible phenotypes are effectively inviable or sterile, then theoretically the rate of change for a recessive resistance gene (r) should be greater (approaching 1 - q per generation, since susceptible homozygotes (RR) and heterozygotes (Rr) will be eliminated) than the rate of change for a dominant resistance gene (R) (approximating - $q^2/1+q$ per generation. q = frequency of the recessive gene in each case)(Mettler and Gregg, 1969). Most factors for resistance exist in the original population at very low frequencies and in the case of a dominant gene, the increase will follow an accelerating rate of increase as q changes from a high value to a moderate

value. In addition to an accelerating rate of increase in resistance there should also on this model be an increased variance in resistance (Crow, 1952). If the resistant factor becomes more common than its allele, the variance and the rate of change will begin to decrease, but this is likely to happen only if resistance depends on one, or at most a very few genes.

Where an advantage is conferred on the organism, resistance will develop at a much greater rate than where the gene is harmful to its host, generally because it is present initially at a high frequency.

2.5 STABILITY OF RESISTANCE

Once a resistant population is developed, there is no hard and fast rule as to whether or not a resistant strain will revert to normal and to how fast it will revert. In order to understand the concept of reversion or stability of resistance, it is necessary to view the words of Lerner (1954) who said, " attempts to shift populations too rapidly and too far from adapted mean values for specific traits, either by artificial selection or by changes in the breeding system, are counteracted by natural selection which is directed towards the maintainance of a phenotypic balance between fitness-determining characters".

Since the genes causing insecticide resistance were at a low frequency in the population before insecticidal selection, it can be viewed that they are to some extent disadvantageous or otherwise they would be common. Therefore, the selection for resistance involves the replacement of the original genes with resistance factors that are theoretically deleterious as far as survival is concerned (excepting

the aspect of insecticide resistance). When the resistant population is not being exposed to an insecticide, it can be expected that reversion to susceptibility will take place; slowly if the resistance factors are only mildly disadvantageous.

The above theoretical case does not in practice apply to all insect populations. Regression to susceptibility when selection for resistance is relaxed is often observed (Pigmentel et al., 1951; 1953; Varzandeh et al., 1954) though sometimes not (Crow, 1954; Lichtwardt et al., 1955; Lindgren and Dickson, 1945; Dittrich, 1961; Saba, 1961). Thus it can be expected that when selection for resistance is accompanied by a great deal of natural selection for general fitness, the only kind of resistance factors that would become frequent in the population would be those that cause very little reduction in fitness. Strains developed in this way would return to susceptibility very slowly in the absence of insecticide. On the other hand, intense selection for resistance under conditions where there is little natural competition (eg. selection in uncrowded laboratory cultures) may result in high resistance, but individuals are poorly adapted for survival. These would revert to susceptibility much more rapidly.

The fact that resistance factors are selected after insecticidal treatment points out clearly that they are not highly detrimental even though there may be an appreciable loss of viability and fertility. Based on this thinking, it can be assumed that reversion to susceptibility should be slow and a short period of insecticide absence is unlikely to lead to a susceptible population. Furthermore, if the population does return to susceptibility it may require a long time for the genes to be carried below a certain frequency, just as it often takes a long time to accomplish the early part of the

increase in resistance. This is because selection in either direction is slow when the gene is rare. Therefore, a susceptible population that has once been resistant is likely to increase rapidly in resistance when the insecticide is again applied. This has frequently occurred in laboratories where a population that had lost some resistance was built up to its former level by a single generation of selection. Such a gene will be neutral or at most very slightly deleterious. Whether reversion occurs in such a case will depend more on other features. In the field, it is highly improbable that resistance will maintain itself in any instance, since the influences of migration and the natural environment are considerable. In the laboratory, a recessive character in homozygous condition, provided it is neutral, should show no tendency to revert. For a dominant gene on the other hand, unless the insecticide discriminates between homozygous and heterozygous resistant forms, in which case homozygosity can be attained, selection will stop to all intents and purposes when the frequency of its recessive allele has become low enough that homozygous susceptibles are unlikely to appear (Milani, 1958b). Thus, reversion is likely to occur, although very slowly at first. With a population of Drosophila, however, Crow (1957) has found that in spite of heterozygosity for resistance factors, reversion did not occur in three years after release from selection pressure. He concludes in this case that the neutrality of the factors is sufficient to maintain resistance.

Severe selection can bring about reversion. Helle (1962) found that a highly organophosphorus resistant population of T.urticae, which showed no signs of reverting over 11 years in the absence of insecticidal treatment showed a considerable decline in vitality after being severely selected to produce a "super-resistant" strain.

Other cases of severe selection with similar results are also noted by various authors (Crow, 1957; Hoskins and Gordon, 1956). Crow's results (1957) seem to indicate that more rapid reversion occurs than would take place under less severe selection, because the selection is taking place under circumstances where there is little natural competition (uncrowded laboratories), so producing highly resistant but very poor and weak specimens.

The resistance gene can be of advantage to its host as in the Dieldrin-resistance gene in mosquito (Brown, 1958; 1961). The linking of the resistance factor with some vital function is an event which is only likely to happen after a long time of continous selection, but its occurrence can be of great practical consequences. It means that, even if the resistant individuals can outbreed through migration with normal individuals, the genes will be present in their favoured linkage in a large number of individuals (Hoskins and Gordon, 1956). Upon the resumption of selection, therefore, resistance will develop extremely rapidly (Crow, 1957; Brown, 1958b). This will hold to a slightly lesser degree only where no advantage is conferred. Since the sorting out and perfecting of the gene has taken place in the first period of selection, then the resistance of a population which has reverted will be restored very rapidly in response to a second period of selection (Milani, 1958b; Hoskins and Gordon, 1956; Unterstenhofer, 1961; Keiding, 1963).

In summary, it appears that although the factors which operate in the laboratory to produce reversion may be quite different from those which operate in the field (Keiding, 1963), a knowledge of the extent and manner in which reversion is likely to occur is of fundamental importance from the point of practical control (Unterstenhofer, 1960).

2.6 THE DOSAGE MORTALITY LINE: INTERPRETATION AND USES

Probably owing to the differences in the physiological and biochemical processes of each individual in a species, the reaction to poison by members of a species will vary. Even in a group selected for uniformity in size, age, rearing, ancestry and other similar basis of classification, response to toxicants still vary from individual to individual.

When the percent mortalities of a homogeneous insect population are continously plotted on a graph paper against varying log doses, the resulting line is that of a sigmoid curve. With a heterogeneous population, the resulting curve is not sigmoid but one which is analogous to a curve derived from several homogeneous sub-populations with different resistance levels (Tsukamoto, 1963).

An estimate of the LD50 from such a procedure is inadequate for two reasons: first, the determination of LD50 is only approximate because the interpolation along a curve is uncertain. Second, information conveyed by the spread of the dosage-mortality relation is neglected.

The transformation of co-ordinates to dosage in logarithms and to percentage mortality in standard deviations or probits derived from them, produces a straight line (called ld-p curve or DM-line) in a homogeneous population. In a very heterogeneous population, the resulting curve may not be a straight line. However, in a majority of cases, the log dosage-probit data determine a straight line over the central region. This provides a more accurate determination of LD50, which is an index of the mean tolerance or the mean resistance of the group tested. The same curve gives a fairer estimation of the slope which measures the diversity of response or the heterogeneity of the

group toward the toxicant used (Hoskins and Gordon, 1956). The slope of the DM-line can be regarded as reflecting a population's reaction to various "internal factors" (eg. the number of factors or mechanisms conferring tolerance or resistance to the insecticide). External factors (eg. test method, chemical used, age and sex differences, etc.) theoretically should not, but in practice influence the slope of the line (Ballantyne, 1966). However, the slope is mainly dependent upon internal factors.

The LD50 of the DM-line provides information on, 1) the tolerance of the population if the organisms have not been exposed to any insecticides (Hoskins and Gordon, 1956) and 2) the measurement of the resistance that has been developed (Hoskins and Gordon, 1956).

However, the LD50 gives little indication of what may be expected. The slope value, on the contrary, provides some information on the future development of the population.

a) steep-slope A steep slope indicates a highly homogeneous population. A slope of this kind also indicates that selection for resistant ones is difficult because the narrow range reflects smaller variability. Even if the possibility of selecting a few very resistant ones (maximum resistance) exists, the resulting population will be low in fertility and viability; consequently the population may die off. The narrow range also indicates that only one or a few factors are probably responsible for resistance (Hoskins and Gordon, 1956; Brown, 1959; Tsukamoto, 1963).

b) low-slope A low slope indicates a wide range of susceptibility and that there are a large number of factors affecting the tolerance of a population to a chemical (Hoskins and Gordon, 1956; Brown, 1959; Tsukamoto, 1963).

The steepness of the slope may indicate to some extent the concentration of toxicant to be used in practical field control. For a population which gives a steep slope, an underestimation will lead to most of the population surviving. On the other hand, an effective dosage will lead to very few survivors which can contribute little to the next generation because of low fertility and natural ingress from untreated areas. With populations showing a small slope, the most casual use of a toxicant will select out a range of the more resistant individuals (Hoskins and Gordon, 1956).

2.6.1 Effect of selection on changes in the DM-line

Changes in slope that occur during the development of resistance are in keeping with the geometric or accelerating period of increase. As the resistance character becomes more common, the DM-line slope flattens, indicating that the variance in resistance is becoming greater and the population more heterogeneous as a consequence of the resistance gene (or genes) segregating (Brown, 1958; 1959). When half the population contains the resistance factor (i.e. maximum rate of resistance development), the slope of the DM-line is at its lowest or flattest level and the variance of resistance at its greatest. As the frequency of the resistance character increases further, the variance begins to decrease and the DM-line becomes steeper again. A decrease in heterogeneity may not occur in all cases, depending upon a number of possible causes, including the number of genes involved (Crow, 1957) and the nature or associations of the resistance genes (Hoskins and Gordon, 1956). The DM-line will not steepen if the resistance genes are associated with secondary harmful effects and a state of heterogeneity is maintained as a result

of a balance being reached between the benefits that the resistance genes provide in their protective role and the vitality depressing effects they confer. This situation is most likely to occur when there are multiple genes for resistance (Hoskins and Gordon, 1956; Crow, 1957). Even if a single gene is the cause for the resistance phenomenon, heterogeneity may not decrease with further selection if secondary harmful effects are present (Hoskins and Gordon, 1956). For a neutral gene, heterogeneity should decrease with continuing selection resulting in a resistant population characterized by a steep DM-line, thus signifying the resistance gene to be in homozygous combination.

2.6.2 Limitations on use of dosage-mortality data

The interpretation of the DM-lines and their slopes is subject to several limitations, particularly when the degree of heterogeneity is considerable (Tsukamoto, 1963). The limitations are: first, they give no precise analysis of the number or nature of the genetic factors involved, nor do they necessarily provide a true indication of the amount of heterogeneity present; second, factors such as the method of testing, the units of dosing (concentration of toxicant, weight of a material per unit of body weight, amount of spray, etc.), age and sex, should not, theoretically, produce changes in the slope of the DM-line, but in practise they do as had been shown by Hoskins and Gordon (1956) and Dittrich (1962) who demonstrated that a spray method gives a flatter slope than a topical application method. Hoskins and Gordon (1956) also show that mixed sexes and ages generally give lower slope values than where single sexes or uniform ages are used; and third, where a large dose of a

toxicant is used, much of the material may be lost and consequently there is false increase in the value of LD50 and a lowered slope value of the DM-line (Hoskins and Gordon, 1956; Busvine, 1956).

2.6.3 Misinterpretation of the response to selection pattern

The pattern of resistances build-up obtained from noting the responses of either the LD50 values or the DM-line (i.e. the exponential increase associated with changes in the degree of variance) has been occassionally accepted as evidence for the polygenic inheritance of resistance (Watson, 1956). In many of these cases, it was proved later that the inheritance was monofactorial. Milani (1958a; 1958b) explained that the changes undergone by the LD50 values and DM-lines refer only to the response to selection, not to the mechanism of inheritance of the selected character.

2.6.4 DM-line in genetic studies of resistance

In spite of the many limitations, the DM-line is still widely employed in genetic research of the resistance phenomenon. Where no mutant markers are available, it is the only acceptable method of analysis. It need be stressed that the DM-line is only useful when it is capable of differentiating distinctively the resistant population from the susceptible group, since vigour factors can contribute to some increase in the total resistance. Where the DM-line is used for genetical analysis, the vigour factors should contribute only a negligible effect on tolerance both in the resistant and susceptible populations.

In normal procedures, straight DM-lines are obtained for susceptible and resistant strains and for their F_1 offspring. In the

case of heterogeneous populations such as those of the F_2 or backcross generation, the DM-lines show various inflexions which provide information on the relative proportion of segregating phenotypes and on possible mechanisms of inheritance (i.e. the number and nature of the genetic factors which confer resistance (dominance and gene interactions are factors that influence the shape of the curve).

2.7 GENETICS OF RESISTANCE IN SPIDER MITES

In studies on resistance to pesticides in spider mites, most attention had been paid to resistance to organophosphate compounds. The spider mite species, Tetranychus urticae and Tetranychus pacificus, are the only ones that have been studied with regard to the genetics of resistance (Helle, 1965a).

The genetics of resistance has to date been studied indirectly through a system of reciprocal crosses and backcrosses. Reciprocal crosses of a resistant and a susceptible strain, in all cases, produced resistant F_1 offspring. This demonstrated that resistance was dominant and transmissible by both sexes. Since the backcrossing of these hybrid females (F_1) to normal males produced an F_2 apparently made up of two classes, a 1:1 ratio of resistant and susceptible individuals, several authors suggested that a single dominant character appeared to be responsible in particular strains of T.urticae and T.pacificus (egs. Taylor and Smith, 1956; Andres and Prout, 1960; Helle, 1962; Herne and Brown, 1969; Ballantyne, 1966).

On the other hand, a series of papers by Dittrich (1961; 1963a; 1963b; 1963c) concluded that resistance could be characterized by dominant semilethal factors and a major recessive Mendelian factor, as manifested in the Leverkusen-R strain (derived from susceptible

T.urticae through demeton selection) which Dittrich used in his study.

The series of studies on the Leverkusen strains were continued by McEnroe (1967). He made inbred lines of T.urticae, developed from a population plateaued by directed selection with methyl demeton. Toxicological responses of the different lines, using methyl demeton as the toxicant, showed the presence of hidden genetic variability and that a dominant major factor was not fixed in the Leverkusen-R strain.

In addition to the major factor contributing to resistance, several other minor factors may contribute towards total resistance. Among them are the cytoplasmic factors, which are one or more tolerance-increasing factors inherited only via the female parent (Overmeer, 1967). Cytoplasmic factors as contributing factors are evident when the reciprocal crosses show differences in toxicant response. Predetermination (the determination of gene-controlled characters by the maternal genotype prior to the fertilisation of the egg cell) was found to affect total resistance (Overmeer, 1967). His S x S^R and S^R x S crosses exhibited different degree of response (S^R is the homozygous backcross strain which is derived cytoplasmically from a susceptible mother and which obtained its resistance factor from a resistant male). Finally, a minor gene or genes, possibly modifiers, were often quoted as contributing factors towards resistance (Schulten, 1966; Helle, 1962; Overmeer and Harrison, 1969). If through repeated backcrossing of the hybrid (R x S) to susceptible males, there is an indication of a decrease in resistance in the homozygous backcross strain, minor genes are involved.

Whether the major factors responsible for many instances of organophosphorus resistance are located on different linkage groups,

on the same linkage group large distances apart, or as closely linked genes or alleles, is uncertain. However, in one instance, Schulten (1968), considered that the organophosphorus resistances in the SD strain (Helle, 1962) and the CP strain were due to different isoalleles.

2.3 BIOCHEMICAL GENETICS OF RESISTANCE

Even though numerous different types of chemicals have been used as insecticides, relatively little is known of their mode of action and, generally, even less is known of the biochemical resistance mechanisms that operate against them.

Although resistance mechanisms can vary, even from strain to strain in some cases, there tends to be a limited number of general resistance mechanisms. In insects, the only general mechanism so far detected is one of detoxification (Oppenoorth, 1958; March, 1958; Lipke and Kearns, 1958; Brown, 1961; March, 1960; Roulston, 1969; Kimura and Brown, 1964; Morello, 1964). In cattle ticks and spider mites, two general mechanisms have been found, viz., insensitive cholinesterase reaction (Smissaert, 1964; Voss and Matsumara, 1964; Roulston et al., 1969; Roulston, 1969; Roulston et al., 1968) and detoxification (Matcumara and Voss, 1964; Herne and Brown, 1969).

In this review, the author wishes to place more emphasis on the biochemical resistance mechanisms of the spider mites, especially with reference to the cholinesterase inhibiting insecticides which are more thoroughly studied than any other groups of insecticides. This group includes the organophophorus compounds and the carbamates, both of which are believed to kill arthropods by inhibiting the enzyme cholinesterase (ChE) with consequent disruption of nervous

activity caused by the accumulation of acetycholine at nerve endings.

Smissaert (1964) points out that in resistant two-spotted spider mites of the Leverkusen-R strain, the cholinesterase enzyme which is normally present in nerve tissue and which is the target for organophosphorus insecticide action, is changed or altered. The change is reflected in a lowered rate of inhibition of the cholinesterase.

By using the same strain, Voss and Matsumara (1964), verified Smissaert's findings but simultaneously they found that in another resistant strain (Blauvelt strain - organophosphate resistant), there is no unusual insensitivity of the cholinesterase enzyme. In this particular strain, however, parathion and malathion hydrolysis was found. They concluded that there are two different mechanisms of resistance in these two independent resistant strains. The mechanism discovered by Smissaert also occur in the 'Baardse' strain of T.urticae (Helle, 1966), in a strain of T.pacificus McGregor (Helle, 1966), and in three New Zealand strains of T.urticae (Ballantyne and Harrison, 1967).

Cross-resistance of organophosphate resistant houseflies to carbamates had been shown to occur (Forgash and Hansens, 1959; Georghiou et al., 1961). If a similar mode of action of organophosphorus compounds occur in mites as in insects, it would be reasonable to assume that there may be some degree of cross resistance between organophosphates and carbamates in mites. This is substantiated further by the organophosphorus resistant Blauvelt strain which showed absence of cross resistance against carbamates (Voss and Matsumara, 1964). In this strain, resistance to organophosphorus was due to detoxification, which would probably explain the absence of cross-resistance.

Ballantyne and Smissaert (personal communication) in a study of response to formetanate (a carbamate) using organophosphorus resistant strains called by Schulten (1968) OP (cross resistant to carbamate CPMC) and SP (Helle, 1962), found that OP showed negatively correlated resistance (x 9.1) while SP only a slight tolerance (x 2), when compared with the Leverkusen normal strain. Probably this can be explained on the ground that the organophosphate resistance in the OP and SP strains is due to different alleles (Schulten, 1968). Thus, it is reasonable to assume that the different alleles which provide the small difference in organophosphorus response in SP and OP, give rise to a larger difference in the response to formetanate. Furthermore, the fact that the OP shows cross resistance to CPMC but negatively correlated resistance to formetanate, points out that allelic difference can also give rise to different response to different carbamates.

In conclusion therefore, different mutations in the cholinesterase enzyme gene can produce structural effects which result in different inhibition rates to a range of chemical types.

2.9 BASIC GENETICS OF SPIDER MITES

Schrader (1923) distinguished cytogenetically two types of eggs and larvae in T.urticae, viz., with three or with six chromosomes. He also observed that the nuclei of the spermatogonia contain three, and those of the oogonia six chromosomes. He concludes that the males are haploid and develop from unfertilised eggs whereas females are diploid and proceed from fertilised eggs. Thus virgin females produce only haploid eggs which will develop into males. Mated females deposit a mixture of unfertilized and fertilized eggs

which result in haploid males and diploid females respectively. Fertilized females can produce offspring (females only) androgenetically if virgin females are exposed to a certain level of X-radiation before fertilisation (Overmeer et al., 1972).

Generative or haploid parthenogenesis, as this form of reproduction is called, is a salient feature of spider mites. Generative parthenogenesis is always facultative and arrhenotokous (male-producing and seems to be the only type of reproduction in the subfamily Tetranychidae (Pritchard and Baker, 1955; Helle and Bolland, 1967). It appears that a normal meiosis takes place during oogenesis while in spermatogenesis only normal mitotic divisions occur (Suomalainen, 1962).

Since mated and unmated females of T.urticae can produce male offspring , one wonders whether the sons of the former are really impaternate. Crosses with marker genes have demonstrated unmistakably, however, that the male offspring always shows the genotype of the mother only (van Zon and Helle, 1966). Under a situation where the females are produced androgenetically, the haploid males derived from the androgenic mothers will not be the genotype of the mother (Overmeer et al., 1972).

Since parent males have no genetic influence on the present generation males, the ratios of phenotypes among haploid offspring will reflect the segregation of genes that occurs during meiosis in the female parent. Moreover, because arrhenotoky allows recessive genes to be expressed immediately in the haploid males, an unfavourable mutated gene will be eliminated more quickly upon exposure to selection than it would in the case of a diploid species. Consequently species with haploid males will tend to be more homozygous than those animals with diploid males. On the other hand, a favourably

mutated gene will have a greater chance to spread and establish than those species with diploid males. From an evolutionary viewpoint, such a species should be less plastic than its normal diploid counterpart (Suomalainen, 1962).

To date, various visible mutants (aberrations in the pigmentation of the haemolymph and/or eyes) have been found (Ballantyne, 1969; Helle, 1969; Helle and Zon Van, 1970; Van Zon and Helle, 1966) and these are useful for establishing a visible linkage map. Such linkage data would be of tremendous value to a genetic analysis of resistance (Oppenoorth, 1965) since present measurements by dosage-mortality tests are restrictive. Since spider mites have only a small number of chromosomes, a few markers would be adequate for the localisation of the resistance gene. Markers might also allow a more positive identification of smaller factors involved in a polygenic system. So far, markers have been isolated for T.pacificus, T.urticae and T.neocaledonius (Schulten, 1968).

In spite of the many advantageous properties of spider mites as research material, such as rapid development rate, high reproductive potential, male haploidy, ease in rearing, small number of chromosomes and short life cycle, they are disadvantageous when compared to the arrhenotokous Hymenoptera (Habrobracon and Mormoniella in particular) because of the small number of suitable markers available.

2.10 INCOMPATIBILITY IN T.URTICAE

In T.urticae, there is frequent occurrence of reproductive barriers between different populations, although they are morphologically indistinguishable. These barriers are seldom complete and in

many cases, they cause partial hybrid sterility (Boudreaux, 1963; Helle and Pieterse, 1965; Overmeer, 1967). The degree of genetic incompatibility most commonly depends upon the strains involved (Keh, 1952; Boudreaux, 1956; Parr and Hussey, 1960; Helle and Van de Bund, 1962). The phenomenon of genetic incompatibility, besides occurring in the crosses between different geographical and morphological strains, also occur between populations of spider mites collected within a limited horticultural area (Helle and Pieterse, 1965), and between substocks derived from a laboratory stock (Schulten, 1968).

It is not unusual to find qualitative differences between reciprocal crosses. Overmeer (1967; 1965a; 1965b) reported situations of genetic incompatibilites in which the percentage of unhatched eggs in the F_1 and offsprings obtained from hybrids of reciprocal crosses between two strains differed considerably. This suggests that the cytoplasm can play a role in the incompatibility phenomenon. Helle and Overmeer (1973) suggested that orientation of bivalents which is dependent on maternally intrinsic properties during meiosis in the structural hybrid female might explain the differences in the degree of hybrid sterility resulting from reciprocal crosses.

The causes of different incompatibility patterns of two-spotted mite populations are not fully understood. The fact that the F_1 eggs are not affected but their offspring and the lethality in the F_2 haploid eggs is greater than in the diploid eggs, suggests that chromosomal alterations (chromosomal rearrangements) are responsible for genetic incompatibility. The chromosomal rearrangements (translocations, inversions) can cause abnormal pairing (failure of pairing and disjunction) during meiosis and as a result give rise to deficiencies and duplications (aneuploidy) of genetic material in the F_2 eggs (Boudreaux, 1963; Helle and Pieterse, 1965). In cases where

there is an exceptionally high percentage of inviable diploid eggs in the F_1, the hybrid itself is affected apparently as a result of the combination of two different gene complements in one genome (Helle and Pieterse, 1965).

Helle (quote from Schulten, 1968) found that different sub-stocks derived from a laboratory stock developed incompatibility barriers between each other and with the mother stock. It also appears that genetic incompatability fluctuates, widely with time (Ballantyne, 1969). Both these observations suggest that chromosomal polymorphism is a more or less regular feature of spider mites. It would be difficult to detect the existence of chromosomal polymorphism in spider mites by means of the usual cytological techniques because of the small size of the chromosomes.

The degree of incompatibility due to cytoplasmic factors are in general comparatively small in comparison to the incompatibility caused by the chromosomes, Thus, cytoplasmic sterility is perhaps secondary to genetic incompatibility.

The occurrence of genetic incompatibility phenomena among the two-spotted spider mites had led many workers to use it as a criterion for differentiating the species into various strains (Keh, 1952; Boudreaux, 1956; Parr and Hussey, 1960, 1961; Helle and Van de Bund, 1962). This phenomenon is not confined only to mites from different geographic origins (Boudreaux, 1963). Sometimes, adjacent populations exhibit nearly complete genetic incompatibility whereas other populations from widely diverse geographic areas show moderate incompatibility (Helle and Pieterse, 1965). The substantial interpopulational divergence among adjacent populations (eg. glasshouses) provide an interesting case for study since it may provide a means for genetic control. The occurrence of incompatibility among these glasshouse

populations, in spite of regular interpopulational exchange by wind, plant materials and phoresis, suggest that the genetic diversity is a projection of the accelerated differentiation of germinal changes in spider mites due to their reproductive rate and male haploidy (Helle and Pieterse, 1965). Favourable mutations (and also chromosome alterations) in spider mites are less liable to dispersive forces and more prone to fixation as compared to animals with diploid sexes (Helle, 1965). As a result, the chance of extinction for favourable mutations is much slighter, and consequently results in a greater mutational yield and subsequent genetic divergence (Helle and Pieterse, 1965). The holokinetic structure of spider mite chromosomes throws light upon the above concept. It means that chromosomal rearrangements such as reciprocal translocations and inversions can be produced without the formation of acentric and dicentric products. If such translocations or inversions are linked with some selective advantage, they become rapidly established in balanced conditions; that is, they remain in the heterozygous state due to lethality of the homozygous combination as do balanced lethal rearrangements in Drosophila. It is necessary to assume of course that they are hemizygous (male) viable. Populations containing rearrangements could retain a great deal of genetic variation hidden in the heterozygous state, especially if the expression of such variation was sex-limited (expressed only in females). All this explains, on the one hand, the observed genetic incompatibilities explained above, and, on the other hand, the enormous wealth of genetic potential that is observed in the course of their evolution, by the rapidity with which a large number of small populations can break away and become genetically distinct from a single parent population.

Besides resulting from the capacity to retain hidden variability, such evolutionary potential is augmented by a high mutation rate averaging 2×10^{-4} per gene (Helle and van Zon, 1967) and by a strong tendency in nature to inbreed (mother x son)(Helle and Overmeer, 1973).

The effect has been found in T.urticae by van Zon and Overmeer (1972) who showed that X-ray treatment followed by mother x son inbreedings gave rise to new races which showed genetic incompatibilities. Apparently, the chromosome mutations are both easily induced (because of the holokinetic structure of the chromosomes) and established (because of the haplo-diploid reproduction).

