

THE USE OF FREQUENCY DIAGRAMS IN THE SURVEY OF RESISTANCE TO PESTICIDES IN TICKS IN SOUTHERN AFRICA

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ABSTRACT

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A method whereby resistance data can be analysed by means of frequency distributions is described. This method established that *Rhipicephalus appendiculatus* and *R. evertsi evertsi* show either little or only developing resistance to the pesticides chlorfenvinphos and dioxathion. *Boophilus* spp., however, show more instances and higher levels of resistance to these 2 pesticides.

Résumé

DE L'USAGE DE DIAGRAMMES DE FRÉQUENCE POUR ÉTUDIER LA RÉSISTANCE DES TIQUES AUX PESTICIDES EN AFRIQUE AUSTRALE

On décrit une méthode d'analyse des données de résistance au moyen de distributions de fréquence. On a montré par cette méthode que *Rhipicephalus appendiculatus* et *R. evertsi evertsi* ne montrent que peu de résistance ou une résistance inchoative seulement aux pesticides chlorfenvinphos et dioxathion. Toutefois les espèces de *Boophilus* montrent de plus nombreux exemples et des niveaux de résistance plus élevés vis-à-vis de ces 2 pesticides.

INTRODUCTION

The phenomenon of field resistance to acaricides in cattle ticks in southern Africa was heralded by the appearance in 1938 of strains of *Boophilus decoloratus* resistant to sodium arsenate (Du Toit, Graf & Bekker, 1941). This was soon followed by the development of field resistance to DDT and the chlorinated hydrocarbons (Whitehead, 1959) and, more recently, to the organophosphate compounds (Shaw, Thompson & Baker, 1967).

These reports of resistance and numerous field observations that tick control was inadequate, even when the dip-wash was maintained at the recommended concentration, suggested that widespread resistance to acaricides could become a serious problem for the livestock industry in southern Africa in the near future. For this reason, and in an attempt to predict future trends, a survey of the susceptibility of ticks to a number of model acaricides was undertaken. In the course of the analysis of the large body of information obtained from this survey, use was made of frequency distributions to simplify the data. This paper describes the preliminary results of this survey and the use of frequency distributions in the analysis of field resistance to acaricides.

METHODS

Collection of ticks

The survey was planned in 1974, when requests were sent to State Veterinarians, Stock Inspectors and other interested parties to make available collections of ticks from farms. Particular attention was paid to those farms where problems in tick control were being experienced. The distribution of the tick collection sites is shown in Fig. 1.

Since the small number of ticks collected and delays in their receipt precluded the carrying out of bioassays on engorged adult ticks, larvae were used for the bioassays.

Bioassays

Engorged female ticks received from the field were washed, identified, placed in glass flasks stoppered with cottonwool and then incubated at 27 °C, 80%

R.H. until the eggs hatched. Fourteen to twenty-eight days after hatching, the larvae were assayed for the their susceptibility to acaricides. The method used was based on that of Shaw (1965) with an extension of the incubation period to 72 hours, as recommended by J. A. F. Baker [Coopers (South Africa) (Pty) Ltd, personal communication, 1974].

As it was not possible to bioassay larvae against all registered ixodidicides, 3 model compounds were used: toxaphene, dioxathion and chlorfenvinphos. When only limited numbers of ticks were available, priority was given to the class of compounds in use at the particular sample site from which they came, but when there were sufficient larvae, all compounds were bioassayed.

Data analysis

The subjective classification of individuals or strains of organisms as being either resistant or susceptible on the basis of an intuitively evaluated factor of resistance was considered inadequate, since this method failed to take into account natural variability, both within a strain and between the strains of a population. An attempt was made, therefore, to evaluate the susceptibility data more simply and objectively. It was felt that such an evaluation should incorporate a simple graphic representation of the data and, of the various systems tried, a frequency diagram showed the most promise.

The results of the bioassays were corrected for control response and a line of probit mortality versus log concentration fitted by means of a probit analysis programme developed by H. van Ark (Department of Agricultural Technical Services, personal communication, 1977). The values for the LC_{50} ; LC_{90} ; LC_{95} ; LC_{99} ; and the $LC_{99.9}$ were calculated in this programme. Frequency diagrams of the LC data, drawn with the aid of a TI 59 programmable calculator, are shown in Fig. 2-9. Each point of these diagrams represents the LC value of a single sample of ticks collected from the field. The recommended concentration for each acaricide is shown by the vertical dotted line. Some points were lost, particularly at the higher LC values, because values higher than 10% active ingredient were ignored in plotting the frequency diagrams. Although a wide range of tick species was received from the field collections, data were only sufficient in the case of *Rhipicephalus appendiculatus*, *R. evertsi evertsi*, *Boophilus* spp. and *Hyalomma* spp.