2.11 SEX-RATIO IN T.URTICAE

In T.urticae, it appears that for a particular population a certain sex-ratio which fluctuates around a mean under normal circumstances of rearing is usually found. The mean sex-ratio can differ from strain to strain (Gasser, 1951) but in general, the number of females surpasses the number of males.

Several workers have tried to explain the variable sex-ratio in the arrhenotokous spider mites (Fisher, 1930; Hamilton, 1967; Hussey and Parr, 1963; Schrader, 1923; Boudreaux, 1963). Early works by Boudreaux (1963) seemed to indicate that there was no normal sex-ratio for spider mites and it was shown that copulatory accidents (interruption of coitus and variable sperm load) could cause females to produce varying frequencies of females. However, if one is to argue from the point that the mating capacity and the sperm supply of one male is sufficient to fertilize several dozen females, it is not at all convincing that mating or sperm supply will influence the sex-ratio in nature despite the occurrence of a higher female:male

ratio in most populations. In support of this is work done by Overmeer and Harrison (1969) and Mitchell (1972).

Overmeer and Harrison (1969) indicated that differences in the sex-ratio among various different homogeneous or heterogeneous populations (with regard to differences in sex-ratio) appeared to be heritable. By inbreeding a number of lines of T.urticae using sib-mating, they showed that the sex-ratio was controlled by the phenotype of the mother. The same result was obtained by Mitchell (1972) using a different strain but he also found that the maternal effect seemed to be limited to a single generation. Overmeer and Harrison (1969) explained the maternal effect as due to a cytoplasmic system or a sex-linked trait. Recently, Mitchell (1972) threw more light on the situation by using inbred lines that differed in the sex-ratio (i.e. selections for high and low sex-ratios - a system different from the haphazard selections adopted by Overmeer and Harrison). He agreed with Overmeer and Harrison about the presence of genetic variability for sex-ratios in a population and put forward the concept that the variability of the sex-ratio in nature would appear to be a genetic polymorphism and the genetic control of sex-ratio is polygenic. Observations that 1) lines could be established that did not diverge in sex-ratio under directed selection, providing evidence for sex-ratio to be under the control of a few locii, and, 2) there was greater variation in the offspring of females from the intermediate range for sex-ratios than from either extreme, providing evidence for heterozygosity at autosomal locii, seemed to rule out the possibility of a hemizygous sex-linked trait mentioned by Overmeer and Harrison (1969).

Since genetic polymorphism and polygenic control are features of sex-ratio in the spider mites, it is also reasonable to

assume that the sex-ratio is determined by selective pressures operating over several generations (Mitchell, 1972). As in the case of the resistance genes, when the alleles for sex-ratios are present at intermediate frequencies, the population can easily respond to natural selection over a very few generations. This concept can probably explain the adaptive role of sex-ratio in determining the success of dispersing mites (Overmeer and Harrison, 1969), and in responding to different selective cultural regimes (Mitchell, 1972).

Thus, it is important to determine the nature of the selective forces that fix the sex-ratio; a long term response study will be more valuable than a simple phenotypic response.

2.12 SEX-DETERMINATION IN T.URTICAE

Sex-determination in arrhenotokous species is difficult to explain but some hypotheses, which are yet to be proven, have been developed to approach the genetics of sex determination.

a) Goldschmidt's theory (1934) Chromosomes bear genes for femaleness while the cytoplasm would carry maleness elements. According to this theory, one set of genes for femaleness would not be enough to outweigh factors for maleness and therefore, haploids would be males. Two sets of chromosomes, however, would be sufficient to dominate cytoplasmic maleness; so diploids would be females.

b) Whiting's theory of multiple-alleles in Habrobracon (1943) Females are heterozygous for one pair of a series of sex-alleles, for example, X_1X_2, X_3X_4, X_4X_7, etc., while males are haploid, for example, X_1, X_2, X_3, X_4, etc.. This theory apparently would not apply

to spider mites, since mother x son crosses result neither in lethal eggs nor in diploid males, although it is possible that some mechanism operates at the time of, or following, fertilisation, such as selective syngamy or heteropycnosis, to preferentially eliminate or inactivate one set (maternal or paternal) of chromosomes. Such mechanisms are known to operate in some scale insects and in the mealy bugs (Markert and Ursprung, 1971).

c) Cunha and Kerr's theory (1957) A series of male-tendency genes, m, and a series of female-tendency genes, f, are considered to be scattered over several chromosomes. The effects of m would be the same in hemizygotes as in diploids and the effect of all m's may be represented as M in both kinds of individuals. The effect of f's would be accumulative and therefore would be F in the haploids and 2F in the diploids. So, sex would be determined by the the equations 2F>M = female and M>F = male.

d) Helle's theory (1968) Helle, from a genetic study of the sex-limited trait, diapause, in spider mites, suggested that sex-determination may depend on either cytoplasmic or chromosomal factors. Regarding the chromosomal factors, he postulated the existence of sex-chromosomes, and that females could be heterogametic, assuming polarised meiosis with selective syngamy. This theory does not seem to be attractive, as androgenetic females, homozygous for the paternal recessive marker, can be obtained as a result of the combination of two identical haploid male chromosome complements, when females are exposed to X-radiation before fertilisation by males carrying a recessive marker (Overmeer et al., 1972).

Earlier (van Eyndhoven and Helle, 1966), it was reported that

as a result of intensive mother x son inbreeding of a large number of lines, all beginning from a single female and continuing for several generations, intersexual forms were produced. These forms appeared in one line only, and included what were described as giant diploid males and haploid females. Possibly these reflect the breakdown by recombination of a sex determining segment that is normally maintained in a balanced heterozygous state by a chromosomal rearrangement (as described earlier).

2.13 METHODS OF GENETIC CONTROL

In the control of insects, approaches involving the use of insecticides are coming under increasing attack by environmentalists because of their ecological side-effects. To replace the use of insecticides, considerations of the use of sterile or genetically incompatible insects, of sex or other pheromones, of attractants in association with traps or poison baits, of hormones or hormone analogue, of physical factors such as sound, shape, colour, and of factors which modify chromosomal segregation, have been made. In this review, the first and the last methods are elaborated; basically these methods constitute genetic control, which has the advantage that its immediate effects are restricted to the species or strains and there are few ecological side-effects beyond those caused by elimination of the pest species itself.

Various techniques of genetic control have been developed. Some have already been applied in the field while others are at the experimental stage.

a) Hybrid sterility Hybrid sterility results from appropriate crosses between various populations of some species-

complexes which give rise to infertile hybrids. This method had been used to control Anopheles gambiae (Wright and Pal, 1967) and it had been suggested that the tzetze fly, Glossina swynnertoni, might be eradicated by introducing Glossina moisifans into its territory. These species mate with each other but only a few offspring are obtained and these are mostly sterile.

b) Cytoplasmic incompatibility In this situation, a cytoplasmic factor is involved. In some species of mosquitoes, crosses in one direction between certain populations give no offspring or a very low number of offspring; the reason is that the cytoplasmic factor kills or eliminates the incompatible sperm after entry into the egg, but before fusion with the egg nucleus. If an embryo develops,it is haploid and non-viable. The reciprocal cross is usually normal. Laven (1967) made use of this phenomenon in Culex pipens in which 20 different crossing types are known. He used a strain of Culex pipens fatigans with cytoplasm from a strain from Paris and genome from a Californian strain. Males of this synthetic strain were shown to be incompatible with females in the wild populations around Rangoon.

c) Sterile males Some factors may be present in the males which when crossed to the females will produce low fertility or sterile offspring. For example, attached $\overline{XY}$ chromosomes are available in Drosophila and males of this constitution, when mated with normal females, produce all sterile male offspring. Sterile factors can also be produced artificially by X or χ radiation. Examples of the use of this technique for control are several: the screw-worm fly, Cochliomyia hominivorax in Curacao and Florida;

the oriental fruit fly, Dacus dorsalis in Guam; and the melon-fly, Dacus cucurbitae. Irradiated males are released into pest infested areas. Chemosterilants, still under experimentation, may also serve as a technique for sterilisation in the future.

d) Introduction of deleterious chromosomes The principle of this method involves the introduction of a number of genes, capable of causing lethality, lowered viability or sterility, into the population. The genes must be recessive and can be introduced via heterozygous males. Repeated releases are often necessary to compensate for selection against the chromosomes. The appropriate genes very often exist at low frequency in natural populations and can be isolated by inbreeding (Wright and Pal, 1967), or induced by X-rays.

A similar method is the introduction of chromosomes carrying reciprocal translocations. Translocation heterozygotes produce zygotes with a lowered viability rate. First suggested by Serebrovsky (1963), their use was experimented by Curtis (1968) with tzetze flies, Glossina species, using cages. He found that half the gametes (theoretically $\frac{2}{3}$ are expected) from the translocation heterozygotes had a chromosomal deficiency or a duplication and produced inviable organisms. Introduction of males homozygous for a translocation will result in the production of many progeny which are heterozygous for the translocation and therefore only partially fertile. Furthermore, translocation heterozygotes will also appear in subsequent generations, thus resulting in a lowered productivity of the population over a considerable period.

e) Factors which modify chromosomal segregation Meiotic

drive is a situation where factors on one chromosome cause it to become incorporated into more gametes as compared to its homologue. Meiotic drive causes chromosomes to spread through a population even though they may carry deleterious genes. If such a factor could be linked to a recessive deleterious gene such as a lethal or a sterility factor, it could cause these genes to spread through the population, which would be eradicated eventually. There is a factor in Aedes aegypti which causes populations to become about 90% male. It is known as "distorter" and is probably an example of a Y chromosome with meiotic drive (Craig and Hickey, 1967).

The general survey of the available techniques in the genetic control of insects may provide some information towards finding a form of genetic control for the two-spotted spider mite, T.urticae. Before any techniques can be recommended, it is essential to know the genetic background of the various strains of T.urticae.

2.14 GENETIC INCOMPATIBILITY AS A POSSIBLE MEANS OF CONTROL IN SPIDER MITES

Since T.urticae is not compatible with other species of spider mites and there is variation in the degree of genetic incompatibility within the species, this phenomenon could be exploited as a means of genetic control (Helle, 1968b).

On a similar line, instead of using naturally occurring incompatible populations, the males of T.urticae can be made incompatible by means of radiation (van Zon and Overmeer, 1972). Radiation induces a considerable number of chromosome mutations which can be made homozygous by following a selection scheme. Through outcrossing with the original mother colony and among strains, it was

shown that in some cases induction of two different chromosome mutations can result in nearly complete reproductive incompatibility.

Genetic control using males of artificially induced chromosomal races can be useful in more or less isolated areas of limited size, like the glasshouses. The introduction of such males which are resistant to a particular toxicant could be appropriate after an insecticidal spray, so that the ratio of introduced males to host males is very high. In a situation such as this, it is necessary that the reproductive incompatibility between the released males and the resistant mites be 100%, so as to avoid the introduction of resistance genes into the glasshouse, along with chromosomal rearrangements which may render subsequent releases of males ineffective van Zon and Overmeer, 1972).

CHAPTER 3

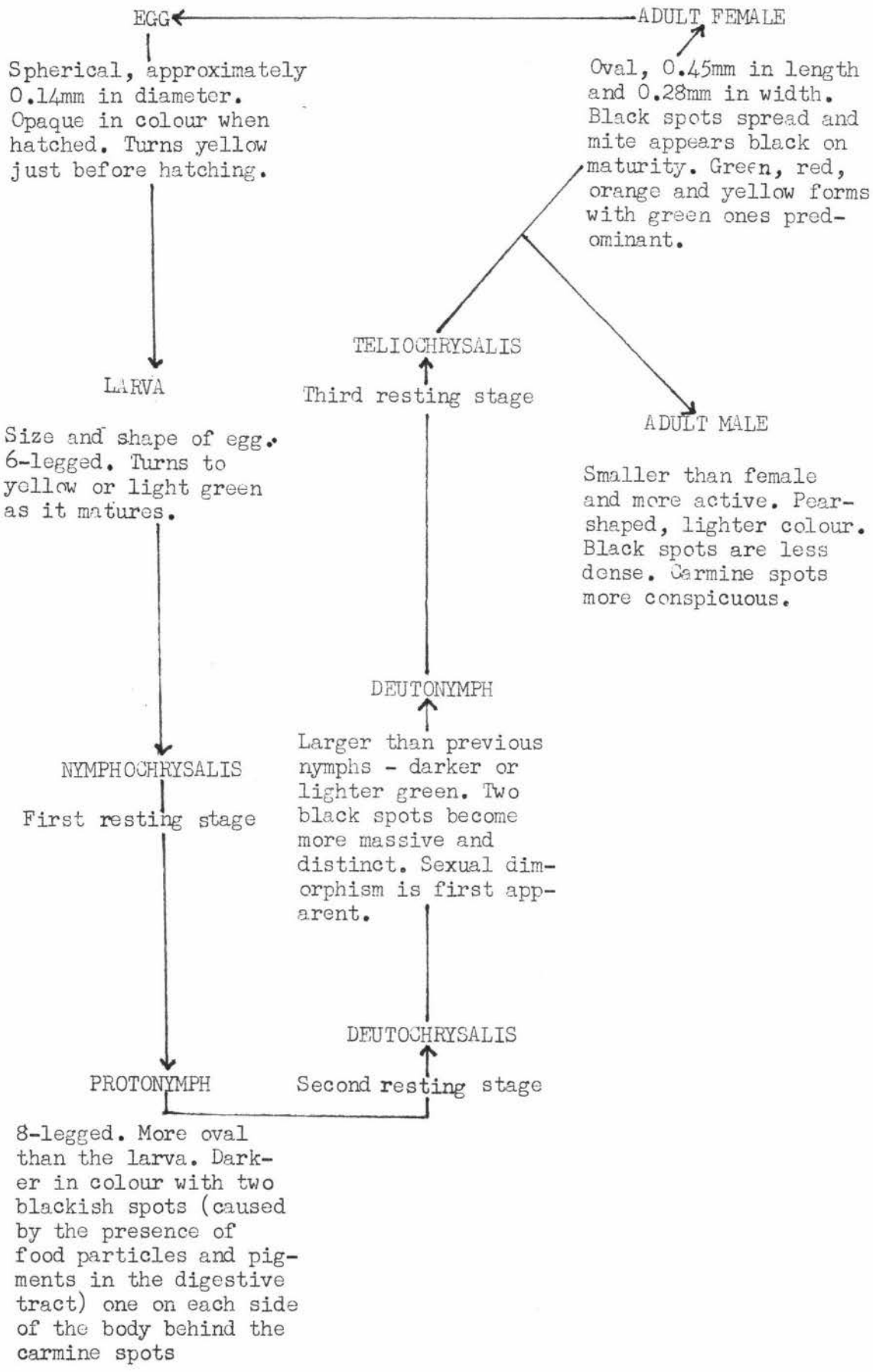
LIFE HISTORY AND BEHAVIOUR OF MITES

Since a genetical study of spider mites involves a great deal of crossing and breeding, it is essential to know the biology and behaviour of the organism. A considerable number of authors have dealt at length with these aspects (Cagle, 1949; Harrison and Smith, 1960; Helle, 1962; Hussey and Parr, 1963). Without going into great detail, it is here intended to briefly present the various facets of the biology and behaviour of the two-spotted spider mite, T.urticae, which are known up to the present date, with special emphasis on those which bear relevance to the present study. Most of the information is summarized from the reviews of various authors (Boudreaux, 1963; Ballantyne, 1966; Unwin, 1971; Helle, 1962; Helle and Overmeer, 1973).

3.1 LIFE CYCLE OF T.URTICAE

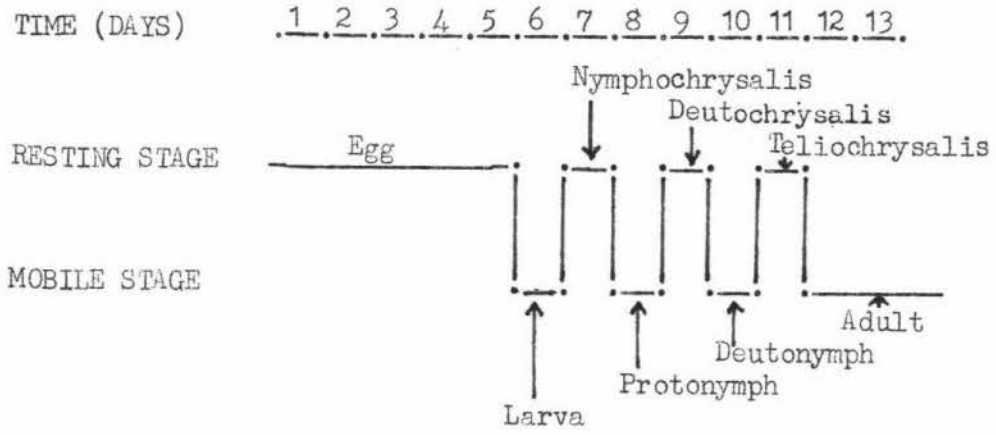
The life cycle of two-spotted spider mites consists of alternating active and quiescent stages: Egg → Larva → Nymphochrysalis → Protonymph → Deutochrysalis → Deutonymph → Teliochrysalis → Adult. The detail descriptions of each stage are presented in the annotated diagram (see page 43).

The time for development from egg to adult averages about 10-12 days at 25°C. Embryonic development (incubation) and post-embyronic development each requires 5-6 days. The time period also varies with different strains but the difference is not more than



ANNOTATED LIFE-CYCLE OF T.URTICAE

three days. The time taken for each stage to develop at 25°C is given below:



Stages of Development of T.urticae at 25°C

From a normal mixture of haploid and diploid eggs, the adult haploid males appear to emerge first. The males usually hover over the female teliochrysalids before any of the latter have emerged. Attraction of males to females is regarded as being due to a pheromone (Cone et al., 1971). Immediately after emergence, the females are fertilized. In copulation, the male crawls bebeath the female from behind, arching the pointed tip of the abdomen to introduce the aedeagus into the female genital opening. Copulation can occur several times with different males but generally only the first mating is successful and necessary, permitting a female to lay diploid eggs for the rest of her life. Many of the eggs remain unfertilized since males always appear among her progeny (Helle, 1967).

Usually, after copulation, females tend to move to new leaves. Migration of this kind (not due to overcrowding or shortage of food) occurs only during the pre-oviposition period (less than one day up to about 8 days). With the strains used in the present study, the trend appears to be between 1-2 days in the normal environment. The

period is variable between strains. There is a clear indication that the mating preference of T.urticae females for males of their own strain (geographical isolate) does occur regardless of whether males of other strains are immediately available (Smith et al., 1969; Dieleman and Overmeer, 1973).

3.2 ENVIRONMENTAL FACTORS AFFECTING DEVELOPMENT AND VIGOUR

Mite outbreaks are favoured by hot, dry weather and are checked by high humidity (Boudreaux, 1963; Bognar, 1960). The optimum temperature for egg production seems to be 30°C. Egg laying is also favoured by a drier surrounding. 30°C is also the optimum temperature for maximum rate of development (Bravenboer, 1959). The two-spotted spider mites seem unable to develop successfully at relative humidities above 80% or below 25% (Boudreaux, 1963). A figure of 30-50% R.H. can be suggested as optimum (Boudreaux, 1963).

Two-spotted spider mites are greatly affected by the nutritional status of their host-plant substrates, as determined by normal or abnormal seasonal changes in plant physiology, by fertilizer practices, or by changes from other causes in the chemical composition of the leaf tissue. Generally, good crop husbrandry encourages maximum populations of two-spotted spider mites. The author's observations have been that the greener the leaves (i.e more nitrogen) and firmer the leaf tissues (i.e. more calcium), the more vigorous will be the mites which feed on them. This is in agreement with experiments which show that any crop management practice, chemical or otherwise, which significantly affects leaf nitrogen, will have corresponding effects on the plant feeding mite population, although the biochemical basis of such effects cannot be

specified.

3.3 SEASONAL LIFE CYCLE AND BEHAVIOUR OF MITES

In early spring, the surviving overwintering females of T.urticae seek out and lay eggs on the new season's foliage, usually on the undersides of susceptible host plants, in response to a negatively geotactic orientation (Foott, 1964). Various authors report egg production per female in the range of 30-118 eggs at a rate of 2-12 per day (Arcanin, 1958; Bohm, 1961; Gasser, 1951; Rambier, 1958). Several broods are produced over the summer. As the season progresses, the mite colonies rapidly reach a state of overcrowding, and dispersal to uncolonized sites on the same plant and to other plants occur fairly rapidly. Mites in the overcrowded state form rope-like structures which are threads of cotton with mites attached to them. These can be several feet in length and allow mites to be dispersed to other leaves, plants and to the ground. In the field, mites are normally dispersed by wind which is capable of dispersing mites at all stages. Within glasshouses, dispersal may be achieved by crawling, falling or air current (Hussey and Parr, 1963).

With the approach of winter, the mite colonies cease feeding and reproducing and undergo a number of changes. All, or most of the males die, and the overwintering forms are usually all adult females (Boudreaux, 1963; Gasser, 1951). In the higher latitudes, where long, extremely cold winters are usual, the mites overwinter in a state of true diapause. In milder districts and in lower latitudes, it seems clear that the overwintering form is in a quiescent or inactive stage rather than in true diapause. Mites in true diapause require a minimum period of chilling before feeding and oviposition

can be resumed (Lees, 1953; van der Bund and Helle, 1960). Photoperiod, temperature and availability of food are each important and interdependent factors in provoking the change from summer to winter body form (Bondarenko, 1958; Bengston, 1965; Gasser, 1953).

The winter females, by a geotactic response, usually descend from the host plants in search of suitable hibernation sites. Favoured locations are beneath bark scales on the lower trunk of deciduous hosts, in cracked or damaged bark, under debris in crotches and under dead leaves and rubbish on the soil surface close to the butts of trees (Jamin, 1971). Burrowing under clods or into loose soil, particularly near the junction of root-stock and soil is usual. In milder climates, where true diapause does not occur, some or all of the mites migrate to altermative host plants where feeding and possibly reproduction may occur at a more or less reduced rate.

In spring, with rising temperatures and necessary daylengths, the winter colonies become active, commence feeding and begin to change colour from red to light green. With the coming of winter, the colour change is reversed, that is, from green to carmine, bright red or orange. In a study of T.cinnabarinus (Veerman, 1970) and T.pacificus (Veerman, 1972), Veerman found that the pigmentation of spider mites is probably due to the presence of esters of keto-carotenoids (3-hydroxy-4-keto-β-carotene, 3-hydroxy-4, 4'-diketo-β carotene, and astaxanthin) which are derived from β-carotene taken up by the mites from the plants. Since pigment analyses demonstrate that the same range of carotenoids occurs in both wild type females of T.cinnabarinus and T.pacificus, Veerman explained that the colour difference is probably due to a quantitative difference in pigment composition, or the presence of different carotenoid-protein complexes

in both species. The same explanation is probably applicable to the colour change in T.urticae during winter and spring.

CHAPTER 4

MATERIALS AND METHODS

4.1 THE STRAINS OF SPIDER MITES

For the present study, the following strains (colonies) of T.urticae are used:

1. Palmerston North Normal (PN) A normal (susceptible) colony collected in Palmerston North, North Island, off garden beans.

2. Havelock North Resistant (HNR) A colony collected from Havelock North, North Island.

3. Levin Resistant (SP) A colony collected from Levin, North Island, off Chrysanthemums.

4. Levin Horticultural Research Station Resistant (LHRS) A colony collected from Levin Horticultural Research Station.

5. Ettrick Resistant (ETTR) A colony collected from Ettrick, South Island, off strawberries.

6. Lines Resistant (LINES) A colony collected from strawberry plants in Levin from the property of Mr. Lines.

4.2 MAINTAINANCE, ISOLATION AND DISPOSAL OF SPIDER MITES

The six strains of T.urticae mentioned above are reared on

dwarf bean leaf cultures (Phaseolus vulgaris) in a thermostatically controlled room of 25°C (24°C - 27°C).

Each leaf culture consists of a primary bean leaf pressed on a ball of cotton-wool which has been dipped in a nutrient solution (which contains 21.3 gm of KNO_3, 12.7 gm of $MgSO_4.7H_2O$, 14.1 gm of KH_2PO_4, 0.5 gm of $(NH_4)_2SO_4$ and 18.6 gm of NH_4NO_3 in 2,500 ml of water). The cotton-wool, contained in a perforated aluminium foil dish, is placed on a plastic tray (Plate 1) which contains some water to keep the cotton-wool moist and the leaf turgid at all times. Whenever the water level is getting low, further water is added.

Since the room is totally closed from sunlight, continous artificial lighting is employed to provide long day environment which provides optimum development for mites (Plate 2). Healthy green leaves are necessary for the culturing of mites (light intensity and quality were found to be important factors in the growing of beans).

To keep a continual and essential supply of bean leaves, primary leaves of beans at various stages are grown. This is achieved by sowing bean seeds at intervals of 3-4 days. For germination of seeds, a peat and vermiculite mixture is used. By trial and error, it is found that application of fertilisers (N,P,K) increase the growing rate and the quality of bean leaves.

As the mites are extremely light animals (5-25ug per organism; Ballantyne, 1966), and can be carried on air currents and clothing especially as a consequence of spinning, the isolation of various colonies and crosses is an important consideration. Movement of individuals from one colony to another can completely alter the original genetical constitution of the strains and to overcome this, several precautions are taken. First, adequate water is added to

plastic trays so that the cotton-wool will be moist all the time and will stop mites from migrating from one aluminium foil dish to another. Second, the marginal edge of each leaf culture is further isolated by a layer of raised cotton-wool. Third, the water in each plastic tray serves as a barrier to the various aluminium foil dishes. Fourth, the culture room is kept draught free.

After 2-4 weeks, new cultures are made to replace old ones. A piece of old leaf of each strain is introduced onto the new leaf culture by means of a hand clipper and a pair of scissor. Mites, after finding that the nutrients are becoming depleted in the old piece, migrate to the new leaf in search of new colonising grounds to lay eggs. As contamination of mites can occur by way of contaminated equipment, precaution in the form of flushing the tools in running tap water are made before and after the introduction of old culture into the new one.

Old cultures are discarded with appropriate precautions taken to ensure that remaining mites are dead.

4.3 TOXICOLOGICAL TESTING

The toxicological method used in this study is the slide-dip method, which was first used by Voss (1961)(Plate 3). A slide consists of an object glass on which a piece of double-sided sticking tape is pressed. On this tape, 30-35 mites are stuck dorsally with the aid of a small brush which is wetted at the tip. The slides are checked 4-6 hours later and those mites which have died or injured owing to handling are removed by means of a needle. Then the slides are dipped into the desired acaricide and concentration for 10 seconds. Immediately after dipping, the slides are dried with small pieces of

Plate 1 Leaf cultures on a plastic tray.

Plate 2 Trays of leaf cultures kept in long daylength environment.

Plate 3 Glass slides with double-sticking tapes containing mites placed dorsally.

filter paper and placed in an incubator at 25°C. Mortality is determined after 24 hours. A mite which moves its leg after being touched with a fine brush is considered to be alive.

For toxicological testing by slide-dipping, age uniformity of mites is an important factor for obtaining reliable results. To achieve this, eggs are laid in waves by fertilised females of desired strains or crosses on several leaf cultures, each containing 50-60 females which laid from 600-1000 eggs. Usually two egg waves are required, each consisting of 2-3 days of egg laying. The second wave of eggs serves as a precaution in case there is some misfortune with the first wave (eg. non-uniform response which requires testing to be repeated, incorrect estimation of concentration range or inadequate number of mites). Normally, one egg wave is adequate to allow testing of approximately 7-12 conventrations involving 630-1080 mites. Just before the mites develop into the adult stages the teliochrysalids are transferred to fresh new cultures where they feed on fresh leaves for 2-3 days. Mites obtained in this way are not only uniform but healthy, the later condition being an important criterion in obtaining uniformity of response. To obtain DM-lines which may later be used for comparative purposes, a more or less standardised level of mite vigour has to be emphasied

4.4 SELECTION TECHNIQUE (LEAF-DIP)

A number of female teliochrysalids are collected and placed on a detached leaf culture. After their emergence, the mites are transferred to another leaf culture which had been dipped in a toxicant of known concentration (a discriminatory dosage which completely kills the normal population but spares the resistant popula-

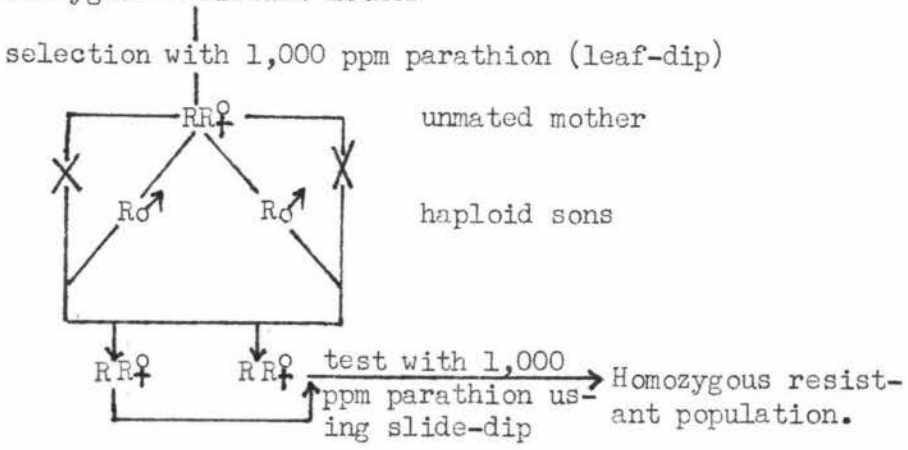
tion). Surviving mites (after 1-2 days of toxicant exposure) are transferred to an untreated leaf culture where males of the desired strain for crossing are introduced. The fertilised females, after the removal of males, are allowed to lay egg waves as described before (4.3).

4.5 MOTHER x SON INBREEDING

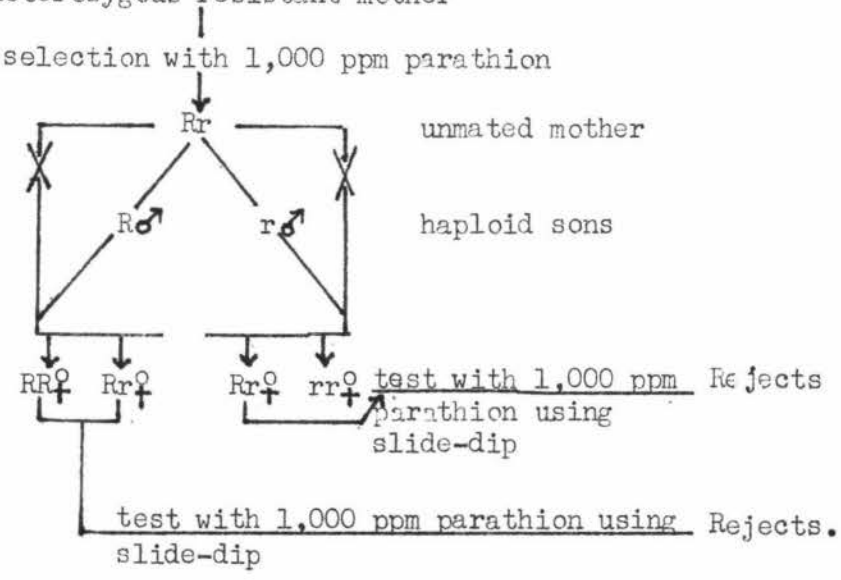
The mother x son inbreeding is performed as follows: About ten unmated females which survived from a 1-2 days exposure to a discriminatory dosage using the leaf-dip technique are isolated singly on leaf cultures. The haploid eggs are allowed to develop to adults such that mating between sons and mothers can take place. The female progeny from the mother x son cross are tested with a discriminatory dosage by using the slide-dip. The populations which give a near 100% survival are selected and bred for further study. Theoretically, the haploid males from the virgin mothers should be tested for full resistance to determine the genotype of the latter before any inbreeding is proceeded. This is due to the fact that in the fertilisation of spider mites, only one mating is usually effective (Helle, 1967) and in the case of an inbreeding starting from a virgin female heterozygous for resistance, there is a 0.5 probability that the mother will be fertilized by a son bearing the R genotype, resulting in the production of RR and Rr individuals. Rr females are heterozygous for resistance and because of the dominant character of the resistance factor, they may be mistaken for RR homozygotes. Despite the attractiveness of the latter procedure, the testing of haploid sons is not possible for two reasons. They are more liable to injuries caused by handling than the females and tend to wander off into the cotton-wool.

Schematically, the mother x son inbreeding is presented below:

A. Homozygous resistant mother



B. Heterozygous resistant mother



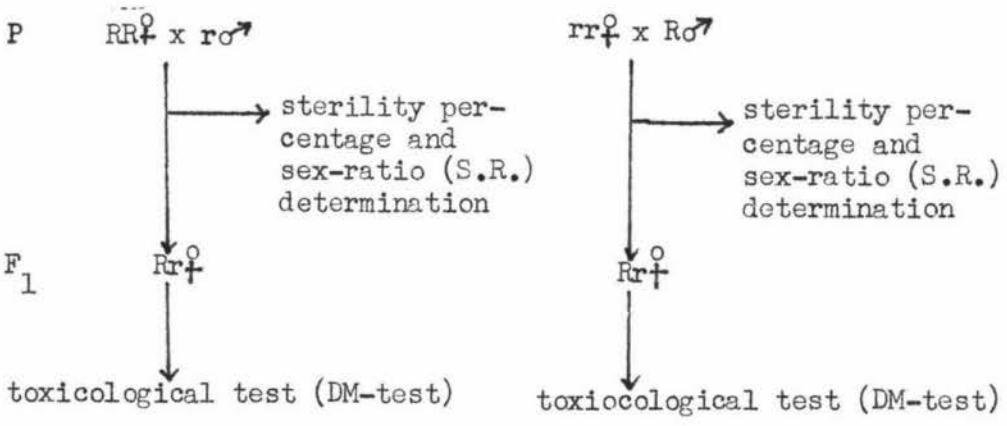
NOTE: Selection using 1,000 ppm of parathion is regarded as vigorous and probably mites showing heterozygosity for resistance will be eliminated. The ones that survive are probably those that are homozygous for resistance. However, the possibility of heterozygous resistant mites being mistaken for homozygous ones, is not totally excluded . The homozygosity of inbred populations, as a result, cannot be confirmed until the DM-lines are obtained.

4.6 MITE CROSSING AND HANDLING METHODS : RECIPROCAL CROSSES

Crosses of mites between strains are performed on single leaf cultures. A number of female teliochrysalids of one strain (30-40) are placed on a leaf culture and males of the opposite strain are introduced. Since the sex-ratio of female to male in the populations of T.urticae fluctuates between 2-3, the males to female ratio used for crossing can be estimated to be 1:2 or 1:3. Thus, for the present experiment, usually fifteen to thirty males are used for crossing with a corresponding number of 30-40 females . Usually, males are introduced to the female teliochrysalids before emergence but this technique is not applicable in instances where the male and female teliochrysalids are not clearly distinctive. When such a situation occurs, the female teliochrysalids are allowed to hatch before males are introduced so as to ensure that the teliochrysalids give rise to females.

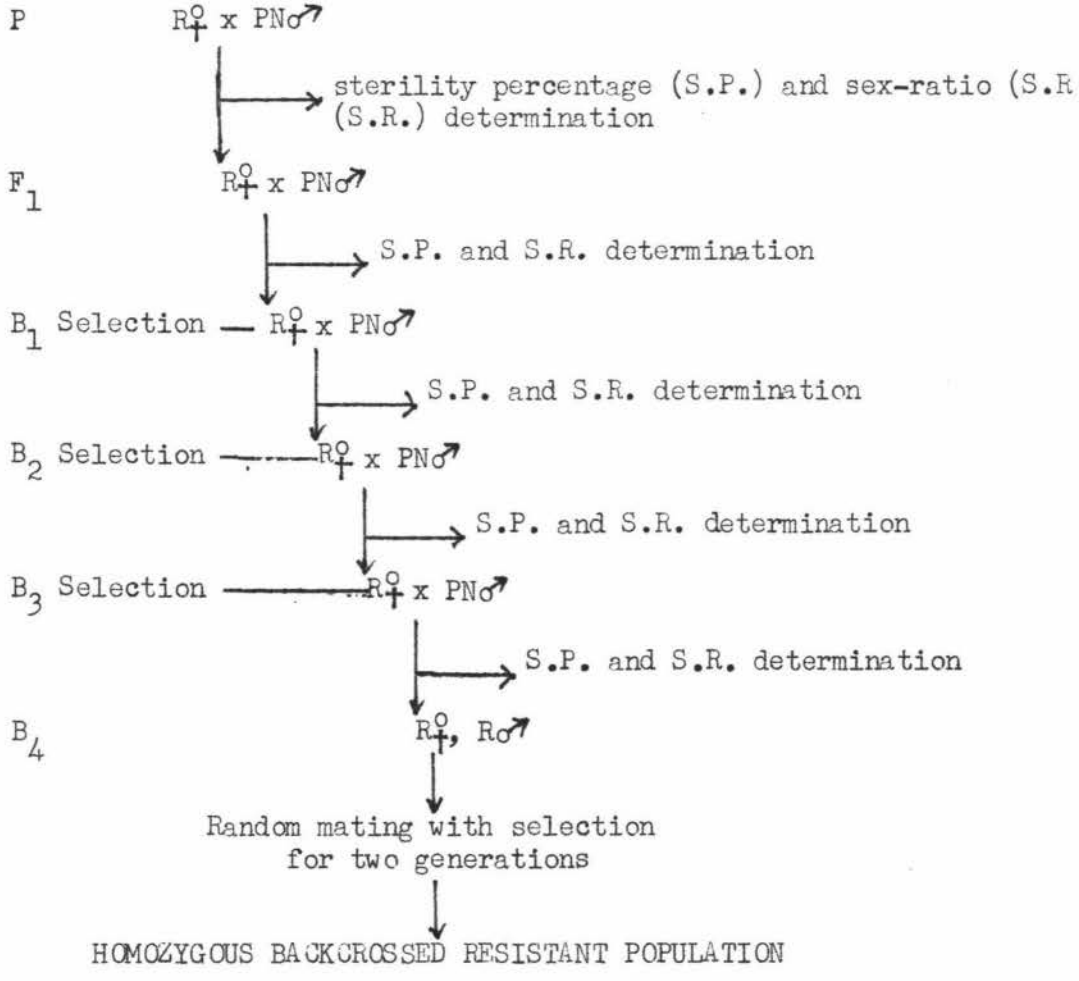
The males and females are mixed for about 48 hours, a time period which is adequate to fertilize all the females. The males are removed and the females are left to lay egg waves.

The reciprocal crosses are carried out as shown below:



4.7 BACKCROSSING WITH SELECTION

Backcrossing with selection serves to substitute alleles of the resistant strain for those of the normal strain excepting the resistance gene which maintains its position due to selection pressure. The end product, after random mating and selection, is a strain with a homogeneous genome but containing the isolated resistance gene. For backcrossing, virgin females of the resistant strains are backcrossed to PN males and selected with parathion-methyl. Owing to the shortage of time, the number of backcrosses was limited to four. The scheme for backcrossing is presented as follows:



4.8 CONSTRUCTION OF DM-LINES

In order to construct the DM-lines, female mites of uniform constitution (2-6 days old) are slide-dipped using five or more concentrations of toxicant, depending on the variability of the response. For each concentration, 90-130 mites are used.

The mortality figures are corrected with the mortality of the control according to Abbot's formula, $\frac{a - b}{100 - b}$ (Abbot, 1925), where "a" is the observed mortality of the concentration and "b" the mortality of the control. Finney's (1952) probit analysis is used to determine the probit regression line and the 95% confidence limit.

Approximately, three to nine points are adequate for the definition of the DM-lines. For more accurate results, the points between mortalities of 85% and 30% are considered for fitting the DM-lines by eye. Mortalities below 30% and above 85% can show some degree of plateau effect which may confuse the true position of the DM-line.

4.9 ACARICIDAL MATERIALS

The following chemicals are usdd for testing and study:

COMMON NAME	TRADE NAME	CHEMICAL NAME	GROUP CLASSIFICATION
Parathion-methyl	Folidol M.50 50% w/w	dimethyl 4-nitrophenylphosphorothionate	Organophosphate
Formetanate	- 92% w/w	3-dimethylaminomethylleneiminophenyl N-methylcarbamate	Carbamate

COMMON NAME	TRADE NAME	CHEMICAL NAME	GROUP CLASSIFICATION
-	Plictran 50% w/p	tricyclohexyltin hydroxide	Ungrouped
Dicofol	Kelthane AP 18.5% w/w	2,2,2-trichloro-1,1-di-(4-chlorophenyl) ethanol	Diphenyl

Kelthane was abandoned later after some preliminary use because the toxicant did not give a uniform kill. Even among the slides which had been slide-dipped in the same concentration, there was very clear indication of variability in response. Another difficulty associated with the use of Kelthane is that the distinction between the dead and living is difficult to ascertain as compared with the other chemicals.

CHAPTER 5

RESULTS

The present study involves three main areas and the results can be conveniently presented under the following headings.

5.1 TOXICOLOGICAL STUDY

This section contains only the essential and most significant numerical facts. The original figures from which all statistical data and DM-lines have been calculated are presented in the Appendix.

5.1.1 Toxicological responses of the original strains

The toxicological responses of the six selected colonies to parathion-methyl, formetanate, tricyclohexyltin hydroxide and dicofol were studied by using the slide-dip technique (4.3).

a) Response to parathion-methyl (Organophosphate) The results for parathion-methyl are presented in Table 1. Their DM-lines are shown in Fig. 1.

The results indicate a wide R:S (i.e. resistant:susceptible) ratio in LD50 values for parathion-methyl. Resistance to parathion appears to be highly developed, indicating that parathion is probably ineffective in the field control of parathion-resistant mite populations.

All the DM-lines except HNR (data not included in Table 1; data are presented in Appendix 1 and the DM-line in Fig.1)

FIG. 1. TOXICITY OF PARATHION-METHYL TO SEVERAL ISOLATES OF T. URTICAE.

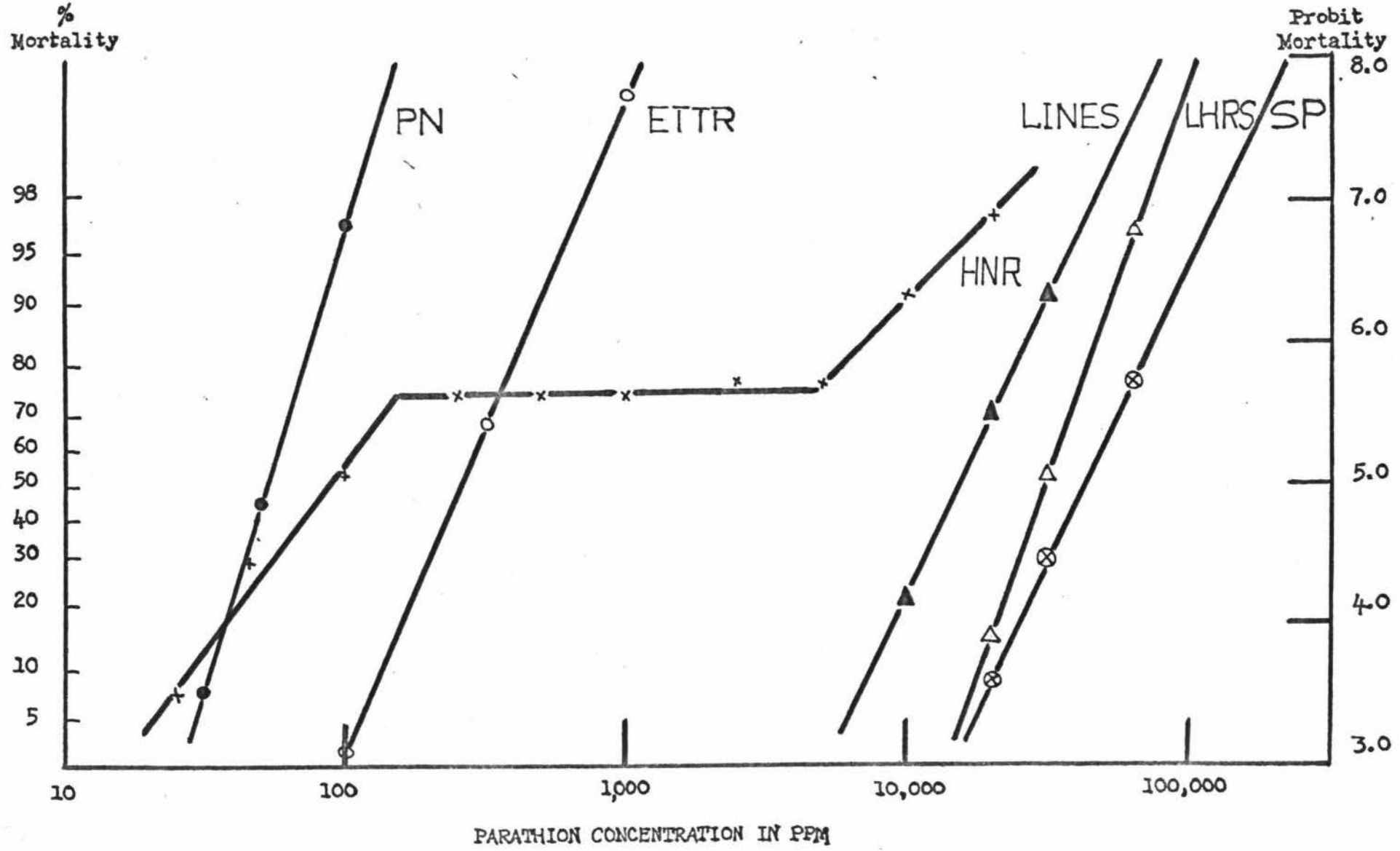


Table 1 Summary of the Dosage-Mortality Response of the PN, ETTR, LHRS, LINES and SP Strains of T.urticae to parathion-methyl.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
PN	52.07	68.4-55.9	6.64	0.73	3.02	
ETTR	254.90	228.9-278.8	4.69	0.49	1.42	4.9
LHRS	30,680.00	32,950-28,220	5.86	0.49	2.60	589.2
LINES	15,260.00	16,500-13,960	4.29	0.35	5.21	293.1
SP	42,440.00	46,140-38,930	4.24	0.45	2.33	815.1

show steep slopes, indicating that all populations except HNR are homogeneous in response to parathion. The high slope values for these homogeneous resistant populations (LINES, LHRS, SP and ETTR) suggest that they are advanced in the development of resistance to parathion, since populations in the process of resistance development usually exhibit low-slope DM-lines.

The step-like DM-line of HNR indicates that the population is heterogenous or segregating for resistance. Two types of mites are present: resistant and susceptible individuals. The fact that the plateau is at the 75% mortality shows that the proportion of susceptible to resistant mites is 3:1. Two explanations are possible: The HNR population may be contaminated by a susceptible population; or the HNR population has reverted.

To investigate these possibilities, and in an attempt to derive a homozygous HNR resistant population, a procedure involving mother x son inbreeding plus selection (1000 ppm) was carried out according to the protocol given previously (4.5). If the proportion of susceptible females in the HNR population is taken as 75%, and

assuming a single dominant gene confers resistance then the genotype distribution amongst females would be expected to approximate a Hardy-Weinberg equilibrium:

Phenotype	susceptible	resistant		Alleles	R	r
Genotype	rr	RR	Rr	Frequency	0.13	0.87
Frequency	0.75	0.02	0.23			

(It is assumed that the male haploid situation parallels that for a sex-linked gene.)

The intense selection (against susceptible individuals and possibly some heterozygotes) together with inbreeding would be expected to result in a population in which the resistant proportion (males and females) was at least 50% and probably approached 75%. However, the plateau for the progeny of the mother x son inbreeding plus selection procedure remained about the same as the original population (25% resistant individuals). Repeated attempts likewise resulted in failure to increase this proportion significantly or to establish a homozygous population. Later, a HNR homozygous resistant population was obtained with difficulty and involving a system of backcrossing to PN with selection. From these findings, it is concluded that certain important vigour factors are probably present in HNR which prevent the attainment of homozygosis. Hence contamination is considered unlikely to be the cause of the apparent segregation in this population.

b) Response to formetanate (Carbamate) The results for formetanate are presented in Table 2. Their DM-lines are given in Fig. 2.

The results indicate high LD50 ratios of R:S but they are not as wide as for parathion. Nor are the DM-lines as steep as those responding to parathion, showing that response to carbamate is not as

FIG. 2. TOXICITY OF FORMETANATE TO SEVERAL ISOLATES OF T. URTICAE

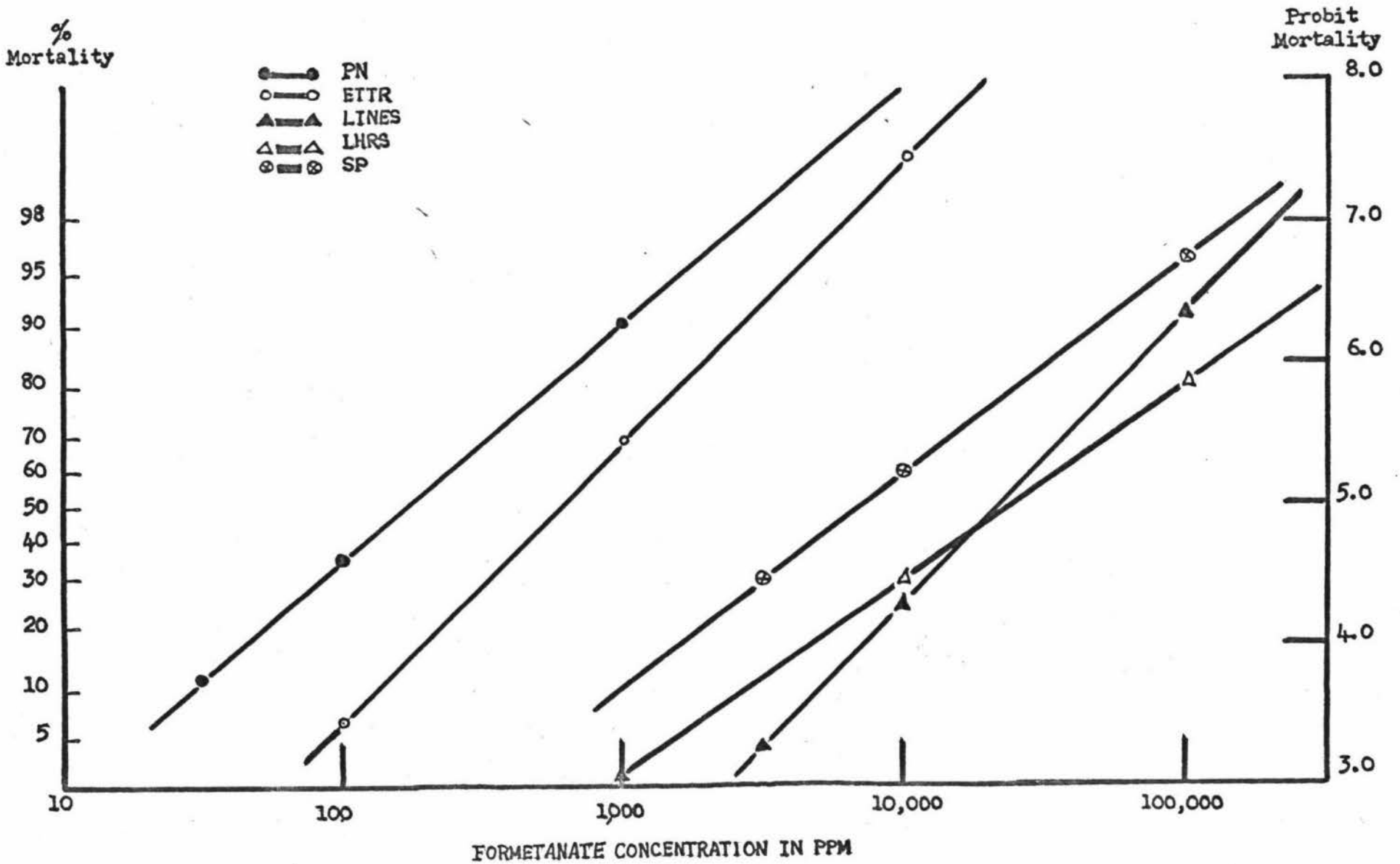


Table 2 Summary of the Dosage-Mortality Response of the PN, ETTR, LHRS, LINES and SP Strains of T.urticae to formetanate.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
PN	166.2	372.3-92.51	1.67	0.13	35.50	
ETTR	595.7	702 -497.4	1.97	0.15	3.99	3.6
LHRS	23,440.00	29,250-18,780	1.41	0.15	4.26	141.0
LINES	21,960.00	26,230-18,850	2.06	0.20	0.15	132.1
SP	7,185.00	9,319-5,049	1.52	0.18	4.26	43.2

homogeneous as that to parathion. The HNR population was not used for study because of its heterogenous response to parathion.

c) Response to tricyclohexyltin hydroxide (Plictran)

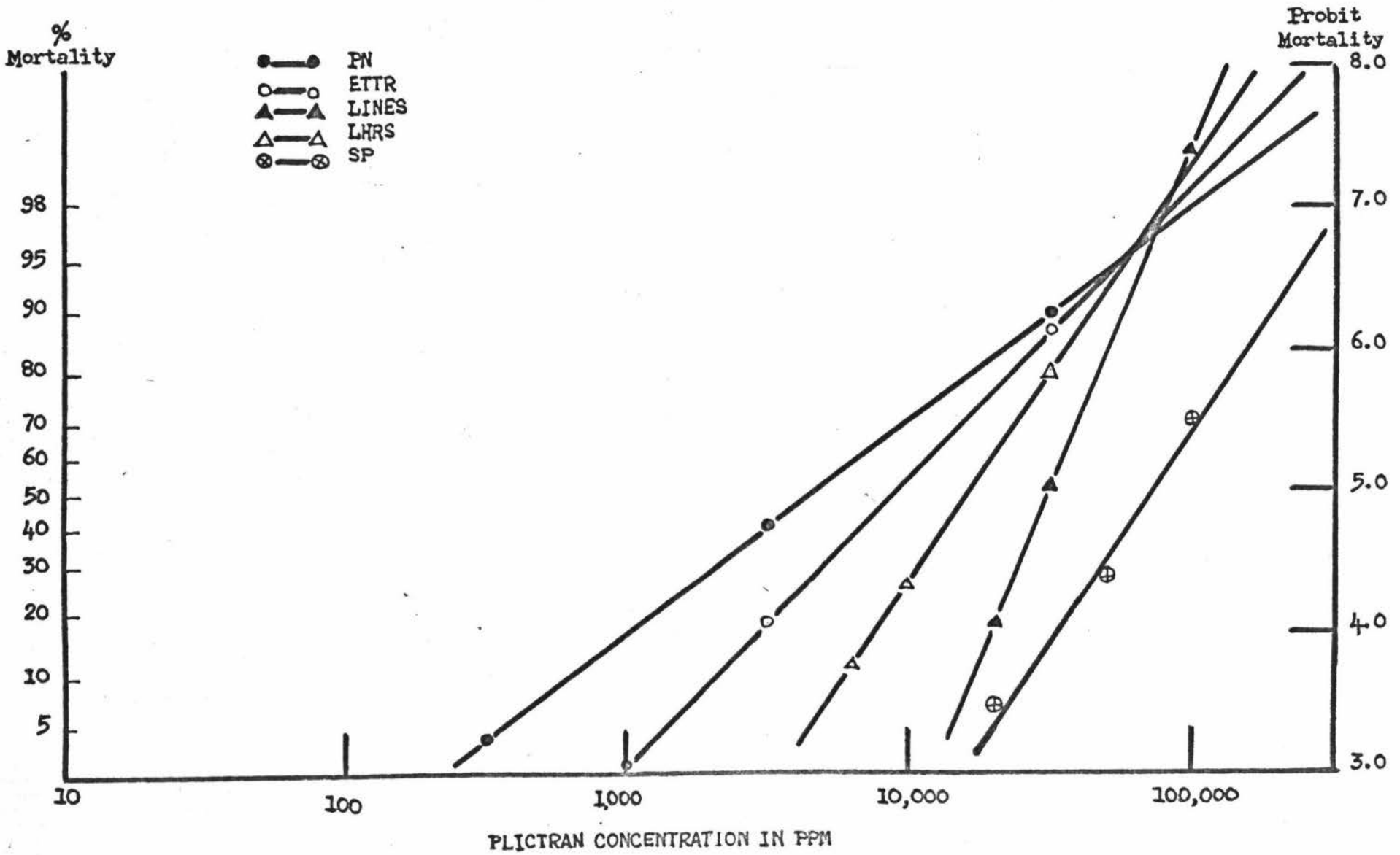
The results for Plictran, an ungrouped compound, are presented in Table 3. The DM-lines are shown in Fig. 3.

Table 3 Summary of the Dosage-Mortality Response of the PN, ETTR, LHRS, LINES and SP strains of T.urticae to Plictran.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
PN	4,688	9,124-2,051	1.49	0.34	26.07	
ETTR	8,880	53,800-4,365	2.06	0.61	66.13	1.9
LHRS	16,270	23,240-11,690	2.94	0.45	12.27	3.5
LINES	31,170	34,990-27,170	4.80	0.53	0.69	6.6
SP*	-	-	-	-	-	-

*Owing to the low number of SP female mites available at the time of toxicological testing, only a small number is used. The results (which are in Appendix 1) are thus not statistically analysed.

FIG. 3. TOXICITY OF TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN) TO SEVERAL ISOLATES OF T. URTICAE



Resistance is only slightly indicated in the LD50 values; probably the small LD50 ratios in R:S values is a reflection of the difference in vigour factors.

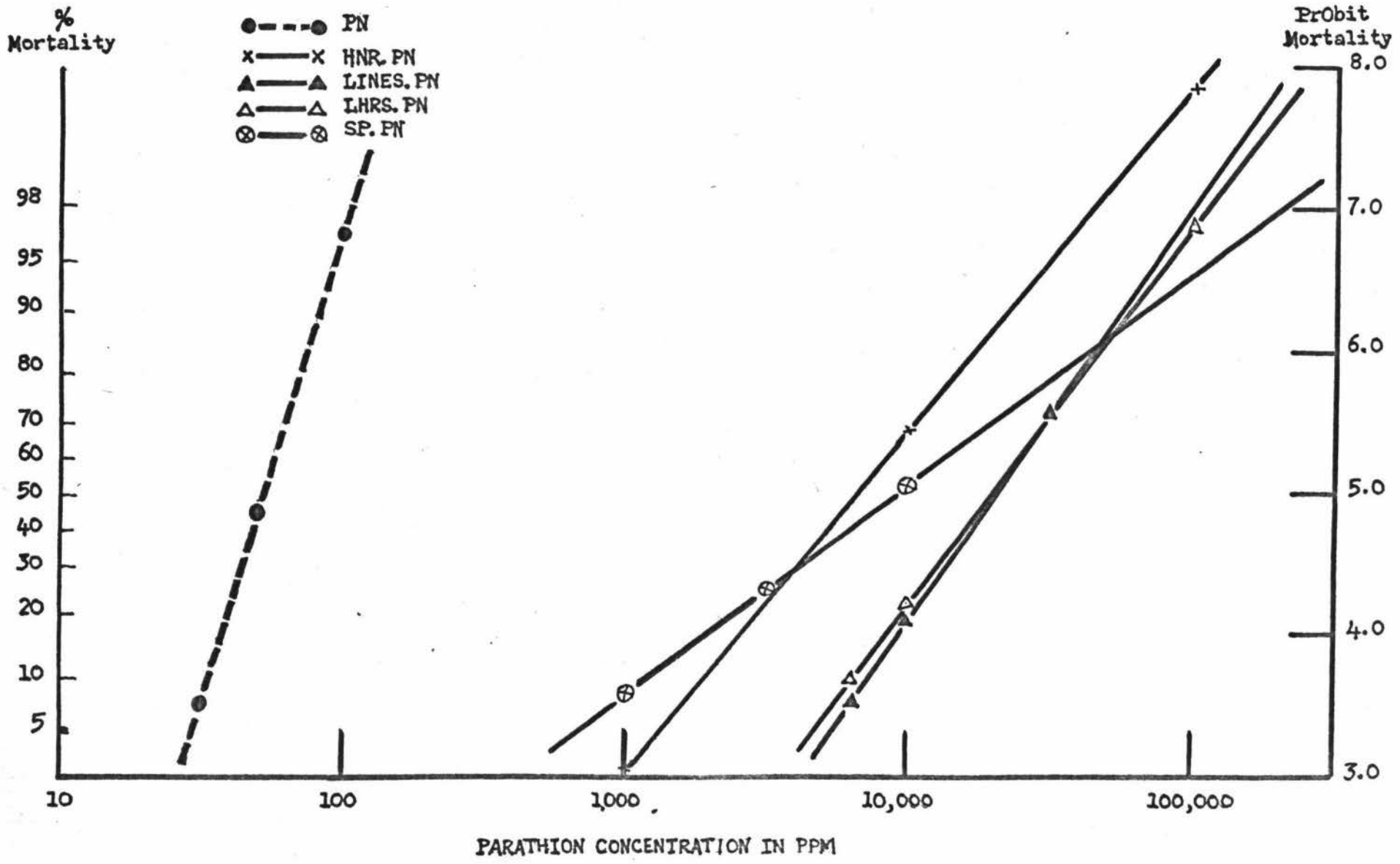
d) Response to dicofol (Organochlorine) Since all the parathion resistant strains exhibit the same response as PN to Kelthane, it can be implied that resistance to Kelthane is non-existent in the strains under study. It is important to note that in comparison with parathion, formetanate and tricyclohexyltin hydroxide, treatment with Kelthane did not produce uniform mortality results, leading to difficulty in drawing accurate DM-lines. In spite of the non-uniform mortality results, the testing system would permit the detection of Kelthane resistance if it is present at all. The non-uniformity in response of mites to Kelthane probably lies in the specific action of Kelthane which causes mites to be morbid over a long period, a condition which is intermediate between dead and living.

The differences that occurred in the resistance levels of the six colonies to the three acaricides, parathion, formetanate and tricyclohexyltin hydroxide indicate that each of these is a toxicologically distinct strain of two-spotted spider mite. If toxicological response is a criterion for delimiting strains, then there is no strain distinction as regard to response to Kelthane. Thus delimitation of strains using toxicological response as a criterion is a matter of convenience. Another criterion based on incompatibility is a more accurate approach to strain delimitation.

5.1.2 Toxicological response of the homozygous backcrossed resistant strains.

These strains were produced by the system of backcrossing and

FIG. 4. RESPONSE OF THE HOMOZYGOUS BACKCROSSED RESISTANT STRAINS TO PARATHION-METHYL



and selection given in chapter 4 (4.7). Each of the original populations underwent four generations of backcrossing to PN and selection. A selection intensity of 250 ppm of parathion was used. Since a concentration of 250 ppm will kill all the F_1 offspring of ETTR x PN cross, the backcross series starting from ETTR was abandoned.

The results for parathion-methyl are presented in Table 4 and their DM-lines in Fig. 4.

Table 4 Summary of the Dosage-Mortality Response of the HNR.PN, SP.PN, LHRS.PN and LINES.PN Backcrossed Strains to parathion-methyl.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio $\frac{\text{R strain}}{\text{S strain}}$
HNR.PN	6,476	3,575-9,506	2.42	0.38	8.63	124.4
SP.PN	9,055	9,610-6,923	1.47	0.22	0.04	173.9
LHRS.PN	19,300	22,550-16,620	2.67	0.29	5.81	370.7
LINES.PN	20,210	27,390-15,590	2.95	0.87	16.04	388.1

Results indicate that resistance for parathion-methyl is maintained. The four strains responded in the same manner (as shown by the slopes and positions of the DM-lines) as the corresponding original strains. The small shifts to the left, when compared to the original populations, are probably due to loss of some vigour factors.

The results for formetanate are presented in Table 5 and their their DM-lines in Fig. 5.

Results indicate that there is no change in the response pattern when compared with the original strains, except for a small

FIG. 5. RESPONSE OF THE HOMOZYGOUS BACKCROSSED RESISTANT STRAINS TO FORMETANATE

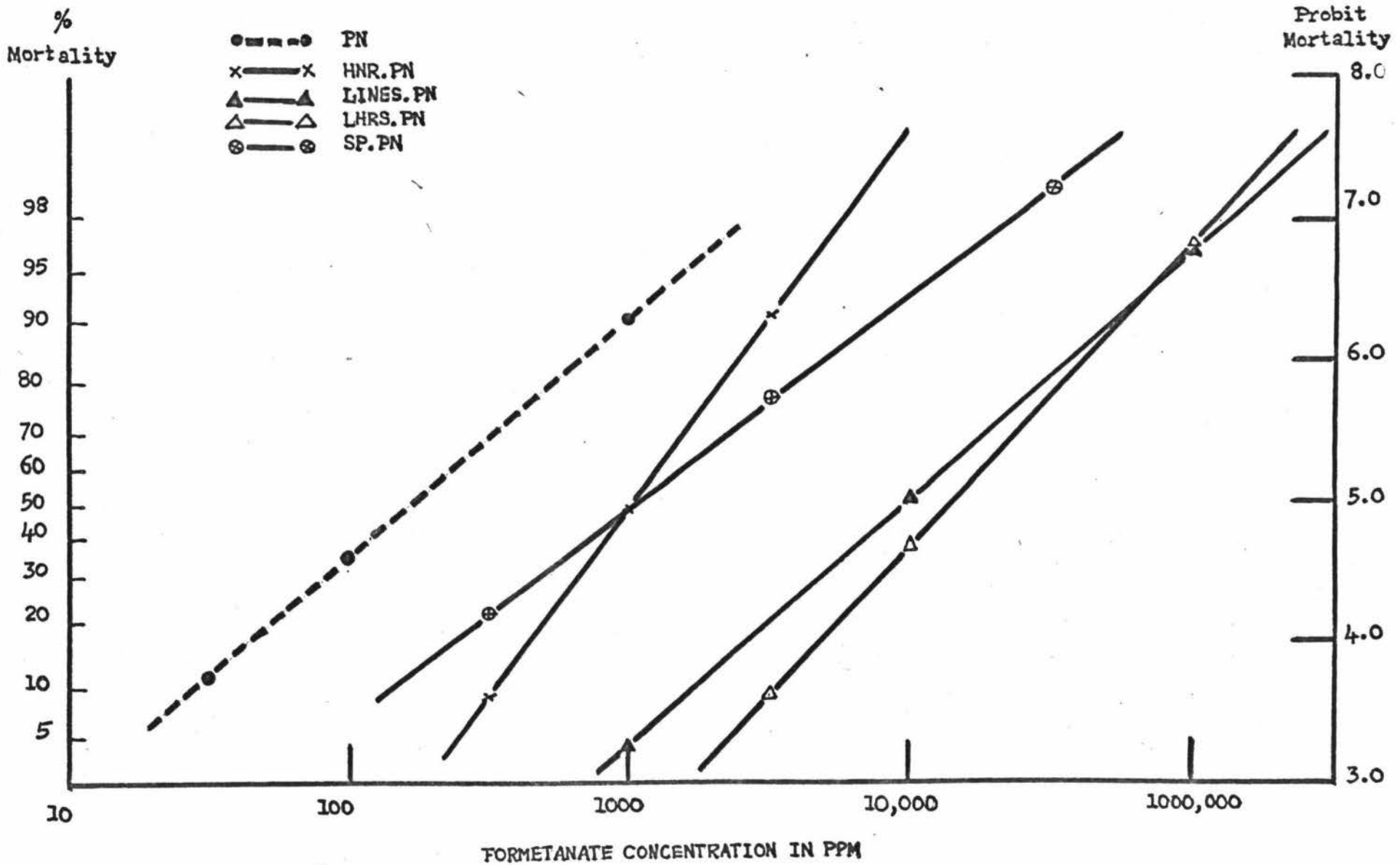


Table 5 Summary of the Dosage-Mortality Response of the HNR.PN, SP.PN, LHRS.PN and LINES.PN Backcrossed Strains to formetanate.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio $\frac{\text{R strain}}{\text{S strain}}$
HNR.PN	1,023.00	2,521-258.5	2.71	0.73	18.09	6.2
SP.PN	1,035.00	1,445-687.9	1.51	0.16	24.94	6.2
LHRS.PN	13,830.00	20,640-8,054	2.13	0.34	10.62	83.2
LINES.PN	9,614.00	15,810-472.0	1.76	0.31	20.19	57.8

shift to the left.

By comparing the toxicological responses of the five original strains (SP, LHRS, LINES, ETTR, PN) to parathion-methyl and formetanate, one deduction can be made: the resistance patterns of the five strains to parathion and formetanate are more or less similar; that is the resistance order for parathion is SP > LHRS > LINES > ETTR > PN, and for formetanate, LHRS > LINES > SP > ETTR > PN, in decreasing order of resistance, with SP alone appearing exceptional. By examining the homozygous backcrossed resistant strains of LHRS, LINES and SP (LHRS.PN, LINES.PN and SP.PN) in their response to parathion and formetanate, the LHRS > LINES > SP in decreasing order of resistance, seems to be operative. This leads the author to conclude that some vigour factors are probably involved in SP in producing small differences in response to a toxicant. Thus, it is logical to say that the pattern of response to formetanate and parathion is of the order LHRS > LINES > SP > ETTR > PN, which means that the response patterns of the various colonies to parathion and formetanate are similar. Thus cross-resistance of parathion and formetanate is implied.

The adoption of a repeated backcross procedure to differen-

tiate whether cross resistance or multi-resistance is operating is based on the assumption that the factors causing multi-resistance are separate distinct entities. If the situation is such that the phenomenon of multi-resistance is due to closely-linked genes, repeated backcrossing will not be able to separate the resistances and multi-resistance can be mistaken for cross resistance.

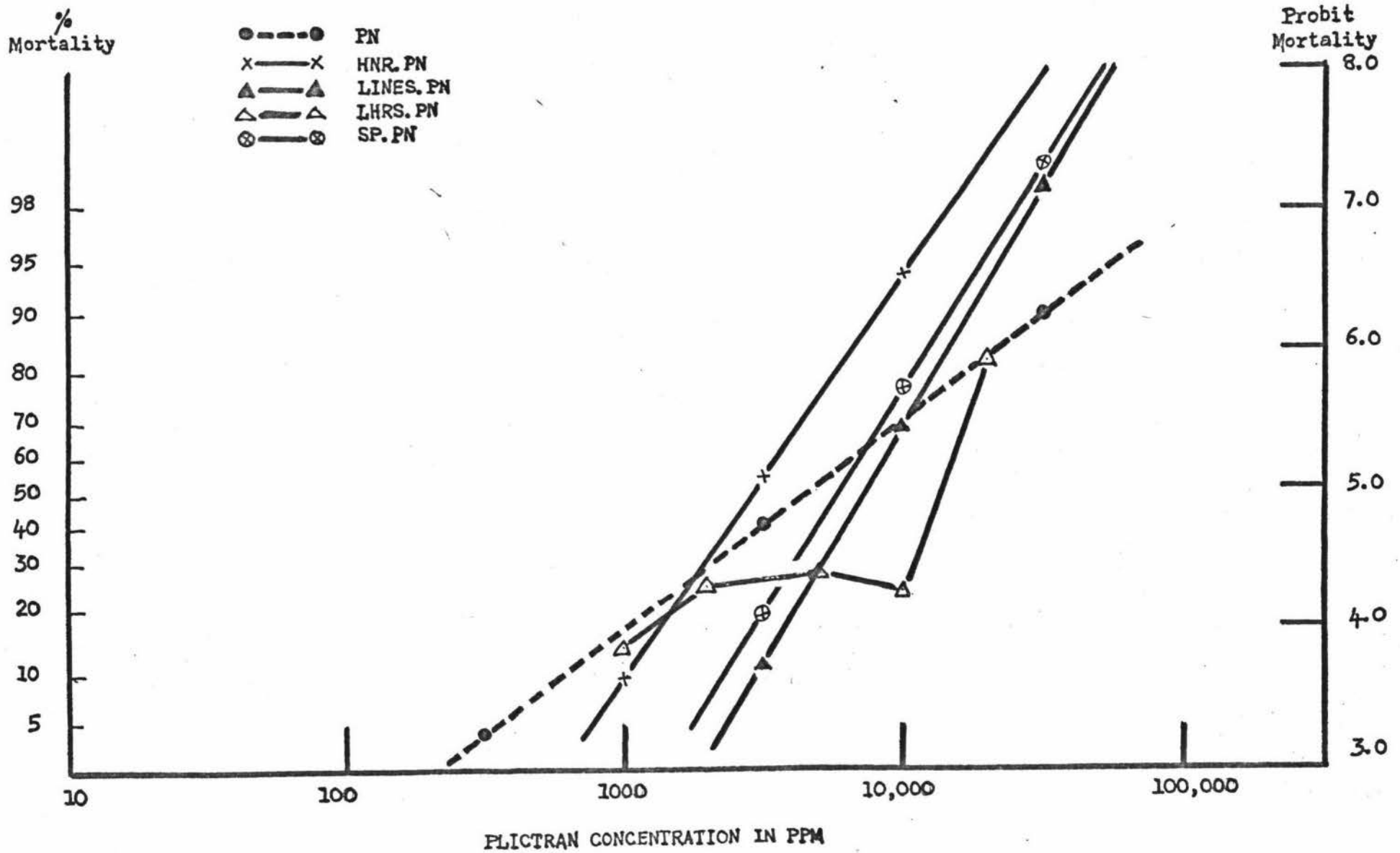
For multi-resistance to be operating in the situation under study, several conditions have to be assumed. First, formetanate must have been used before in all the areas from where the colonies were collected and, at the moment of collection, formetanate resistance had already developed; second, formetanate resistance would have had to develop proportionately to the degree of parathion resistance; and third, if the degree of formetanate resistance development is dependent on selection pressure, the number of generations per year, mite number, etc., these factors must be operating in the right combinations to give a formetanate resistance pattern of LHRS > LINES > SP > ETTR, an occurrence which is very unlikely.

The results of the response of LHRS.PN, LINES.PN, SP.PN and HNR.PN to tricyclohexyltin hydroxide (Plictran) are presented in Table 6. The DM-lines are shown in fig. 6.

Table 6 Summary of the Dosage-Mortality Response of the HNR.PN, SP.PN, LHRS.PN and LINES.PN Backcrossed Strains to Plictran.

Strain	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio $\frac{\text{R strain}}{\text{S strain}}$
HNR.PN	2,915	3,493-2,306	2.90	0.34	1.75	0.6
SP.PN	5,988	6,861-5,139	3.23	0.37	2.57	1.3
LHRS.PN	DM-line shows high heterogeneity.					
LINES.PN	7,256	8,260-6,197	3.42	0.45	0.17	1.6

FIG. 6. RESPONSE OF THE HOMOZYGOUS BACKCROSSED RESISTANT STRAINS TO TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)



Results indicate that resistance to Plictran is not detectable. The fact that a small degree of tolerance or resistance is implicated in the original populations and not in the homozygous backcrossed resistant populations, serve to indicate that the small resistance exhibited by the original populations is due to some vigour factors which had been removed by repeated backcrossing.

5.1.3 Toxicological response of the homozygous backcrossed strain of HNR

It can be recalled that an attempt to obtain a homozygous resistant population from HNR had ended in failure (5.1.1.a). However, when HNR was repeatedly backcrossed to PN, a homozygous backcrossed resistant strain of HNR was obtained without the occurrence of reversion. The fact that a stable population can be obtained by crossing HNR with PN points out that some factors from PN must be involved in producing stability in HNR.PN. That 'vital factors' are involved can be substantiated by the observations that the original HNR population and the HNR inbred populations are less vigourous than the HNR.PN (i.e. longer development period, higher adult mortality and lower number of eggs produced). Possibly the factor for parathion resistance in HNR is deleterious in the absence of the 'vital factors' and thus cannot exist in the homozygous condition. Recessive lethal or semi-lethals can only persist in the male haploid system when the frequency of homozygous resistant individuals is less than 2%.

5.2 INHERITANCE OF RESISTANCE

Of the four acaricides (parathion, formetanate, tricyclo-

hexyltin hydroxide, and dicofol) used in the present study, parathion appears to be the most appropriate toxicant for the genetical studies of resistance (as indicated by the homogeneity and steep slopes of the DM-lines).

5.2.1 Reciprocal crosses between resistant and normal strains

The object of reciprocal crosses between individuals of the R and S strains is to determine the degree of dominance of the genetic factor or factors for resistance, to ascertain whether both sexes can transmit the resistance character, and to observe any cytoplasmic influences.

The reciprocal crosses were carried as described before (4.6). For the reciprocal crosses, three parathion resistant populations were selected for study (SP, LINES and LHRS). Owing to the low number of mites available in the F_1 generation (as a result of interpopulational infertility), only two replicates of each concentration were used for toxicological determination.

The results of the reciprocal crosses of LINES, LHRS and SP are presented in Tables 7, 8 and 9 and their DM-lines in Figs. 7, 8 and 9 respectively.

Table 7 Summary of the Dosage-Mortality Response of the F_1 Progeny From the Reciprocal Crosses of LINES and PN to parathion.

Cross	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
F_1 ♀♀s from LINES ♀ x PN ♂	12,460	14,360-10,920	4.67	0.65	0.08	239.3
F_1 ♀♀s from LINES ♂ x PN ♀	3,967	4,729-3,226	2.51	0.27	0.07	76.2

FIG. 7. DOSAGE-MORTALITY LINES FOR THE F_1 PROGENY LINES x PN RECIPROCAL CROSSES

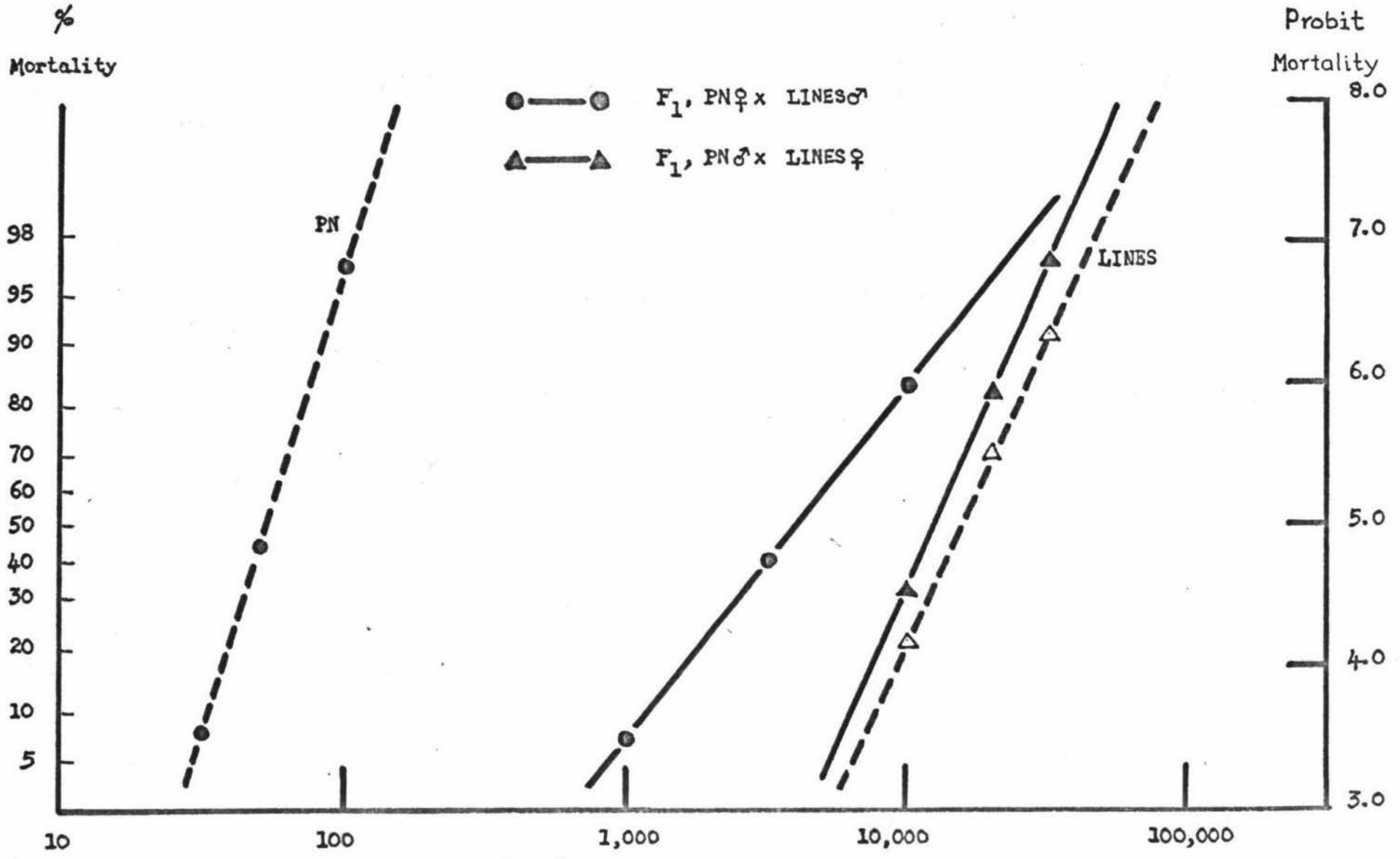


FIG. 8. DOSAGE-MORTALITY LINES FOR THE F_1 PROGENY

LHRS x PN RECIPROCAL CROSSES

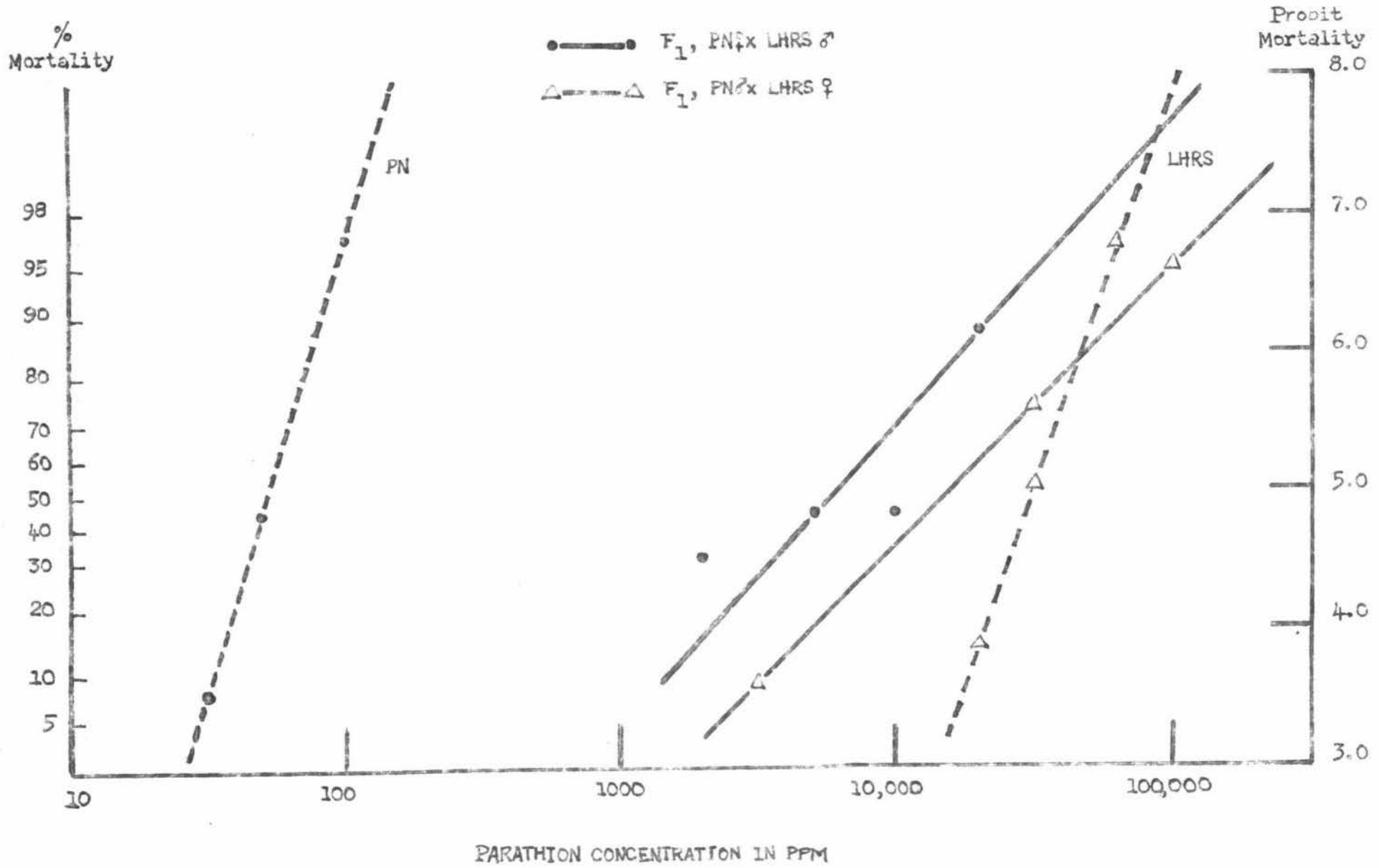


FIG. 9. DOSAGE-MORTALITY LINES FOR THE F_1 PROGENY

SP X PN RECIPROCAL CROSSES

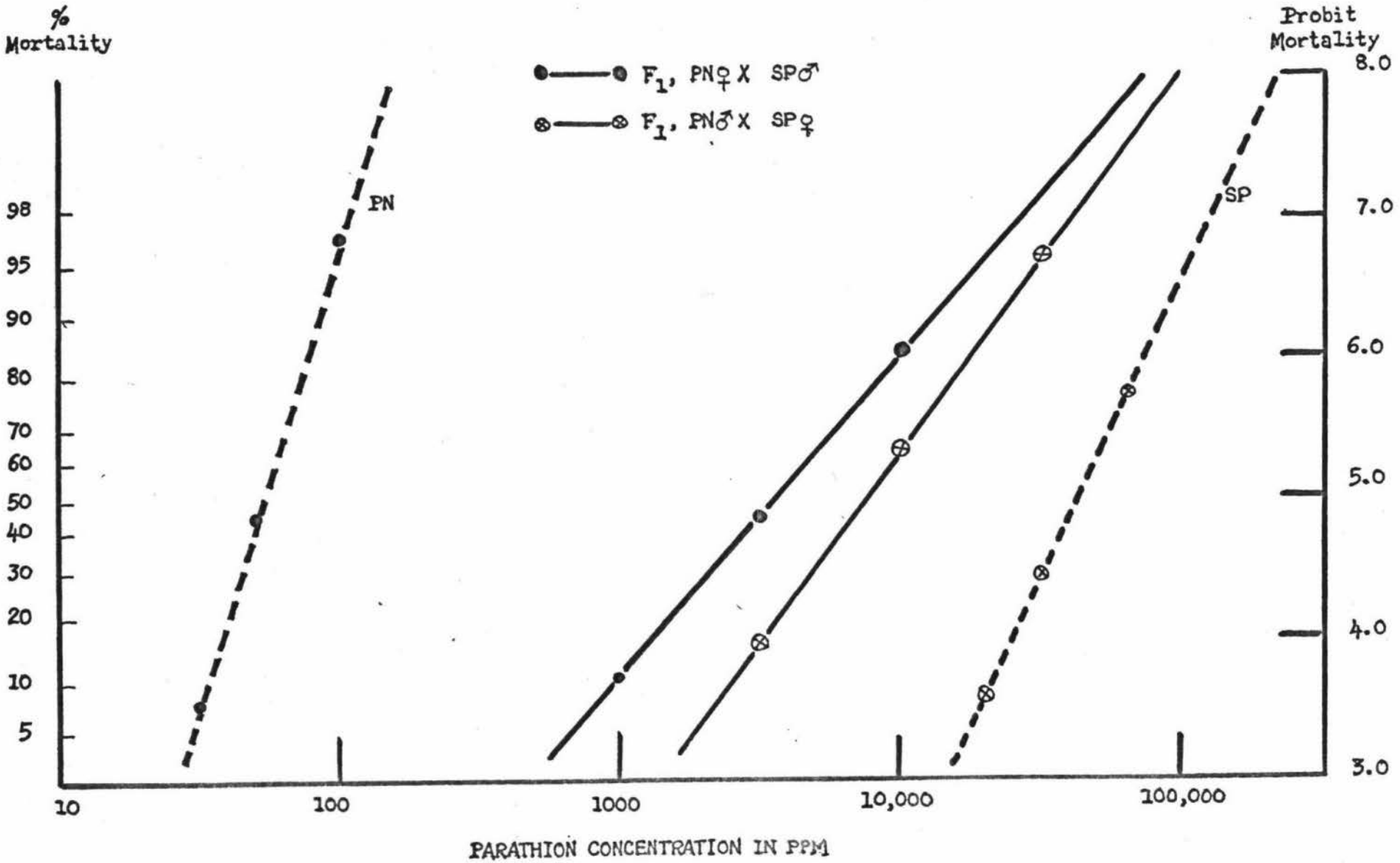


Table 8 Summary of the Dosage-Mortality Response of the F_1 Progeny From the Reciprocal Crosses of LHRS and PN to parathion.

Cross	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
F_1 ♀s from LHRS♀ x PN♂	15,120	31,440-7,069	2.03	0.49	44.59	290.4
F_1 ♀s from LHRS♂ x PN♀	Owing to the high value of 'g', the data are not statistically analysed					

Table 9 Summary of the Dosage-Mortality Response of the F_1 Progeny From the Reciprocal Crosses of SP and PN to parathion.

Cross	LD50 in ppm	95% Confidence Limits	Slope b	SE (b)	χ^2	LD50 Ratio R strain / S strain
F_1 ♀s from SP♀ x PN♂	7,457	11,980-2,440	2.76	0.66	18.01	143.2
F_1 ♀s from SP♂ x PN♀	3,252	6,700-203.4	2.33	0.58	9.43	62.5

The data show that the progeny of all reciprocal crosses are semi-dominant or dominant. The character can be transmitted equally well by both sexes. A slight influence of the cytoplasm is apparent but the degree is not great. The difference may be due to cytoplasmic vigour tolerance factors or to nuclear-cytoplasmic interactions. Anyway, the DM-lines of the reciprocal crosses seem to indicate that resistant heterozygotes containing cytoplasm from the resistant strains have a higher resistance than the resistant heterozygotes with

cytoplasm from the PN strain. This is probably due to predetermination.

A certain degree of incompatibility is observed in the reciprocal crosses. This effect will be the next field of study (5.3).

5.2.2 Backcrosses between the resistant F_1 and normal

This is to determine whether the backcross progeny would segregate into ratios suggestive of Mendelian inheritance of resistance. The reciprocal backcross between the resistant F_1 and normal is outlined in chapter 4 (4.6). The backcross female progeny (B_1) were subjected to a full DM-test in each case. For a test, about 12-14 concentrations were used. Owing to the high percentage of dead eggs, 1-2 replicates were used for each concentration.

The results for the backcrosses are presented in Appendix 1 and their DM-lines are in Figs. 10, 11 and 12.

The backcross progeny have segregated into two classes, resistant and susceptible, signifying a population which is mixed or heterogeneous.

The clear cut 1:1 segregation of R and S phenotypes in the backcross F_2 generation implies that resistance may be attributable to a single Mendelian factor.

However, it is still possible that other modifying genes may play a considerable role in the expression of the resistance character. It is also possible that a polygenic system is "mimicing" simple Mendelianism.

5.2.3 Repeated backcrosses with selection

The partition ratio of the first backcross is suggestive of

FIG. 10. DOSAGE-MORTALITY LINES FOR THE B_1 PROGENY

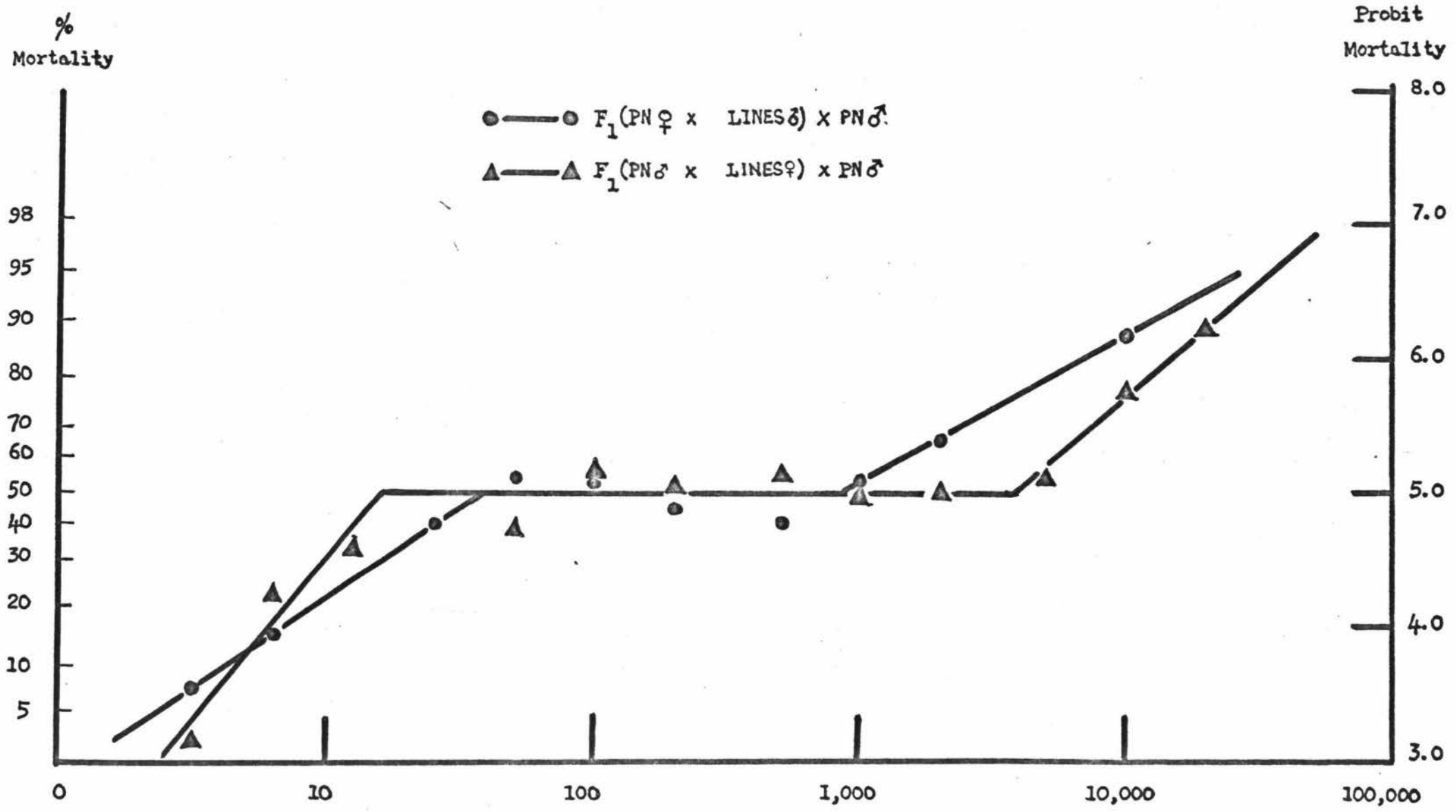


FIG 11 DOSAGE-MORTALITY LINES FOR THE B_1 PROGENY

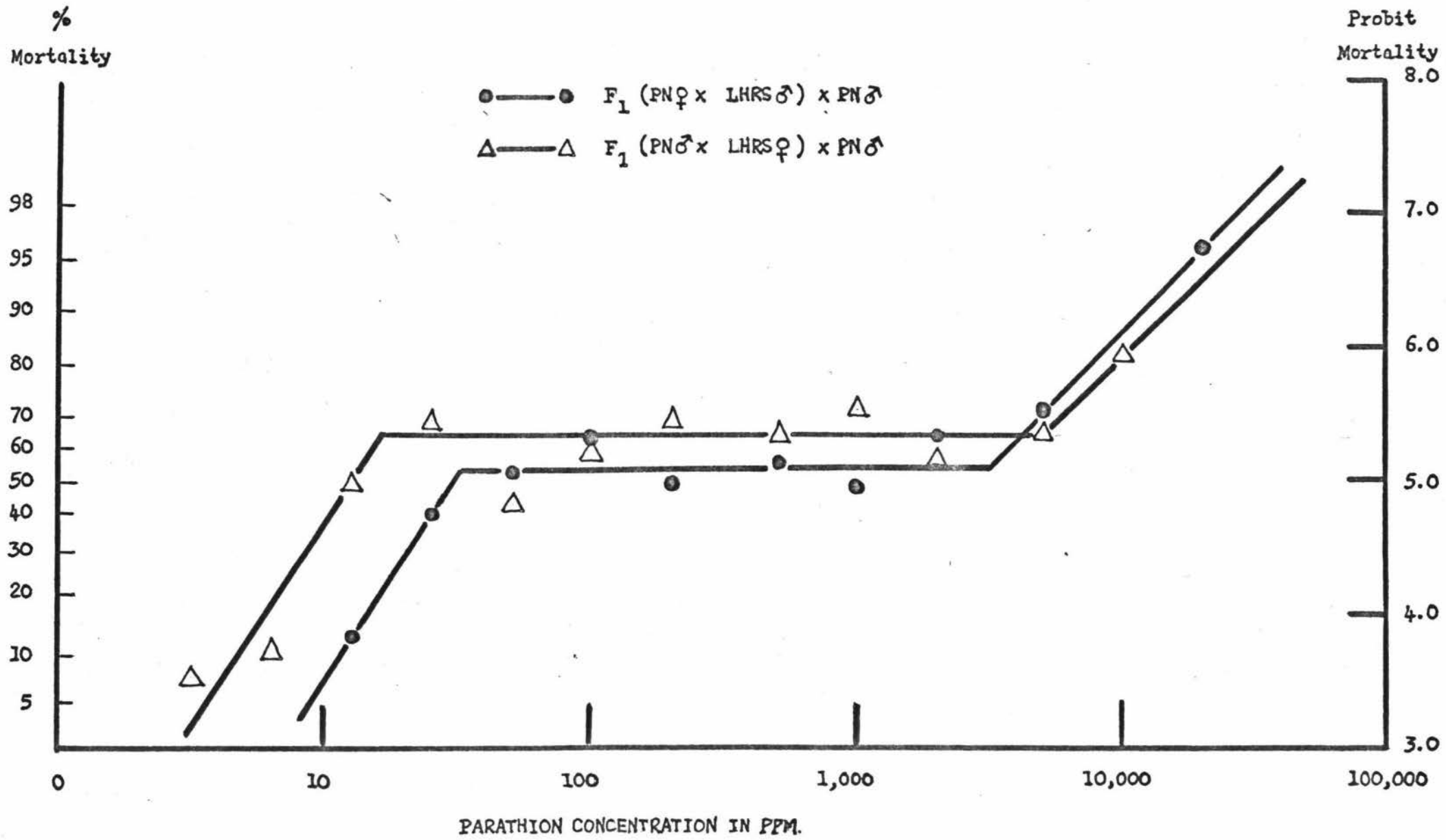
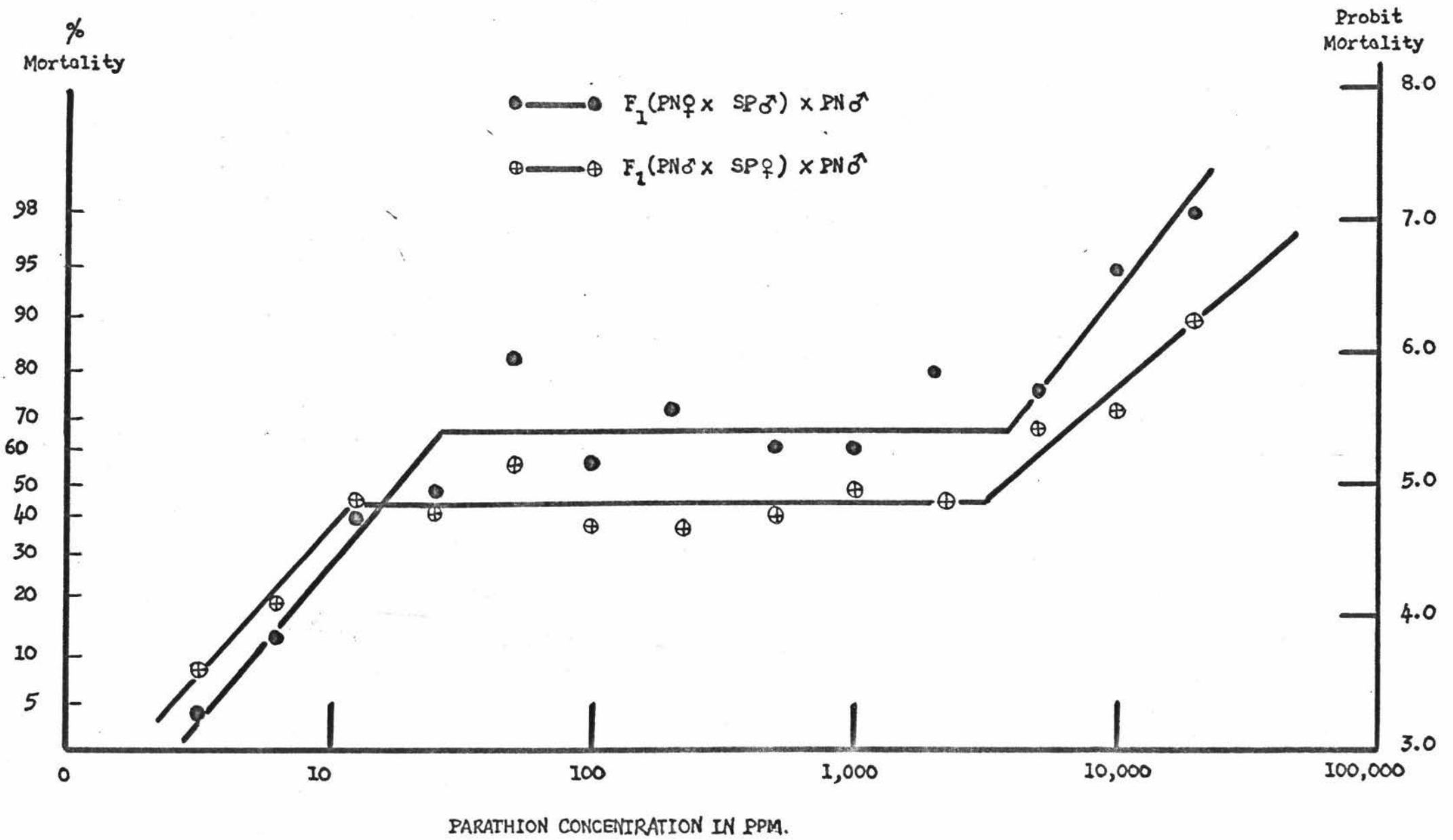


FIG. 12. DOSAGE-MORTALITY LINES FOR THE B_1 PROGENY



a single major gene controlling resistance. To confirm this conclusion and to eliminate the likelihood of a polygenic system behaving in a similar manner as a monogenic system, repeated backcrossing of resistant strains to PN with selection at each generation was undertaken. The procedure is the same as outlined for the backcross scheme in the first part of the experiment (4.7). The results are presented in Table 4 and their DM-lines in Fig. 4.

The homozygous backcrossed resistant populations (LINES.PN, LHRS.PN and SP.PN) did not show any significant decrease in resistance (less than 50%). Therefore, it suggests that monogenic control of resistance holds in the three strains of two-spotted spider mite.

5.3 INCOMPATIBILITY

When different isolates of two-spotted spider mites from either widely separate or adjacent geographical regions are crossed, it is often observed that various degrees of infertilities (as expressed by the percentages of dead eggs) occur. To determine the degree of incompatibility (degree of infertility, egg mortality, or sterility percentage) among the six mite colonies and the nature of the incompatibility factor or factors, the following study was undertaken.

5.3.1 Intrapopulational crosses

Crosses were made between individuals within each of the six strains according to the scheme described before (4.6). The egg mortality was determined for the F_1, F_2(haploid and diploid) and F_2(haploid) generations. The results are presented in Table 10.

The results indicate that crosses with mites from the same

Table 10 Intrapopulational and Interpopulational Crosses of Six Strains of T. urticae.

	% of Unviable Eggs of Mass Crosses Between Different Populations F_1						% of Unviable Eggs Produced By Hybrids From Mass Crosses Between Different Populations F_2(haploid and diploid)						% of Unviable Eggs Produced by Teliochrysalids From Mass Crosses Between Different Populations F_2(haploid)					
♀♀ ♂♂	PN	HNR	LHRS	SP	ETTR	LINES	PN	HNR	LHRS	SP	ETTR	LINES	PN	HNR	LHRS	SP	ETTR	LINES
PN	4.0 (2.2)	17.5 (0.9)	39.1 (0.9)	22.8 (1.0)	5.5 (1.9)	16.3 (1.9)	5.4 (3.2)	6.7 (2.6)	62.3 (2.0)	66.2 (2.1)	32.5 (3.2)	92.0 (5.5)	5.1 -	10.3 -	73.4 -	81.9 -	58.6 -	96.1 -
HNR	1.9 (1.6)	2.5 (4.0)	2.3 (2.5)	12.0 (0.7)	6.3 (3.2)	4.1 (3.7)	10.5 (5.0)	4.1 (6.9)	62.5 (1.9)	65.4 -	14.7 (1.6)	55.8 (1.7)	39.4 -	22.4 -	77.2 -	55.7 -	41.4 -	59.8 -
LHRS	8.4 (0.8)	7.4 (1.9)	10.8 (2.1)	53.7 (1.4)	10.0 (2.7)	15.5 (1.0)	51.1 (6.7)	40.2 (4.6)	26.1 (1.4)	94.3 (2.0)	69.5 (1.5)	71.9 (1.6)	73.6 -	63.9 -	19.2 -	- -	79.5 -	40.6 -
SP	2.6 (0.9)	42.7 (0.9)	43.7 (1.9)	4.1 (2.2)	16.6 (0.7)	31.7 (0.9)	53.6 (4.7)	70.4 (1.2)	47.4 (3.5)	7.2 (2.6)	47.4 (4.3)	25.1 (11)	85.4 -	70.3 -	71.5 -	20.3 -	88.1 -	83.0 -
ETTR	17.5 (2.5)	45.9 (0.3)	34.1 (0.2)	32.8 (0.6)	11.4 (2.9)	23.9 (0.6)	13.7 (4.1)	18.9 (4.0)	73.7 (11)	52.3 (1.5)	6.5 (2.6)	46.3 (4.5)	47.9 -	9.4 -	94.6 -	91.6 -	19.3 -	62.8 -
LINES	5.0 (0.9)	17.4 (0.6)	21.7 (1.6)	35.9 (1.1)	7.1 (0.9)	4.7 (1.6)	47.5 (7.9)	24.6 (5.2)	23.0 (4.6)	61.4 (2.3)	50.5 (2.6)	21.1 (2.3)	82.6 -	71.7 -	- -	78.2 -	83.8 -	17.4 -

Note: Values within brackets refer to sex ratios.

population do not produce any significant egg mortality in the F_1 and F_2 generations, viz. from 2.5% - 11.4% in the F_1 generation; 4.1% - 26.1% in the F_2(haploid and diploid) generation; and 5.1% - 22.4% in the F_2(haploid) generation . By comparing the egg mortalities of the intrapopulational crosses in the F_1 and F_2(haploid and diploid) generations, which are biologically the same, it can be deduced that egg mortality does fluctuate considerably. Low haploid egg mortalities in the F_2(haploid) generation suggest that meiotic divisions are occurring normally.

Since HNR is toxicologically a heterogeneous population (thought to contain deleterious factors), it may be expected that the intrapopulation cross will produce a high F_1 and F_2(haploid) egg mortality. However, this did not occur, indicating that HNR individuals, although consisting of resistant and susceptible genotypes (with reference to parathion), are chromosomally compatible with each other. Thus, it can be said that chromosomally HNR is one strain but toxicologically it exhibits polymorphism. This also serves to indicate that probably the resistance factor is not involved in the phenomenon of interpopulational or intrapopulational sterility (5.3.3). It is however, probable that the genome originally associated with the resistance phenotype was not compatible with the normal genome but that continous outbreeding had produced new gene combinations which broke down the incompatibility barrier (5.3.3). Inbreeding on the other hand leads to incompatibility (Schulten, 1968), indicating that other factors are also involved.

5.3.2 Interpopulational Crosses

The six colonies of T.urticae were crossed reciprocally acc-

ording to the procedure outlined in chapter 4 (4.6). The egg mortalities and sex-ratios for the interpopulational crosses are presented in Table 10.

5.3.2.1 Infertility of F_1 generation Generally, the F_1 interpopulational crosses show higher infertility than the F_1 intrapopulational crosses. This can be attributed to the higher degree of different gene combinations (heterozygosity) in one genome indicating that the six mite colonies are partially distinctive genetic races or strains.

The interpopulational crosses of LHRS and LINES (which are collected from Levin) exhibit higher sterility values than the intrapopulational crosses; and this indicates that mite colonies within even a limited area of distribution can undergo microevolution which results in the formation of reproductive incompatibility.

5.3.2.2 Reciprocal crosses In some reciprocal crosses, the degree of intersterility depends on the direction of the cross. In two instances, the reciprocal difference in sterility is very marked. They are:

1. PN♀ x SP♂.....22.8% (sterility percentage)
   PN♂ x SP♀ ..... 2.6% (sterility percentage)
2. PN♀ x LHRS♂.....39.10%(" "))
   PN♂ x LHRS♀ ..... 8.40%(" ")

The reciprocal differences cannot be accounted for by the fluctuation in the sterility percentage (which is 2-5 times in the intrapopulational crosses) and must be attributable to some cytoplasmic factor(s) or nucleo-cytoplasmic interactions.

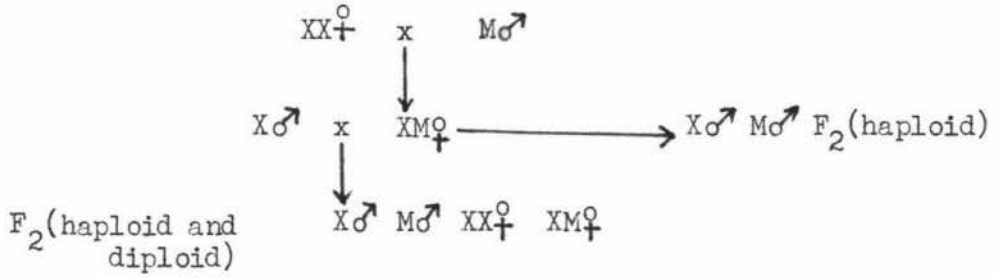
In many other instances, there are indications of reciprocal differences in sterility percentage but whether the values are a

reflection of cytoplasmic factor(s) or nucleocytoplasmic interactions is doubtful, as such deviations come within the range of intrapopulational fluctuations.

5.3.2.3 Sex-ratio of F_1 In many crosses, the sex-ratios (females:males) in the F_1 generations are smaller than either of the two parent strains. If sex-ratios are inherited, then they should approximate those of the parents rather than becoming lower (Mitchel, 1972). Since there is some degree of egg mortality, the lowered sex-ratio can be traced to the death of diploid eggs. It has been shown that there is a clear mating preference of T.urticae females for males of their own strain, regardless of whether males of other strains are immediately available (Smith et al., 1969; Dieleman and Overmeer, 1973; Helle and Overmeer, 1973) but whether this phenomenon contributes to a decrease in sex-ratio is doubtful.

5.3.2.4 F_2(Haploid and Diploid; Haploid) Generations

Diagrammatically, the scheme for F_2(haploid and diploid) and F_2(haploid) generations is represented as follows:



In general, the data indicate that there is an increase in the F_2 sex-ratio compared with that in the F_1. At the same time, the egg mortality is much higher in the F_2(haploid and diploid) than in the F_1. Several conclusions are possible:

1. The haploid eggs are affected more than the diploid eggs

as shown by the high sterility percentage in the F_2(haploid) generation. This strongly suggests that chromosomal rearrangements (translocations, inversions, etc.) causing abnormal pairing relationships at meiosis may be responsible.

2. Strain discrimination in mating behaviour may have broken down giving rise to more diploid eggs.

3. Nucleocytoplasmic interactions are probably involved. This is substantiated by the observable differences in sterility percentages in the F_2(haploid and diploid) generations of the interpopulational reciprocal crosses. It is quite conceivable that the orientation of bivalents during meiosis in the strain hybrid female is dependent on maternally inherited intrinsic properties (Helle and Overmeer, 1973).

There are cases where the F_2 sex-ratio decreased when compared to the sex-ratio of F_1. In these cases, the change is probably a result of normal fluctuation in sex-ratios (5.3.3).

5.3.3 Repeated backcrossing with selection

It has been observed that a resistant population obtained from intensive selection of a susceptible population to Tedion can develop an incompatibility with the susceptible population from which the resistant population is derived (Overmeer, 1965a; 1965b). Overmeer showed that the resistance factor and incompatibility factor are not correlated. To show whether the same phenomenon is operating in the New Zealand strains, repeated backcrossing to PN, with selection at each backcross, was carried out. Schematically, the the procedure was similar to the backcrossing procedure used in the determination of a major factor in resistance. The sex-ratios and

and sterility percentages were determined at each backcross.

The results of sterility percentage and sex-ratio are presented in Appendix 2 and graphically they are shown in Figs. 13 and 14.

Results show that with increasing number of backcrosses, the sterility percentage appears to decrease while the resistance factor is maintained, indicating that the incompatibility factor is not associated with the resistance factor. If the development of an incompatibility barrier due to selection pressure (Overmeer, 1965a) is not associated with the selection for the resistance factor, it is possible to deduce that any acaricide selection pressure will select not only the resistant character but also other characters which will enable the resistant individuals to adapt to the new environment. With inbreeding the resistant individuals with new genotypes can give rise to a new strain. This would probably explain why Overmeer's Tedion resistant strain was incompatible with his susceptible strain. This process has occurred to different extents in the present strains as revealed by the incompatibility data. Since the strains are collected from the fields, selection pressures other than acaricide selection (temperature, light, geographical factors and etc.) are very likely to be involved in the microevolutionary process leading to the development of new strains and consequently of incompatibility barriers.

The increase in fertility in each backcross is an indication that incompatibility is governed by many factors as might be expected.

As the sterility percentage increases, the sex-ratios of some backcrosses (LHRS.PN, PN.LHRS, PN.LINES and PN.SP) increase,

FIG. 14. GRAPH SHOWING THE CHANGE IN SEX-RATIOS (FEMALES:MALES) WITH INCREASING NUMBER OF BACKCROSSES

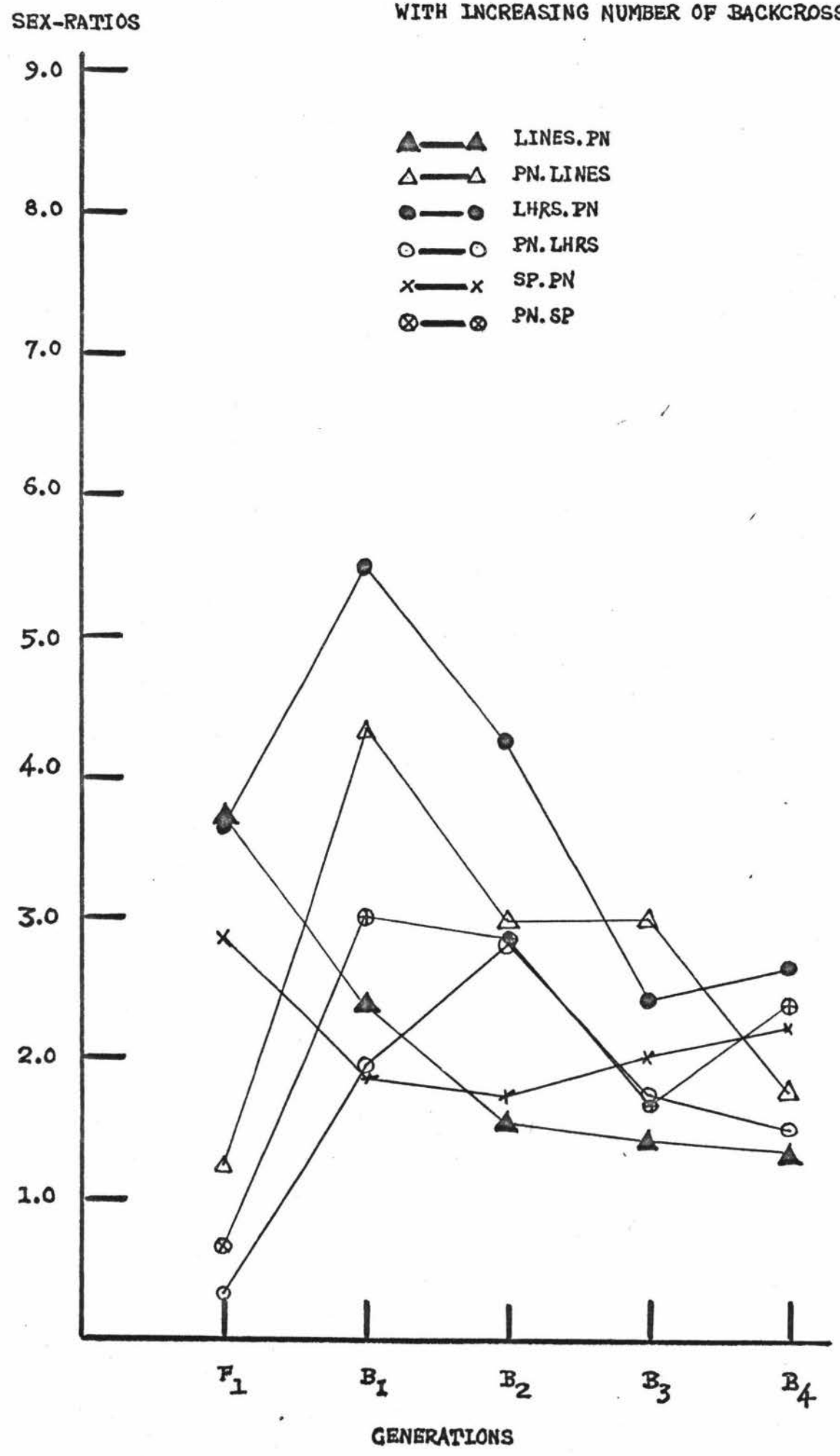
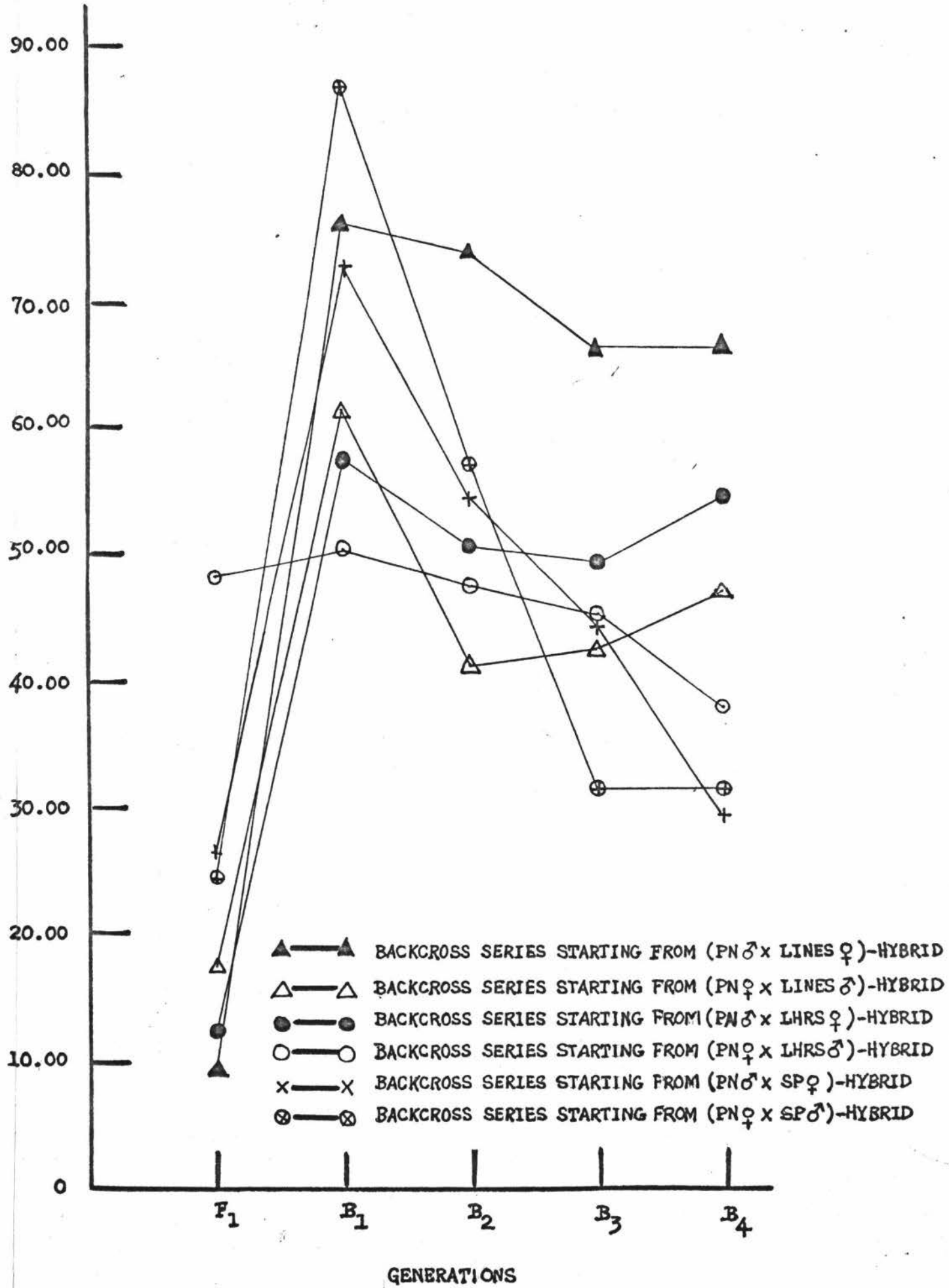


FIG. 13.

GRAPH SHOWING THE CHANGE IN PERCENTAGE EGG STERILITY WITH INCREASING NUMBER OF BACKCROSSES



suggesting that proportionately haploid eggs are affected more than the diploid eggs. In contrast, there are some backcrosses (LINES.PN and SP.PN) whose sex-ratios decrease with increase in sterility percentage. The decrease in the sex-ratio with increase in sterility percentage in the backcrosses of LINES.PN and SP.PN is probably the product of normal fluctuation in the sex-ratios. This is substantiated by the low sex-ratio values in the F_1 generation of similar crosses in Table 10.

Lethality in backcross eggs is probably due to the formation of unbalanced gametes. If the unbalanced gametes are not fertilized (or fertilised) the resulting haploids (or diploids) will end in death. If on the other hand, balanced gametes are formed, the resulting eggs whether diploid or haploid will give rise to viable mites.

By B_2, the sex-ratios approach somewhere between 1.3 and 2.6, values which are approximately the average of the two parents. Although the sex-ratio approximates that of the original parents, the sterility percentage is still not that of the original parents, suggesting that the mortalities in haploid and diploid eggs are occurring at almost the same ratio as that of the original parental eggs.

Of all the backcrosses, only the SP.PN and PN.SP have reached a sterility percentage of approximately 30% by B_4. The other backcrosses have sterility percentages between 38% - 69%; values which are nowhere near the sterility percentage of the parental strains. The data appear to show that the sterility percentage approaches a constant value at B_3 and B_4 and it is likely that further backcrossing may not reduce the level of sterility percentage, with the exception of SP.PN whose graph still shows a declining curve.

5.3.4 Intrapopulational and interpopulational crosses of homozygous backcrossed resistant strains

If the resistance factor is not directly associated with the incompatibility factors, then it also means that the homozygous backcrossed resistant strains, if crossed interpopulationally, will not produce an abnormal degree of egg mortality in the F_1 and F_2. This is based on the assumption that by repeated backcrossing, the resulting offspring will possess a PN genome with the exception of the resistance factor. To show whether the principle is operating in the various homozygous backcrossed resistant strains, selected crosses (i.e. corresponding crosses which showed high egg mortalities in the F_2 haploid generation in Table 10) were undertaken. For the sake of comparison, the intrapopulational crosses were also included.

The results of the intrapopulational and interpopulational crosses are presented in Tables 11 and 12.

The results show that in the intrapopulational crosses, there are various degrees of infertilities which can be considered as normal. However, in the interpopulational crosses, the high mortality in the F_2 haploid eggs show what there is, as before, failure of pairing at meiosis. The reasons are probably due to one or more of the following:

1. Incomplete substitution of normal alleles There is a possibility that the high F_2 egg mortality is due to incomplete substitution of normal alleles. Substitution of normal alleles is probably incomplete in all the homozygous backcrossed resistant strains (LHRS.PN, PN.LHRS, PN.SP, LINES.PN and PN.LINES) with the exception of SP.PN which shows, in contrast to the others, normal sex-ratio and egg sterility in the F_1 and F_2 generations of the int-

Table 11 Intrapopulational and Interpopulational Crosses of Selected Homozygous Backcrossed Resistant Strains of T. urticae.

	% of Unviable Eggs of Mass Crosses Between Different Populations of Homozygous Backcrossed Resistant Strains F_1					% of Unviable Eggs Produced By Teliochrysalids From Mass Crosses Between Different Populations of Homozygous Backcrossed Strains F2(haploid)				
♀♀ \ ♂♂	PN	HNR.PN	LHRS.PN	SP.PN	LINES.PN	PN	HNR.PN	LHRS.PN	SP.PN	LINES.PN
HNR.PN	- -	8.6 (3.7)	6.6 (1.3)	- -	- -	- -	3.8 -	54.1 -	- -	- -
LHRS.PN	18.6 (1.4)	- -	11.8 (3.4)	12.9 (1.3)	- -	51.6 -	- -	13.1 -	72.6 -	- -
SP.PN	4.2 (2.7)	49.8 (0.6)	36.1 (0.9)	4.2 (3.4)	26.1 (1.4)	4.5 -	9.1 -	64.4 -	4.6 -	62.7 -
LINES.PN	2.8 (1.5)	1.9 (1.1)	- -	3.5 (0.9)	2.9 (3.8)	55.2 -	54.9 -	- -	80.9 -	2.2 -

Note: Values within brackets refer to sex ratios

Table 12 Intrapopulational and Interpopulational Crosses of Selected Homozygous Backcrossed Resistant Strains of T. urticae.

	% of Unviable Eggs of Mass Crosses Between Different Populations of Homozygous Backcrossed Resistant Strains F_1					% of Unviable Eggs Produced by Teliochrysalids From Mass Crosses Between Different Populations of Homozygous Backcrossed Strains F_2(haploid)				
♀♀ ♂♂	PN	PN. HNR	PN. LHRS	PN. SP	PN. LINES	PN	PN. HNR	PN. LHRS	PN. SP	PN. LINES
PN. HNR	– –	5.9 (3.1)	8.9 (0.6)	– –	– –	– –	7.9 –	50.7 –	– –	– –
PN. LHRS	13.0 (1.8)	– –	8.9 (2.3)	22.3 (1.7)	– –	64.9 –	– –	– –	80.9 –	– –
PN. SP	21.8 (0.9)	29.9 (0.6)	18.0 (0.2)	2.8 (1.9)	19.2 (0.9)	47.9 –	52.0 –	77.4 –	10.7 –	78.5 –
PN. LINES	10.9 (2.2)	7.9 (2.1)	– –	10.2 (2.4)	2.4 (4.0)	57.0 –	59.3 –	– –	78.8 –	22.8 –

Note: Values within brackets refer to sex ratios.

erpopulational cross with PN. The incomplete substitution is also indicated in the graphs of Fig. 13 which shows that, with the exception of SP.PN, the other backcrosses show a constant sterility percentage of 30% - 70% after the B_3 backcross, probably signifying that substitution cannot proceed any further.

2. New gene combinations New gene combinations which occurred in meiosis in the hybrids and were retained during the backcrossing can possibly contribute to the high F_2 egg mortalities of the interpopulational crosses. The low F_2 egg mortalities in the intrapopulational crosses appear to substantiate this; the new gene combinants seem to be self-compatible. In other words, new genetic strains have been formed in each homozygous backcrossed resistant population and substitution for normal alleles is not complete. If it was complete, probably the incompatibility would disappear since it is assumed that all the homozygous backcrossed resistant strains would then have the same PN genome.

If the backcrossing procedure will not decrease the sterility percentage to normal (as shown by LINES.PN, PN.LINES, PN.SP, LHRS.PN and PN.LHRS), then this may serve an important tool for biological control in the glasshouse. By introducing normal males after an acaricide spray (assuming residual effects are not present), mite numbers can be further reduced. This can be carried on indefinitely if the sterility percentage does not decrease to normal. In the field field, this technique may not be useful because several other strains of mites may be present. The introduction of normal alleles to SP will decrease the degree of sterility percentage and hence will not serve a useful method of biological control.

CHAPTER 6

DISCUSSION

6.1 TOXICOLOGICAL REACTIONS

The various DM-lines are very obvious indications that high levels of parathion-methyl resistance have been developed in many commercial fruit growing areas of New Zealand. Since the mite populations are collected from several areas stretching from Hawkes Bay to Central Otago, the resistance pattern also indicates that the resistance to parathion-methyl in the two-spotted spider mite, T.urticae, is widespread in the country. Considering the fact that mites resistant to one organophosphate chemical are also likely to be resistant to another of the same group (Garman, 1950; Jeppson and Jesser, 1962), the use of organophosphate compounds generally, may not be effective as a means of mite control. With respect to a susceptible population, such as PN, parathion-methyl remains a comparatively effective acaricide. Since this latter population is a suburban garden population however, it would be fair to conclude that the days of effective control by organophosphates in commercial areas are numbered.

The fact that all the DM-lines, with the exception of HNR, show comparatively steep slopes, demonstrate that the various populations are very homogeneous in response to parathion-methyl. Such homogeneity, which can only be reached after considerable selection, no doubt reflects the fact that parathion-methyl has been in commercial use longer than any of the other acaricides. The following

data (Pesticide Manual, 1971) provide some idea of the time of introduction of the chemicals that are used for the present study.

CHEMICAL	YEAR INTRODUCED*
1. parathion-methyl (Folidol)	1949
2. dicofol (Kelthane)	1955
3. formetanate	1967
4. tricyclohexyltin hydroxide (Plictran)	1968

* The year refers to the time the chemical was first produced commercially, not the year the chemical was introduced in New Zealand.

The DM-lines for formetanate are not as steep as those of parathion-methyl. The populations, therefore, do not react as homogeneously as to parathion-methyl.

Considering that formetanate is a recent chemical, the resistance situation may not be one due to formetanate selection. Since the changes in the patterns of resistance to formetanate between the various original strains and those of the homozygous backcrossed resistant strains are very similar to those obtained for parathion-methyl, it is possible to deduce that resistance to the two groups of chemicals is a phenomenon of cross-resistance and not multi-resistance. Cross-resistance between organophosphates and carbamates had also been reported by Hansen et al. (1963). Since cross-resistance between organophosphate and carbamate does occur, probably due to the same mode of cholinesterase inhibition, any pest control programme must surely be carefully thought out if organophosphates and carbamates are involved.

Formetanate was initially selected for the present study because of reports (2.8) that organophosphate resistance in some strains provide cross-resistance to formetanate but in other strains

clear negative correlation has been found. These different responses appeared to be due to allelic differences at the resistance gene (Schulten, 1968) which specifies the cholinesterase enzyme, such that some isozymic variants provide resistance both to organophosphate and carbamate, while other variants are distinctive in their tolerances to the acaricides. It was thought that from amongst five populations selected from different areas of New Zealand for organophosphate resistance, one or more might exhibit negative correlation with formetanate. The possibilities for improved control through alternate use of organophosphate and formetanate might then be considered in the particular cases of such populations. However, the data in the present study give no indications that negative correlation exists between organophosphate and formetanate in any of the strains used.

The low R:S LD50 ratios of the original and the homozygous backcrossed resistant strains with respect to Plictran and Kelthane are probably strong indications that for organophosphate resistant mites, the two groups are still practical and effective in spider mite control in New Zealand.

So far, there is no report of Plictran resistance. Laboratory selection experiments have shown no resistance development even after 42 generations over a period of 14 months and after 29 applications (Allison et al., 1968). Although Plictran resistance did not develop in the laboratory after repeated pressures, this fact should not be taken to mean that resistance would not develop if a larger genetic pool were sampled, such as a field population.

In New Zealand, there is some indication that Kelthane resistance had developed (Harre, 1971; Paice, 1971; MacKenzie, 1971) but

positive evidence is lacking. Up to date, several cases of Kelthane resistance had been reported elsewhere (Zil'bermints et al., 1968; 1969; Helle, 1965a), so probably it will just be a matter of time before Kelthane resistance may appear in New Zealand.

With Kelthane, there is a high number of mites which show a moribund condition, which makes the distinction between dead and living difficult, even after pricking of the mites with a sharp needle (Thiele and Harrison, 1969). The difficulty in distinguishing between living and dead can be attributed to the type of chemical, method of testing, type of solvent and variation in the intrapopulational response. Moreover, Kelthane, when dissolved in water, leaves a heavy deposit which may affect the uniformity of the results. In spite of the many limiting factors in obtaining uniform DM-lines with Kelthane for comparisons of interpopulational tolerances, the present method does serve the purpose of being able to distinguish a resistant population from a susceptible one.

In discussing the toxicity of acaricides, it would be incorrect to assume that one acaricide is better than another for mite control through deductions of the results derived from the slide-dip technique. To confirm the value of each acaricide, a field trial would have to be performed.

Furthermore, the slide-dip method does not allow the commercial potential of the material to be assessed on all stages of mites by systemic, contact and residual actions. The differential effect of acaricides on different mite stages may influence the total effectiveness of a toxicant in practical mite control.

On the other hand, it is clear that accurate laboratory screening tests of acaricides under carefully controlled conditions

can provide a sound basis on which to develop final testing under field conditions. This would save a tremendous amount of effort which is at present being expended on time consuming and sometimes inaccurate and non-quantitative field demonstrations of acaricide performance.

Thus, until such time that a correlation is found between the effectiveness of a chemical as indicated by a practical laboratory testing method and its performanace in the field, the efficacy of an acaricide cannot be adduced purely on responses based on DM-lines derived from any particular laboratory testing method.

In spite of the many disadvantages of the slide-dip technique being used for screening acaricides, it has, at the same time, a number of advantages. These include 1) comparatively less work is involved than with, for instance, topical application methods; 2) standardisation of treatment and post-treatment are not too difficult; 3) no plant materials are involved; 4)the method is also applicable outside the laboratory. For the study of resistance, the slide-dip technique is sufficiently accurate.

Attempts to obtain DM-lines by using the leaf-dip method was unsuccessful. The reason was due to lack of uniformity in the results, caused by a combination of several factors, such as lack of complete randomisation, the gathering of the toxicant along the midrib, which is a preferred site of mites, and loss of mites from the treated surface. In spite of the variable results it produces, the leaf-dip still serves its purpose of acting as a selection technique. A discriminatory dosage, which kills the normal mites completely without affecting the mortality of the resistant mites, can still be obtained.

6.2 GENETICS OF RESISTANCE AND INCOMPATIBILITY

Toxicological tests on the progeny of crosses involving three strains highly resistant to parathion-methyl (LHRS, LINES and SP) and PN revealed certain aspects of the resistances of the strains concerned. Reciprocal crosses between resistant individuals (LHRS, LINES and SP) and PN (normal) individuals produce resistant progeny, indicating that both sexes are capable of transmitting the resistance character. Differences in toxicolgical responses of reciprocal crosses to parathion-methyl implicate cytoplasmic factors in resistance or tolerance. The segregation of the first backcross progeny into a distinct 1:1 ratio of resistant:normal individuals suggests that a single Mendelian gene is the major factor in resistance. Through a process of backcrossing of resistant strains to a susceptible strain, with mild selection of parathion-methyl at each backcross, the resistance factor is not broken down, confirming that resistance is monogenic. A small shift to the left of the DM-lines of the homozygous backcrossed resistant strains suggests that some minor genes may be involved in resistance. Their effects are small and are removed by backcrossing.

The results of the study on resistance factor are in accord with several workers: Taylor and Smith (1956) for Malathion resistance in T.telarius; Andres and Prout (1960) for parathion resistance in T. pacificus; Helle (1962) for parathion resistance in T.urticae; Overmeer (1967) for tedion resistance to T.urticae; Ballantyne and Harrison (1967) for parathion resistance to T.urticae; Schulten (1968) for azinphosmethyl, demeton-S-methyl, parathion and diazinon resistance to T.urticae; Herne and Brown (1969) for parathion resistance to T.urticae; Overmeer and Harrison (1969a) for demeton-

S-methyl resistance to three independent strains of T.urticae; Overmeer and Harrison (1969b) for tetradifon resistance to T.urticae; all of whom conclude that the resistance is due to a major gene.

On the other hand, the results are in contrast to those of Dittrich (1963a; 1963b) and Zil'bermints et al. (1968; 1969). Dittrich concluded that resistance to TEPP and systox was multigenic and characterized by dominant semi-lethal factors and a major recessive Mendelian factor. Criticism of the results of Dittrich has been put forward by Helle (1965a), Ballantyne (1966) and Schulten (1968). Zil'bermints et al. found that their Kelthane reistant strain is due to a recessive gene.

Failure to develop a stable resistant population from the HNR strain by mother x son inbreeding after selection suggests that some factors are present (or absent) in the original HNR population. The presence of semi-lethals or absence of sub-vitals in the HNR are probably the causes for the reversion of the inbred populations to a situation when the susceptible to resistant individuals reaches an approximate ratio of 3:1. Longer development period, higher adult mortality and lower egg numbers in the original HNR population seems to substantiate that vital factors are involved. The homozygous backcrossed resistant strain of HNR (HNR.PN) do not appear to exhibit the phenomenon of reversion and the reason is probably due to the introduction of vital factors from PN.

The results on incompatability threw light on several biological aspects of the two-spotted spider mite in New Zealand. The degrees of sterility or infertility observed, while never complete, indicate that the New Zealand species is divided into many strains which hold sub-specific status. The infertilities among the inter-

populational crosses are apparently caused by the bringing together of two different chromosome complements in a common cytoplasm. The chromosomal basis of sterility is to be inferred most clearly from the high F_2 haploid inviabilities. Being essentially the gametes of their hybrid mothers, the cause of this inviability must be gene deficiencies and duplications generated during meiosis. Such deficiencies and duplications (unbalanced gametes) themselves result from abortive pairing and non-disjunction between homologous chromosomes heterozygous for chromosome rearrangements. It can be concluded that chromosomal polymorphism is a constant and regular factor in spider mite evolution.

The fact that interpopulational crosses also lead to a reduction in the sex-ratio (females:males) of the F_1 compared to the sex-ratio of either of the parents, indicates that diploid eggs are also affected, and suggests that heterozygosity itself (two different gene complements in one genome) may be lethal at some stage of development. This is more difficult to understand, however, since all F_1 hybrids are genetically uniform, and the best explanation seems to be that the cytoplasm is intimately involved. The important role of the cytoplasm in determining degree of resistance, or pigmentation (Ballantyne, 1969) has been demonstrated. It seems that substances, perhaps RNA, is carried over in the egg and can have a pronounced effect on development. In the present study of incompatibility, the significant differences in the F_1 sterility percentages between reciprocal crosses clearly points to the involvement of cytoplasmic factors, or nucleocytoplasmic interactions, in the expression of infertility. Such nucleo-cytoplasmic interactions can also apparently extend their effect to the next generation, as

indicated by the measurable differences in sterility in the F_2 (haploid and diploid; haploid) generations of the interpopulational reciprocal crosses.

In analysing the actual sterilities, it could be shown that the incompatibility factor(s) (e.g. rearrangement) are not associated with the resistance factor. By backcrossing the resistant strains to a common susceptible strain (PN), with mild selection by parathion-methyl at each backcross, the degree of incompatibility is found to decrease. The slow decrease in sterility percentage with each backcross indicates that incompatibility between two strains is not due to one major factor, but several or many (polygenic). Since resistance is not associated with the incompatibility factor(s), it implies that the same selection pressure (i.e. acaricide) can simultaneously select resistant and chromosomally different individuals. Similar to the above have been found by Overmeer (1965a; 1965b).

As hybrid genetic material is replaced by PN in the backcrosses, there is generally an increase in the sex ratio and a decrease in sterility percentage. There are some backcrosses, however, (LHRS.PN, PN.LHRS, PN.SP, LINES.PN and PN.LINES), however, which still maintain a significantly high egg mortality (40-70%) at the B_3 and B_4 generations. The return to normal sex-ratio without a decrease in egg mortality in these cases suggest that the haploid and diploid eggs are affected in the same proportions as the parental populations. In contrast to these backcross series, the SP.PN backcross (showing normal sex-ratio), exhibits declining egg sterility. Even at the B_4 generation, the egg sterility is still declining and it is predictable that the egg mortality will reach the figure of the parental populations.

The contrasting results in egg mortality with regard to the SP.PN backcross and the other backcross series (LHRS.PN, PN.LHRS, LINES.PN, PN.LINES and PN.SP) may provide some useful information as to the biological control of two-spotted spider mites. The biological control of a strain such as SP by the introduction incompatible genes from a different strain (e.g. PN) may not be a worthwhile proposition for two reasons: First, the SP strain may in the long run, become compatible with the recurrent parent. Second, since there is no complete egg mortality in the F_1 (nor in the B_1, B_2, ...etc.), the new strain hybrids or backcross hybrids may form new gene combinations. Such combinations could remain hidden in balanced chromosomal rearrangements (2.10), enabling these strains to achieve greater resistance levels and adaptability to other chemicals and environments. Greater mutability due to hybridisation has also been suggested (Helle and Overmeer, 1973).

In contrast, biological control based on the same principle may be attractive for LHRS and LINES. The constant egg mortality in the backcross generations may imply that the introduc tion of normal alleles (PN males) after an acaricide spray (assuming zero residual effect of toxicant) would theoretically reduce the mite number through biological control. This system of integrated control may be attractive in the glasshouse, where usually only one type of strain is present, but not feasible in the fields where several or many strains may be present.

The egg mortalities of Overmeer's backcrosses (1967) showed some degree of contrast to the results obtained from the backcrosses of LHRS.PN, PN.LHRS, PN.SP, LINES.PN and PN.LINES. In his backcross series starting with (S x T)-hybrids, the mortalities of the untreated

eggs decreased after the B_3 backcross and remained constant in the subsequent backcrosses (averaging 5%). In the backcross series starting with the (T x S)-hybrids, the proportion of non-viable eggs maintained themselves at a level of about 18%. In the backcross series of the present experiments (LHRS.PN, PN.LHRS, PN.SP, LINES.PN, and PN.LINES), the egg mortalities decreased up to the B_3 backcross, and remained constant at a level of 30%-70%, depending on the backcross series. The SP.PN backcross, an exception, exhibits a low egg mortality at the B_4 generation. It is also expected that with increasing number of backcrosses of SP to PN, the egg mortality will reach a constant low value.

The ability of the homozygous backcrossed resistant populations (LHRS.PN, PN.LHRS, LINES.PN, PN.LINES, SP.PN and PN.SP) to multiply randomly without any significant egg mortality indicates that they have recovered a genic balance or harmony which was lacking in the F_1 of the backcross series. The question arises, therefore, as to whether this balanced combination of genes corresponds to the balance which existed initially in PN or whether it represents a new balance. Crosses between the homozygous backcrossed resistant populations and PN males showed high egg mortalities in the haploid F_2, except for the SP.PN x PN. Thus the homozygous backcrossed resistant populations could be considered as the initial stocks of new strains.

The homozygous backcrossed resistant population of SP.PN, when crossed to PN, produces normal F_1 and F_2 egg mortalities. This is probably due to the complete substitution of normal alleles and chromosomes in the backcrossing procedure. With the other backcrosses, there is still indications of high egg mortalities in the

F_1 and F_2 eggs, suggesting that substitution of normal alleles and rearrangements is still incomplete. For the latter backcrosses, the high constant level of egg sterility at and after the B_3 generation may imply that further substitution cannot proceed; otherwise the graph would show a declining sterility percentage curve.

The ability to adapt to acaricide pressure, and the rapid divergence of one population into several strains which bear different degrees of reproductive infertility with one another, signify that the two-spotted spider mite is very polymorphic. As has been shown in insects (e.g. Drosophila), the more polymorphic the species, the larger their geographical distribution. They would generally be capable of adapting to the varying kinds of environments within any distribution area. Species of chromosomal uniformity have limited genetic versatility and tend towards evolutionary stability. The chromosomally polymorphic species on the other hand, have several levels of balance between genetic stability and versatility. Within any particular area, relative uniformity can be sustained, particularly in the absence of strong selective forces; but in the presence of such forces, or under changed environmental conditions, the capacity to create new adaptive types exists, by virtue of the store of genetic variation that has accumulated and been maintained in balanced chromosomal rearrangements. As discussed earlier (2.10), sex limited variation would be especially beneficial, as would be a high mutation rate. There is strong evidence for the existence of both (sex limited traits and a high mutation rate) in spider mites. The tendency to inbreeding in spider mites could work both ways: to maintain uniformity, or to force the break up of balanced rearrangements.

Such a picture accords with the observed situation in the two-spotted spider mite. As far as intrapopulational variability is concerned, the genetic variability (or chromosomal polymorphism) is small, but with regard to the interpopulational variability, the genetic variation is large (high egg sterilities). The reasons must be due to many interacting factors: the haploid-diploid sex determination, a high mutational rate, a high recombination index, a high degree of inbreeding without depressive effects, the holokinetic chromosome structure, the production of new gene combinations by hybridisation. These various factors contribute greatly to the mastery of the two-spotted spider mites in adapting to new environments and ecological niches.

CHAPTER 7

CONCLUSIONS

From the results obtained in the present investigation, the following conclusions can be reached:

1. Resistance to parathion-methyl in the two-spotted spider mite, Tetranychus urticae, seems to be widespread in the commercial fruit growing areas of New Zealand. If the development of parathion-methyl resistance does lead to cross-resistance to other organophosphorus compounds, then alternative organophosphates as acaricides will not be effective for the control of spider mites.

2. Resistance to formetanate shown by strains resistant to parathion-methyl is probably a reflection of the phenomenon of cross-resistance between organophosphates and carbamates. Therefore, the use of carbamates as alternatives to organophosphates in a situation where organophosphate resistance had already developed may not be attractive.

3. Plictran (tricyclohexyltin hydroxide) and Kelthane (dicofol) are still effective acaricides for non-resistant and organophosphate resistant mites. Parathion-methyl is still the most effective acaricide in comparison with the other three acaricides (formetanate, tricyclohexyltin and dicofol) for the control of non-resistant mites under the conditions of testing used in the present study.

4. The parathion resistance in the strains used for the present study is due to a semi-dominant or dominant major gene which is transmissible by both sexes. Cytoplasmic factors or nucleo-cytoplasmic interactions are probably involved to a slight degree in the expression of total resistance. Minor genes which are removable by repeated backcrossing are also probably involved.

5. Reversion which occurs in the HNR population is probably due to the absence of some vital factors.

6. The two-spotted spider mites in New Zealand appear to be comprised of various strains. Intrapopulational crosses of two-spotted spider mites produce progeny which show normal egg sterility and sex ratio in the F_1 and F_2 generations but these values are liable to some degree of fluctuations.

7. Interpopulational crosses of the six strains of T.urticae give rise to various degrees of egg infertilities in the F_1 generation (caused probably by different gene complements in one genome) and high egg mortalities in the F_2 generations (caused probably by chromosomal rearrangements resulting in abnormal pairing at meiosis). Cytoplasmic factors or nucleo-cytoplasmic interactions can be involved to a small extent in the total expression of incompatibility.

8. A reduction in the sex-ratio (females:males) of F_1 in the interpopulational crosses is probably due to the mortality of diploid eggs.

10. Incompatibility between colonies is multifactorial.

11. Some strains, by repeated backcrossing with normal males,

are not completely substituted by normal alleles. If this is the case, this concept can be useful as a means of integrated control of spider mites in enclosed areas (e.g. glasshouses).

12. Strain or backcross hybrids, on allowing to multiply randomly, are capable of forming new gene combinations to become self-compatible. Through the same process, the hybrids can give rise to new strains which are totally different from the parents.

REFERENCES

ABBOTT, W.S. 1925.
A method of computing the effectiveness of an insecticide.
J. Econ. Entomol. 18: 265-267.

ALLISON, W.E., POTY, A.E., HARDY, J.L., KENAGA, E.E. and WHITNEY, W.K. 1968.
Laboratory evaluations of Plictran miticide against two-spotted spider mites.
J. Econ. Entomol. 61: 1254-1257.

ANDRES, L.A. and PROUT, T. 1960.
Selection response and genetics of parathion resistance in the Pacific Spider Mite, Tetranychus pacificus.
J. Econ. Entomol. 53:626-30.

ARGANIN, B. 1958.
The bionomics of the red spider mites on beans.
Rev. Appl. Ent. (A), 49: 510, 1961.

ASCHER, K.R.S. and BERGMANN, E.D. 1962.
Ent. exp. & appl. 5: 88-
Cited in Georhiou (1965).

AUERBACH, C. 1956.
"Genetics in the Atomic Age", Oliver & Boyd. 157 pp.

BALLANTYNE, G.H. 1966.
Genetics of resitance in the spider mite, Tetranychus species.
Thesis for M. Agric. Sci., Lincoln College, New Zealand.

BALLANTYNE, G.H. and HARRISON, R.A. 1967.
Genetic and biochemical comparisons of organophosphate resistance between strains of spider mites (Tetranychus species: ACARI).
Ent. exp. & appl. 10: 231-9.

BALLANTYNE, G.H. 1969.
Genetic fine structure and complementation at the albino locus in spider mites.
Genetica 40: 289-323.

BEARD, R.L. 1952.
Effect of sub-lethal doses of toxicants on susceptibility of insects to insecticides.
J. Econ. Entomol. 45: 561-67

BENGSTON, M. 1965
Overwintering behaviour of Tetranychus telarius (L) in the Stanthorpe district, Queensland.
Old. J. Agric. Anim. Sci. 22: 169-176.

BOGNAR, S. 1960.
Megfigyelesek a gyumoksfakon elo takaccatkafajok populacios dinamikajat befolyasolo tenyezokkel kapcsolatban, kulonos tekintettel a DDT tartalmu keszitmenyekre (Observations on the effect of various factors on the dynamics of red-spider populations, with special reference to preparations of DDT).
Rev. App. Ent. (A) 49: 665, 1961.

BOHM, H. 1961.
Untersuchungen uber die Spinnmilbenbiozonose (Tetranychidae) au Obstgeholzen in Osterreich (Investigations on the spider mite biocoenosis (Tetranychidae) on fruit trees and bushes in Austria.
Rev. App. Ent. (A) 51: 472, 1963.

BONDARENKO, N.V. 1958.
Characteristics of the diapause in Tetranychus urticae Koch.
Rev. App. Ent. (A) 48: 395, 1960.

BOUDREAUX, H.B. 1956.
A revision of the two-spotted spider mite complex, Tetranychus telarius L.
Ann. Ent. Soc. Amer. 49: 43-48.

BOUDREAUX, H.B. 1963.
Biological aspects of some phytophagous mites.
Ann. Rev. Entomol. 8: 137-54.

BRAVENBOER, L. 1959.
De chemische en biologische bestrijding van de spintmijt Tetranychus urticae Koch (In Dutch, English summary).
Diss. Landbouwhogesch. Wageningen.
Cited in Vrie et al. (1972).

BROWN, A.W.A. 1958.
Insecticide resistance in Arthropods.
WHO (Geneva) Monograph Series No.38. 240 pp.

BROWN, A.W.A. 1959.
Inheritance of insecticide resistance and tolerance.
Misc. Publ. Ent. Soc. Amer. 1:20-26.

BROWN, A.W.A. 1960.
Mechanisms of resistance against insecticides.
Ann. Rev. Ent. 5: 301-326.

BROWN, A.W.A. 1961.
a. The challenge of insecticide resistance.
Bull. Ent. Soc. Am. 7: 6-19.

b. Studies on the biochemistry and genetics of insecticide resistance in mosquitoes.
A progress report. Proc. 48th Ann. Meeting New Jersey Mosquito Exterm. Assoc.

b.Protective mechanisms against nerve poisons in insects. Trans. Roy. Soc. Canada 55: 25-35.

BROWN, A.W.A. 1972.
Insecticide resistance comes of age.
In "Readings in Entomology" Ed. P. Barbosa and T.M. Peters. p. 410-416.

BUND, C.F. van de and HELLE, W. 1960.
Investigations on the Tetranychus urticae complex in North-west Europe.
Ent. exp. & appl. 3: 142-156

BUSVINE, J.R. 1956.
Resistance of insects to insecticides: Occurrence and status of insecticide resistant strains.
Chem. & Ind. p. 1190-1032.

CAGLE, L.R. 1949
Life history of the two-spotted spider mite.
Tech. Bull. Va. Agric. Expt. Sta. 113: 1-31

COMPTON, C.C. and KEARNS, C.W. 1937.
Improved control of red spider on greenhouse - with sulphur and cyclohexylamine derivatives.
J. Econ. Entomol. 30: 512-522.

CONE, W.W., McDONOUGH, L.M., MAITLEN, J.C. and BURDAJEWICZ, S. 1971.
Pheromone studies of the two-spotted spider mite.
1. Evidence of a sex hormone.
J. Econ. Entomol. 64: 355-58

CRAIG, G.B. and HICKEY, W.A. 1967.
Genetics of Aedes aegypti.
In "Genetics of Insect Vectors of Diseases". Ed. J.W. Wright & Pal. Chap. 3:67-128. Amsterdam: Elsevier. 794 pp.

CROW, J.F. 1952.
Bull. Natl. Research Council (U.S.), No. 219, 72-75.
Cited in Crow (1957).

CROW, J.F. 1954.
Analysis of a DDT-resistant strain of Drosophila.
J. Econ. Entomol. 47: 393 -98.

CROW, J.F. 1957.
Genetics of insect resistance to chemicals.
Ann. Rev. Entomol. 2: 227-260.

CUNHA da, A.B. and KERR, W.E. 1957.
A genetical theory to explain sex determination by arrhenotokous parthenogenesis.
Forma et Functio 1:33-36.
Cited in Kerr (1962).

CURTIS, C.F. 1968.
A possible genetic method for the control of insect pests, with special reference to tzetze flies (Glossina species)
Bull. Entomol. Res. 57: 509-523.

DIELEMAN, J. and OVERMEER, W.P.J. 1973.
Preferential mating hampering the possibility to apply a genetic control method against a population of Tetranychus urticae Koch by means of release of incompatible males.
Z. Angew. Entomol. In press - cited in Helle & Overmeer (1973).

DITTRICH, V. 1961.
Populations genetische Untersuchungen on normalen und phosphorsaureester-resistenten stammen von Tetranychus urticae Koch.
Z. Angew. Entomol. 48: 34-57.
Cited in Ballantyne (1966).

DITTRICH, V. 1962.
A comparative study of toxicological test methods on a population of the two-spotted spider mite, Tetranychus telarius.
J. Econ. Entomol. 55: 644-648.

DITTRICH, V. 1963.

a. Genetics and dynamics of o-p resistance in strains of Tetranychus urticae K. (=telarius)
Adv. in Acarol. 1: 238-247.

b. A recessive factor of o-p resistance in population of Tetranychus urticae Koch.
J. Econ. Entomol. 56: 182-184.

c. Investigations of o-p resistance of two genetically differing populations of T.urticae Koch.
Ent. exp. & appl. 6 : 10-20.

EYNDHOVEN van, G.L. and HELLE, W. 1966.
Sex abnormalities in the common spider mite, Tetranychus urticae.
Ent. Ber., Amst. 26: 204-208
Cited in Overmeer (1967).

FINNEY, D.J. 1952.
"Probit Analysis". Cambridge University Press. 318 pp.

FISHER, R.A. 1929
"The Genetical Theory of Natural Selection". Clarendon Press. Oxford. 141 pp.

FOOTT, W.H. 1964
Geotactic responses of the two-spotted spider mite, Tetranychus urticae Koch (ACARINA: Tetranychidae). Proc. ent. Soc. Ont. 95: 106-108.
Rev. App. Ent. (A) 54:350, 1966.

FORGASH, A.J. and HANSENS, E.J. 1959.
Cross resistance in a Diazinon resistant strain of Musca domestica (L.)
J. Econ. Entomol. 52: 733-739.

GARMAN, P. 1950.
Parathion-resistant red spider.
J.Econ. Entomol. 43: 53-56.

GASSER, R. 1951.
a. Zur Kenntnis der gemeinen Spinnmilbe Tetranychus urticae Koch. 1. Mitteilung: Morphologie, Anatomie, Biologie und Oekologie. (Contributions to knowledge of the common spider mite, T. telarius.
First communicatkon: Morphology, Anatomy, Biology and Ecology.
Rev. App. Ent. (A) 41: 398, 1953.

b. Zur Kenntnis der gemeinen Spinnmilbe Tetranychus urticae Koch.
Mitt. Schweiz. ent. Ges. 24: 217-262.
Cited in Overmeer (1967).

GASSER, R. 1953.
Uber ein neues Insektizid mit breitem Wirkungsspektrum (On a new insecticide with a wide range of effectiveness)
Rev. App. Ent. (A) 41: 399, 1953.

GEORGHIOU, G.P. 1965.
Genetic studies on insecticide resistance.
Adv. Pest Control. Res. 6: 171-230

GEORGHIOU, G.P., METCALF, R.L. and MARCH, R.F. 1961
The development and characterization of resistance to carbamate insecticides in the house-fly, Musca domestica
J. Econ. Entomol. 54: 132-140.

GOLDSCHMIDT, R. 1934.
The influence of the cytoplasm upon gene controlled heredity.
Am. Natur. 68: 5-23.

HADAWAY, A.B. 1956
Cumulative effect of sub-lethal doses of insecticides on houseflies.
Nature (Lond) 178: 149-150

HAMILTON, W.D. 1967.
Extraordinary sex-ratios.
Science 156: 477-88.

HANSEN, C.O., NAEGELE, J.A., and EVERETT, H.E. 1963.
Cross-resistance patterns in the two-spotted spider mite, Tetranychus telarius (L.)
Adv. in Acarol. 1: 257-75.

HARRE, A.J. 1971
Horticultural Advisory Officer, N.Z. Dept. Agric., Nelson.
Personal communication.

HARRISON, R.A. and SMITH, A.G. 1960.
Effect of relative humidity on hatching and on toxicity of acaricides to eggs of the red spider mite, Tetranychus telarius (L.) (Acarina: Tetranychidae).
Nature (Lond) 186: 409-

HELLE, W. 1962.
Genetics of resistance to o-p compounds and its relation to diapause in Tetranychus urticae Koch (Acari).
Tijdschr. Plziekt 68: 155-197

HELLE, W. 1965.
a. Insecticide resistance in Acarina : mites
Adv. in Acarol. 2: 71-93.

b. Inbreeding depression in an arrhenotokous mite, Tetranychus urticae.
Ent. exp. & appl. 8: 299-304.

c. Population genetics of arrhenotokous mites.
Boll. Zool. Agr. Bachicolt Serie 11, 7: 219-25

HELLE, W. 1966.
Personal communication.
Cited in Schulten (1968).

HELLE, W. 1967.
Fertilization in the two-spotted spider mite.
Ent. exp. & appl. 10: 103-10.

HELLE, W. 1968.
a. Genetic variability of photoperiodic response in an arrhenotokous mite (Tetranychus urticae Koch)
Ent. exp. & appl. 11: 101-13.

b. New developments toward biological control of the two-spotted spider mite by incompatible genes.
Rev. App. Ent. (A) 58: 590, 1970.

HELLE, W. 1969.
Genetics and cytogenetics of spider mites.
Proc. Int. Congr. Acarol., 2nd , Sutton, Bonington, 1967, 479-83. Cited in Helle and Overmeer,(1973).

HELLE, W. and BOLLAND, H.R. 1967.
Karyotypes and sex determination in spider mites (Tetranychidae).
Genetics 38: 43-53.

HELLE, W. and van de BUND, C.F. 1962.
Crossbreeding experiments with some species of the Tetranychus urticae group.
Ent. exp. & appl. 5: 159-165.

HELLE, W. and PIETERSE, A.H. 1965.
Genetic affinities between adjacent populations of spider mites (Tetranychus urticae).
Ent. exp. & appl. 8: 305-308

HELLE, W. and OVERMEER, W.P.J. 1973.
Variability in Tetranychid mites.
Ann. Rev. Entomol. 18: 96-120.

HELLE, W. and van ZON, A.Q. 1967.
Rates of spontaneous mutation in certain genes of an arrhenotokous mite, Tetranychus pacificus.
Ent. exp. & appl. 10: 189-193.

HELLE, W. and van ZON, A.Q. 1970.
Linkage studies in the pacific spider mite, Tetranychus pacificus. II. Genes for white eye, lemon and flamingo.
Ent. exp. & appl. 13: 300-6.

HERNE, D.H.C. and BROWN, A.W.A. 1969.
Inheritance and biochemistry of o-p resistance in a New York strain of the two-spotted spider mite.
J. Econ. Ent. 62: 205-9.

HOSKINS, W.M. and GORDON, H.T. 1956.
Arthropod resistance to chemicals.
Ann. Rev. Entomol. 1: 89-122.

HUSSEY, H.W. and PARR, W.J. 1963.
Dispersal of the glasshouse red spider mite, Tetranychus urticae Koch (Acarina: Tetranychidae).
Ent. exp. & appl. 6: 207-214.

JEPPSON, C.R. and JESSER, M.J. 1962.
Laboratory studies on resistance of the pacific spider mite to acaricides.
J. Econ. Entomol. 55: 78-82.

KEH, B. 1952.
Mating experiments with the two-spotted spider mite complex.
J. Econ. Entomol. 45: 308-312.

KEIDING, J. 1963.
Possible reversal of resistance.
Bull. Wld. Hlth. Org. No. 29 p. 51-62.

KELLY-GARVERT, F. and LEGATOR, M.S. 1973.
Cytogenetic and mutagenic effects of DDT and DDE in a Chinese Hamster cell line.
Mutation Resdarch 17: 223-229.

KERR, W.E. 1962.
Genetics of sex determination.
Ann. Rev. Entomol. 7: 157-176.

KIMURA, T. and BROWN, A.W.A. 1964.
DDT-dehydrochlorinase in Aedes aegypti.
J. Econ. Ent. 57: 710-6.

KING, J.C. 1954.
The genetics of resistance to DDT in Drosophila melanogaster.
J. Econ. Entomol. 47: 387-93.

KING, J.C. 1955.
Integration of the gene pool as demonstrated by resistance to DDT.
Am. Natur. 89: 39-46.

LAVEN, H. 1963.
Eradicating mosquitoes using translocations.
Nature (Lond) 221: 958-959.

LAVEN, H. 1967.
Eradication of Culex pipens fatigans through cytoplasmic incompatibility.
Nature (Lond) 216: 383-384

LEES, A.D. 1953.
Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite, Metatetranychus ulmi Koch.
Ann. Appl. Biol. 40: 449-486

LERNER, I.M. 1954.
"Genetic Homeostasis". John Wiley & Sons, Inc., N.Y. 134 pp.

LICHTWARDT, E.T., BRUCE, W.N. and DECKER, G.C. 1955.
Notes on the inbreeding of houseflies.
J. Econ. Entomol. 48: 301-303.

LINDGREN, D.L. and DICKSON, R.C. 1945.
Repeated fumigation with HCN and the development of resistance in the Californian Red Scale.
J. Econ. Entomol. 38: 296-99.

LIPKE, H. and KEARNS, C.W. 1958.
Specificity of housefly. DDT-dehydrochlorinase.
Bull. Ent. Soc. Amer. 4: 95-

Lloyd, C.J. anc PARKIN, E.A. 1963.
Further studies on a pyrethrum-resistant strain of granary weevil, Sitophilus granarius (L.).
J. Sci. Food Agric. 14: 655-663.

MacKENZIE, D.J. 1971.
Technical Adviser, N.Z. Fruitgrowers' Federation Limited.
Personal communication.

MAELZER, D.A. and KIRK, R.L. 1953.
A preliminary study of the genetics of DDT resistance in houseflies.
Aust. J. Biol. Sc. 6: 244-256.

MARCH, R.B. 1958.
The chemistry and activity of acaricides.
Ann. Rev. Ent. 3: 355-376.

MARCH, R.B. 1960.
Biochemical aspects of organophosphorus resistance.
Misc. Publs. ent. Soc. Am. 2: 139-144.

MARKERT, C.L. and URSPRUNG, H. 1971.
"Developmental Genetics". Prentice-Hall. 214 pp.

MATHER, K. 1943.
Polygenic inheritance and natural selection.
Biol. Rev. (Cambridge) 18: 32-64.

MATSUMARA, F. and VOSS, G. 1964.
Mechanisms of malathion and parathion resistance in the two-spotted spider mite, Tetranychus urticae.
J. Econ. Entomol. 57: 911-917.

McENROE, W.D. 1967.
Genetic variation in a two-spotted spider mite, Tetranychus urticae, population plateaued by directed selection (Acarina: Tetranychoidae).
Ann. Entomol. Soc. Amer. 60: 1080-83.

McENROE, W.D. 1969.
The evolutionary significance of arrhenotoky: The Matherian population.
Advan. Acarol. 3: (in press).
Cited in McEnroe and Lakocy (1969).

McENROE, W.D. and HARRISON, R. 1968.
The role of linkage in the response of an arrhenotokous spider mite, Tetranychus urticae, to directed selection.
Ann. Entomol. Soc. Amer. 61: 1239-47.

McENROE, W.D. and KOT, J. 1968.
Evolution of o-p resistance and fitness in a hybrid swarm of the two-spotted spider mite, Tetranychus urticae.
Ann. Entomol. Soc. Amer. 61: 1255-9.

McENROES, W.D. and LAKOCY, A. 1969.
The development of Pentac resistance in an outcrossing swarm of the two-spotted spider mite.
J. Econ. Entomol. 62: 283-286.

MCENROE, W.D. and NAEGELE, J.A. 1968.
The co-adaptive process in an organophosphorus-resistant strain of the two-spotted spider mite, Tetranychus urticae.
Ann. Entomol. Soc. Amer. 61: 1055-9.

MELANDER, A.L. 1914.
Can insects become resistant to sprays?.
J. Econ. Entomol. 7: 167-173.

MERELL, D.J. and UNDERHILL, J.C. 1956.
Selection for DDT resistance in inbred laboratory and wild stocks of Drosophila melanogaster.
J. Econ. Entomol. 49: 300-6.

METTLER, L.E. and GREGG, T.G. 1969.
"Population Genetics and Evolution". Prentice-Hall. 212 pp.

MILANI, R. 1958.
a. Genetics of insecticide resistance in the housefly.
WHO seminar on the susceptibility of insects to insecticides, New Delhi, pp. 62-68.

b. Genetical considerations on insect resistance to insecticides,
WHO seminar on the susceptibility of insects to insecticides. Panama pp. 193-207.

MITCHELL, R. 1972.
The sex-ratio of the spider mite, Tetranychus urticae.
Ent. exp. & appl. 15: 299-304.

MORELLO, A. 1964.
Role of DDT-hydroxylation in resistance.
Nature 203: 785-6.

NAEGELE, J.A. and JEFFERSON, R.N. 1964.
Floricultural Entomology.
Ann. Rev. Ent. 9: 319-331.

OPPENOORTH, F.J. 1958.
A mechanism of resistance to parathion in Musca domestica (L.).
Nature (Lond.) 181: 425-426.

OPPENOORTH, F.J. 1965.
Biochemical genetics of insecticide resistance.
Ann. Rev. Entomol. 10: 185-206.

OVERMEER, W.P.J. 1965.
a. Intersterility as a consequence of tedion selections in Tetranychus urticae Koch.
Boll. Zool. Agri. Bachicolt, Serie 11, 7: 227-31

b. Peculiarities of resistance to tedion in Tetranychus urticae Koch.
Meded. Landbouwhogesch. Opzoekingssta Staat Gent.
30: 1400-2.

OVERMEER, W.P.J. 1967.
Genetics of resistance to tedion in Tetranychus urticae Koch.
Arch. Neerlandaises de zoologie 17: 295-349.

OVERMEER, W.P.J. and HARRISON, R.A. 1969.
a. Genetical studies of resistance to organophosphate in New Zealand populations of Tetranychus urticae Koch (Acarina: Tetranychidae).
N.Z. Jl. Sci. 12: 889-903

b. Genetical studies of resistance to tetradifon in New Zealand populations of Tetranychus urticae Koch (Acarina: Tetranychidae)
N.Z. Jl. Sci. 12: 904-919.

c. Notes on the control of the sex-ratio in populations of the two-spotted spider mite, Tetranychus urticae.
N.Z. Jl. Sci. 12: 920-928.

OVERMEER, W.P.J., van ZON, A.Q. and HELLE, W. 1972.
Androgenesis in spider mites.
Ent. exp. & appl. 15: 256-58.

PAICE, E.J. 1971.
Senior Field Representative. N.Z. Fruitgrowers' Federation Limited. Auckland.
Personal communication.

PALMER, K.A., GREEN, S. and LEGATOR, M.S. 1972.
Cytogenetic effects of DDT and derivatives of DDT in a cultured mammalian cell line.
Toxicol. Appl. Pharmacol. 22: 355-364.

PARR, W.J. and HUSSEY, N.W. 1961.
The specific status of some European strains of the glasshouse red spider mite.
Report Glasshouse Crops Res. Inst. 111 pp. 91-93.

PARR, W.J. and HUSSEY, N.W. 1960.
Further studies on the reproductive isolation of geographical strains in the Tetranychus telarius complex.
Ent. exp. & appl. 3: 137-141.

PESTICIDE MANUAL 1971
2nd Edition. Issued by British Crop Protection Council.
Ed. Hubert Martin.

PIMENTEL, D., DEWEY, J.E. and SCHWARDT, H.H. 1951.
An increase in the duration of the life-cycle of DDT-resistant strains of the housefly.
J. Econ. Entomol. 44: 477-81.

PIMENTEL, D., DEWEY, J.E. and SCHWARDT, H.H. 1953.
Development and loss of insecticide resistance in the housefly.
J. Econ. Entomol. 46: 295-98.

PRITCHARD, A.E. and BAKER, E.W. 1955.
A revision of the spider mite family Tetranychidae.
Mem. Ser. vol. 2. Pacific Coast Ent. Soc. San Francisco.

RAMBIER, A. 1958.
Les tetranyques nuisibles a la vigne en France continentale.
Rev. Zool. agric. 57: no. 1-3 pp 1-20.
Rev. App. Ent. (A) 48: 108, 1960.

ROULSTON, W.J. 1969.
Mechanisms of insecticide resistance.
Australian applied entomological research conference
8: 1-6

ROULSTON, W.J., SCHNITZERLING, H.J. and SCHUNTER, C.A. 1968.
Acetylcholinesterase insensitivity in the Biarra strain of the cattle tick Boophilus microplus as a cause of resistance to organophosphate and carbamate acaricides.
Aust. J. biol. Sci. 21: 759-67.

ROULSTON, W.J., SCHUNTNER, C.A., SCHNITZERLING, H.J. and WILSON, J.T. 1969
Detoxification as a mechanism of resistance in a strain of the cattle tick, Boophilus microplus (Canestrini) resistant to organophosphate and carbamate compounds.
Aust. J. biol. Sci. 22: 1585-9.

SABA, F. 1961.
Uber Entwicklung und Ruckgang von Gifftresistenz bei Tetranychus urticae Koch und dersen Abhangigkeit von der wirtspflanze (in German, English summary).
Z. Angew. Entomol. 48: 265-293.

SCHULTEN, G.G.M. 1966.
Genetics of resistance to parathion and demeton-S- methyl in Tetranychus urticae Koch (Acarina).
Genetica 37: 207-217.

SCHULTEN, G.G.M. 1968.
Genetics of organophosphate resistance in the two-spotted spider mite, Tetranychus urticae Koch.
Koninklijk Instituut Voor De Tropen.

Schrader, F. 1923.
Haploidie bei einer spinnmilbe.
Arch. Mikrosk. Anat. Entwicklungmech. 97: 610-622.
Cited in Helle (1962).

SEREBROVSKY, A.S. 1940.
Cited in Laven (1969)

SMISSAERT, H.R. 1964.
Cholinesterase inhibition in spider mites susceptible and resistant to organophosphates.
Science 143: 129-131

SMITH, A.J. 1946.
Farming in South Africa. 20:753-760.
Cited in Hoskins and Gordon (1956).

SMITH, F.F., BOSWELL, A.L. and WEBB, R.E. 1969.
Segregation between strains of carmine and green two-spotted spider mites.
Proc. Int. Congr. Acarol., 2nd, Sutton Bonington 1967 pp 155-59.
Cited in Helle and Overmeer (1973).

SUOMALAINEN, E. 1962.
Significance of parthenogenesis in the evolution of insects.
Ann. Rev. Entomol. 7: 349-366.

TAYLOR, E.A. and SMITH, F.F. 1956.
Transmission of resistance between strains of two-spotted spider mites.
J. Econ. Entomol. 49: 858-859.

THIELE, G.F. and HARRISON, R.A. 1969.
Toxicity of selected acaricides to susceptible and resistant strains of Tetranychus urticae Koch.
N.Z. Jl. Sci. 12: 380-394.

THODAY, J.M., GIBSON, J.B. and SPICKETT, S.G. 1964.
Regular response to selection. 2. Recombination and accelerated response.
Genet. Res. 5: 1-19.

TSUKAMOTO, M. 1963.
The log-dosage-probit mortality curve in genetic researches of insect resistance to insecticides.
Botyu-Kagaku 28: 91-98.

UNTERSTENHOFER, G. 1960.
The development of resistance to insecticides and acaricides.
Hofchen-Briefe 13: 141-150.

UNTERSTENHOFER, G. 1961.
Development of spider mite resistance to acaricides.
Hofchen-Briefe 14: 1-6.

UNWIN, B. 1971.
Biology and control of the two-spotted spider mite, Tetranychus urticae Koch.
J. Aust. Inst. Agric. Sci. 37: 192-211.

VARZANDEH, M., BRUCE, W.N. and DECKER, G.C. 1954.
Resistance to insecticides as a factor influencing the biotic potential of the housefly.
J. Econ. Entomol. 47: 129-134.

VEERMAN, A. 1970.
The pigments of Tetranychus cinnabarinus Boisd. (Acari: Tetranychidae).
Comp. Biochem. Physiol. 36:749-763.

VEERMAN, A. 1972.
Carotenoids of wild-type and mutant strains of Tetranychus pacificus McGregor (Acari: Tetranychidae).
Comp. Biochem. Physiol. 42B: 329-340.

VOSS, G. 1961.
Populationsgenetische Untersuchungen an normalen und phosphorsaureester-resistenten Stamen von Tetranychus urticae.Koch.
Zeitschr. ang. Ent. 48: 34-57.

VOSS, G. and MATSUMARA, F. 1964.
Resistance to organophosphorus compounds in the two-spotted spider mite: Two different mechanisms of resistance.
Nature (Lond.) 202: 319-320.

VRIE van de, McMURTRY, J.A. and HUFFAKER, C.B. 1972.
Ecology of tetranychid mites and their natural enemies: A review. III. Biology, Ecology and Pest Status and Host-Plant Relations of Tetranychids.
Hilgardia 41: 343-432.

WATSON, D.L. 1956.
Some genetic aspects of resistance to parathion in the two-spotted spider mite, Tetranychus telarius (L.)
Dissertation abstract 16: 216-217.

WATSON, D.L. and NAEGELE, J.A. 1960.
The influence of selection pressure on the development of resistance in populations of Tetranychus telarius (L.)
J. Econ. Entomol. 53: 80-84.

WHO EXPERT COMMITTEE ON INSECTICIDES. 1957.
WHO Tech. Rept. Serv. 7th Rept. 125.

WHITING, P.W. 1943.
Multiple alleles in complementary sex determination of Habrobracon.
Genetics 28: 365-82.

WRIGHT, J.W. and PAL, R. 1967.
Genetics of insect vectors of disease. Elsevier.
Amsterdam.

ZON van, A.Q. and HELLE, W. 1966.
a. Albinism as a marker in Tetranychus pacificus.
Ent. exp. & appl. 9:206-9

b. Pigment mutations in Tetranychus pacificus.
Ent. exp. & appl. 9: 402-403.

ZON van, A.Q. and HELLE, W. 1967.
Linkage studies in the pacific spider mite, Tetranychus pacificus. 1. Genes for pigmentless, white-eyed, stork and organophosphate resistance.
Ent. exp. & appl. 10: 69-74.

ZON van A.Q. and OVERMEER, W.P.J. 1972.
Induction of chromosome mutations by X-irradiation in Tetranychus urticae Koch (Acarina: Tetranychidae) with respect to a possible genetic method of control.
Ent. exp. & appl. 15: 195-203.

ZON van, A.Q., OVERMEER, W.P.J. and HELLE, W. 1964.
Resistance to tedion in haploid and diploid offspring of Tetranychus urticae Koch.
Ent. exp. & appl. 7: 270-6.

ZIL'BERMINTS, I.V., FADEEV, Y.N. and ZHURAVLEVA, L.M. 1968.
On the inheritance of Kelthane resistance in the two-spotted spider mite.
Selskohozyaistwennaya Biologiya 3: 125-32- in Russian.
Cited in Helle and Overmeer (1973).

ZIL'BERMINTS, I.V., FADEEV, Y.N. and ZHURAVLEVA, L.M. 1969.
Investigation of the genetics of resistance in the laboratory strains of spider mite, Tetranychus urticae Koch.
Genetika Akad. Nauk. SSSR. 5: 96-106 - in Russian.
Cited in Helle and Overmeer (1973).

Appendix 1 Results of the Dosage-Mortality Response of T. urticae to parathion-methyl, formetanate and tricyclohexyltin hydroxide

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	PN	100	131	98.00	98.00
		50	129	46.00	44.00
		25	129	6.00	2.00
		0	129	4.00	0
PARATHION	ETTR	1,000	132	100.00	100.00
		500	134	91.00	91.00
		250	130	52.00	49.00
		100	65	8.00	3.00
		0	131	5.00	0
PARATHION	LINES	40,000	132	99.00	99.00
		30,000	132	88.00	87.00
		20,000	147	70.00	69.00
		10,000	128	24.00	22.00
		5,000	67	6.00	3.00
		0	129	3.00	
PARATHION	LHRS	80,000	64	100.00	100.00
		60,000	132	95.00	95.00
		40,000	132	80.00	78.00
		20,000	131	18.00	12.00
		0	127	6.00	0

TOXICANT	STRAIN, CROSS, OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	SP	60,000	127	78.00	77.00
		40,000	121	42.00	40.00
		20,000	132	14.00	10.00
		0	131	4.00	0
PARATHION	HNR	20,000	131	96.94	96.77
		10,000	133	91.73	91.20
		5,000	130	77.69	76.21
		2,500	129	77.52	76.03
		1,000	125	74.73	73.05
		500	123	74.57	72.88
		250	118	75.43	73.79
		100	127	56.69	53.78
		50	124	33.87	29.40
		25	64	12.50	6.57
		0	126	6.35	0
FORMETANATE	PN	1,000	96	100.00	100.00
		500	65	82.00	80.00
		200	61	38.00	33.00
		100	192	31.00	26.00
		50	188	30.00	25.00
		25	140	22.00	16.00
		25/2	96	11.00	5.00
		0	86	7.00	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
FORMETANATE	ETTR	10,000	67	100.00	100.00
		5,000	63	92.00	92.00
		2,000	95	88.00	88.00
		1,000	98	69.00	68.00
		500	93	48.00	46.00
		200	91	19.00	16.00
		100	91	8.00	4.00
		0	100	4.00	0
FORMETANATE	LINES	50,000	90	77.00	77.00
		20,000	117	49.00	48.00
		10,000	117	25.00	24.00
		5,000	123	11.00	10.00
		2,000	81	1.00	0
FORMETANATE	LHRS	100,000	96	79.00	77.00
		50,000	94	74.00	71.00
		20,000	121	56.00	51.00
		10,000	94	32.00	24.00
		5,000	89	27.00	19.00
		2,000	85	14.00	4.00
		0	101	10.00	0
FORMETANATE	SP	100,000	91	95.00	94.00
		50,000	96	91.00	90.00
		20,000	91	85.00	83.00
		10,000	87	60.00	53.00
		5,000	97	48.00	40.00
		0	79	14.00	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	PN	20,000	122	87.00	85.00
		10,000	132	60.00	54.00
		5,000	119	67.00	62.00
		2,000	115	47.00	39.00
		1,000	124	17.00	4.00
		500	56	18.00	5.00
		200	57	14.00	1.00
		0	122	13.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	ETTR	40,000	96	100.00	100.00
		20,000	95	92.00	91.00
		10,000	131	50.00	49.00
		5,000	131	15.00	13.00
		2,000	130	15.00	13.00
		1,000	131	11.00	9.00
		500	59	8.00	6.00
		0	126	2.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	LINES	100,000	100	100.00	100.00
		50,000	92	84.00	82.00
		20,000	89	28.00	19.00
		0	94	12.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	LHRS	100,000	102	100.00	100.00
		50,000	101	96.00	96.00
		20,000	98	55.00	53.00
		10,000	99	25.00	22.00
		5,000	99	16.00	13.00
		2,000	97	7.00	3.00
		0	103	4.00	0

TOXICANT	STRAIN CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
TRICYCLOHEXYLTIN HYDROXIDE	SP	100,000 50,000 20,000 0	30 48 64 62	70.00 27.08 7.81 3.22	
PARATHION	HNR.PN	50,000 20,000 10,000 5,000 2,000 0	98 96 128 102 98 96	100.00 82.00 76.00 47.00 15.00 9.00	100.00 80.00 74.00 42.00 7.00 0
PARATHION	LINES.PN	50,000 20,000 10,000 5,000 2,000 0	96 98 88 60 97 94	98.00 47.00 24.00 15.00 11.00 7.00	98.00 43.00 18.00 8.00 4.00 0
PARATHION	LHRS.PN	50,000 20,000 10,000 5,000 0	96 97 99 99 96	91.00 47.00 35.00 13.00 8.00	90.00 42.00 29.00 5.00 0
PARATHION	SP.PN	20,000 5,000 2,000 0	123 98 88 90	72.00 42.00 24.00 9.0	69.00 36.00 16.00 0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
FORMETANATE	HNR. PN	5,000	93	99.00	99.00
		2,000	93	82.00	81.00
		1,000	93	44.00	40.00
		500	92	24.00	19.00
		200	101	19.00	13.00
		0	97	6.00	0
FORMETANATE	LINES. PN	100,000	96	92.00	90.00
		50,000	96	92.00	90.00
		20,000	99	88.00	86.00
		10,000	99	57.00	50.00
		5,000	91	41.00	31.00
		2,000	103	16.00	2.00
		1,000	105	17.00	3.00
		0	101	14.00	0
FORMETANATE	LHRS. PN	100,000	95	94.00	93.00
		50,000	97	91.00	89.00
		20,000	100	78.00	74.00
		10,000	101	47.00	38.00
		5,000	94	23.00	8.00
		2,000	87	18.00	4.00
		0	97	15.00	0
FORMETANATE	SP. PN	100.000	102	100.00	100.00
		50,000	105	99.00	99.00
		20,000	109	96.00	96.00
		10,000	106	96.00	96.00
		5,000	139	94.00	93.00
		2,000	201	59.00	56.00
		1,000	185	61.00	59.00
		500	99	27.00	22.00
		200	102	25.00	20.00
		0	102	6.00	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	HNR.PN	20,000	93	100.00	100.00
		10,000	82	94.00	91.00
		5,000	77	84.00	80.00
		2,000	96	45.00	30.00
		0	94	21.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	LINES.PN	20,000	98	95.00	94.00
		10,000	99	71.00	67.00
		5,000	96	39.00	30.00
		0	101	12.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	LHRS.PN	50,000	97	100.00	100.00
		20,000	98	84.00	82.00
		10,000	95	66.00	65.00
		5,000	99	31.00	28.00
		2,000	99	28.00	25.00
		1,000	99	16.00	13.00
		500	33	6.00	2.00
		0	100	4.00	0
TRICYCLOHEXYLTIN HYDROXIDE (PLICTRAN)	SP.PN	20,000	64	95.00	94.00
		10,000	101	84.00	81.00
		5,000	101	46.00	34.00
		2,000	125	18.00	9.00
		0	102	18.00	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	F_1, PN♂ x LHRS♀	80,000	99	98.00	98.00
		40,000	102	86.00	86.00
		20,000	106	46.00	44.00
		10,000	102	35.00	33.00
		5,000	65	25.00	22.00
		2,000	62	11.00	8.00
		1,000	68	7.00	4.00
		0	64	3.00	0
PARATHION	F_1, PN♀ x LHRS♂	40,000	32	100.00	100.00
		20,000	29	90.00	88.00
		10,000	32	50.00	44.00
		5,000	32	50.00	44.00
		2,000	33	40.00	32.00
		0	29	10.00	0
PARATHION	F_1, PN♂ x LINES♀	40,000	99	99.00	99.00
		10,000	103	38.00	33.00
		5,000	62	10.00	2.00
		0	65	8.00	0
PARATHION	F_1, PN♀ x LINES♂	40,000	29	100.00	100.00
		20,000	69	96.00	96.00
		10,000	62	84.00	84.00
		5,000	65	62.00	61.00
		2,000	66	23.00	22.00
		0	68	2.00	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	F_1, PN♂ x SP♀	80,000	94	100.00	100.00
		40,000	67	99.00	98.00
		20,000	97	95.00	95.00
		10,000	96	53.00	50.00
		5,000	31	42.00	38.00
		2,000	27	26.00	20.00
		0	65	6.00	0
PARATHION	F_1, PN♀ x SP♂	40,000	32	100.00	100.00
		20,000	32	100.00	100.00
		10,000	32	94.00	94.00
		5,000	33	48.00	46.00
		2,000	32	44.00	42.00
		0	26	4.00	0
PARATHION	F_1(PN♂ x LINES♀) x PN♂	40,000	32	100.00	100.00
		20,000	67	89.55	89.23
		10,000	32	78.11	77.47
		5,000	31	54.84	53.47
		2,000	31	51.61	50.14
		1,000	28	50.00	48.49
		500	30	56.67	55.36
		200	32	53.13	51.71
		100	28	57.14	55.84
		50	32	40.63	38.83
		25	-	-	-
		25/2	31	35.48	33.53
		25/4	29	24.14	21.84
		25/8	32	6.25	3.41
		0	34	2.94	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	$F_1(PN♀ \times LINES♂) \times PN♂$ (B1)	20,000	34	100.00	100.00
		10,000	61	88.52	87.73
		5,000	31	100.00	100.00
		2,000	94	67.02	64.75
		1,000	94	56.38	53.37
		500	69	43.48	39.58
		200	62	48.39	44.83
		100	91	56.04	53.01
		50	60	56.67	53.68
		25	59	44.07	40.21
		25/2	62	40.32	36.21
		25/4	63	19.05	13.47
		25/8	46	13.04	7.04
		0	31	6.45	0
PARATHION	$F_1(PN♂ \times LHRS♀) \times PN♂$ (B1)	20,000	33	100.00	100.00
		10,000	63	84.13	83.04
		5,000	66	66.67	64.37
		2,000	61	59.02	56.19
		1,000	32	71.88	69.94
		500	57	64.91	62.49
		200	33	69.70	67.61
		100	64	60.94	58.25
		50	62	46.77	43.10
		25	30	70.00	67.93
		25/2	34	52.94	49.70
		25/4	32	15.63	9.81
		25/8	32	12.50	6.47
		0	31	6.45	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	$F_1(PN♀ \times LHRS♂) \times PN♂$ (B1)	40,000	32	100.00	100.00
		20,000	30	96.67	96.44
		10,000	62	85.48	84.48
		5,000	68	72.06	70.13
		2,000	61	65.57	63.20
		1,000	60	51.67	48.34
		500	47	57.45	54.52
		200	61	52.46	49.18
		100	65	63.08	60.53
		50	64	54.69	51.57
		25	59	44.07	40.21
		25/2	61	18.03	12.38
		25/4	62	6.45	0
		0	31	6.45	0
PARATHION	$F_1(PN♂ \times SP♀) \times PN♂$ (B1)	40,000	32	100.00	100.00
		20,000	61	90.16	89.40
		10,000	63	73.01	70.93
		5,000	66	68.18	65.73
		2,000	62	50.00	46.16
		1,000	97	51.54	47.81
		500	33	42.42	40.15
		200	31	41.93	37.00
		100	64	62.50	37.47
		50	64	57.81	54.57
		25	58	44.82	40.58
		25/2	30	50.00	46.16
		25/4	29	24.13	18.30
		25/8	62	14.51	7.94
		0	70	7.14	0

TOXICANT	STRAIN, CROSS OR BACKCROSS	CONCENTRATION OF ACTIVE INGREDIENT IN PPM	NUMBER OF MITES TESTED	24 HOUR % MORTALITY	CORRECTED MORTALITY
PARATHION	B_1 $F_1(PN♀ \times SP♂) \times PN♂$	40,000	60	98.33	98.16
		20,000	63	98.41	98.25
		10,000	91	95.60	95.14
		5,000	60	78.33	76.09
		2,000	62	82.26	80.42
		1,000	34	64.71	61.32
		500	61	70.49	61.06
		200	62	74.19	71.52
		100	50	60.00	55.86
		50	33	84.85	83.28
		25	25	52.00	47.03
		25/2	31	45.16	39.48
		25/4	32	21.88	13.79
		25/8	31	12.90	3.88
		0	32	9.38	0

Appendix 2 Sterility Percentages and Sex-Ratios of the F_1, B_1, B_2, B_3 and B_4 Generations In the Backcross Series

GENERATION	BACKCROSS SERIES	STERILITY PERCENTAGES	SEX-RATIO
F_1	LINES.PN	9.58	3.72
	LHRS.PN	12.36	3.68
	SP.PN	26.85	2.88
	PN.LINES	17.60	1.29
	PN.LHRS	48.29	0.32
	PN.SP	24.88	0.65
B_1	LINES.PN	76.31	2.40
	LHRS.PN	57.51	5.50
	SP.PN	72.93	1.86
	PN.LINES	61.37	4.35
	PN.LHRS	50.42	1.97
	PN.SP	87.05	3.02
B_2	LINES.PN	73.97	1.57
	LHRS.PN	50.91	4.29
	SP.PN	54.67	1.75
	PN.LINES	41.49	3.01
	PN.LHRS	47.54	2.81
	PN.SP	57.29	2.85
B_3	LINES.PN	66.70	1.44
	LHRS.PN	49.67	2.43
	SP.PN	44.41	2.09
	PN.LINES	42.80	3.03
	PN.LHRS	45.32	1.75
	PN.SP	31.70	1.72
B_4	LINES.PN	66.79	1.35
	LHRS.PN	54.85	2.68
	SP.PN	29.71	2.24
	PN.LINES	47.37	1.78
	PN.LHRS	38.11	1.51
	PN.SP	31.80	2.40