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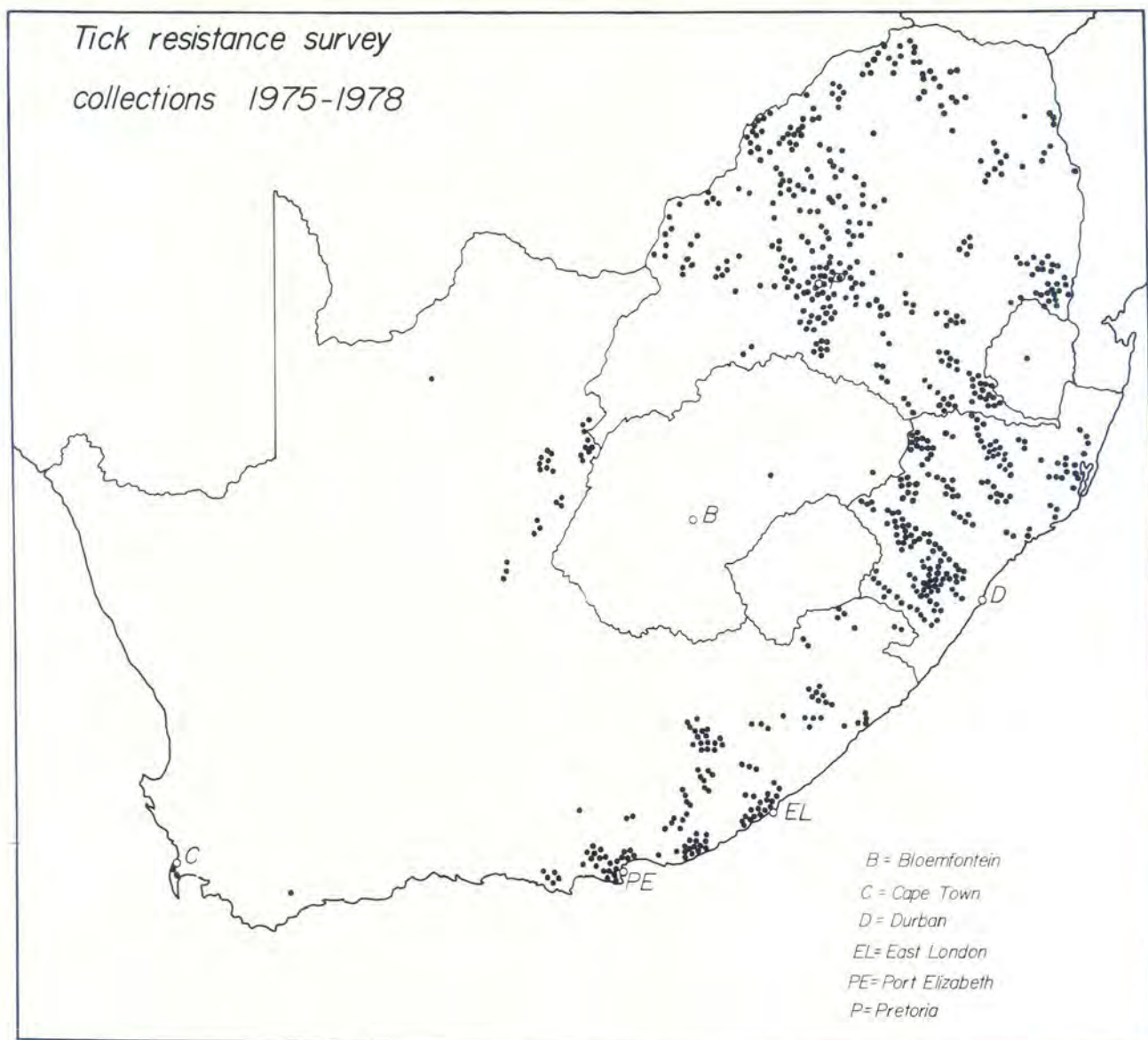


FIG. 1 Collection of ticks for the resistance survey, January 1975 to June 1978. Each dot represents the collection of a sample of a single species

RESULTS AND DISCUSSION

The classification of LC data into frequency distributions was based on a number of assumptions. Firstly, just as a heterogeneous population of individuals shows a normal distribution of any parameter, so will a population of strains of individuals similarly show a normal distribution of a mean parameter such as an LC value. Coupled with this is the assumption that, in the process of selection for resistance in a susceptible population, a second population is selected in which the selected parameter is also normally distributed. A second assumption in this type of analysis is that, just as the response to a toxicant in a population of individuals is proportional to the logarithm of its concentration, the mean response in a population of strains of individuals is also proportional to the log of the concentration of the toxicant.

Support for these hypotheses is given by the results shown in Fig. 2-9, in which the distribution of the LC values closely approximates a normal distribution when the LC value is plotted on a log scale. With dioxathion the response of *R. appendiculatus*

(Fig. 2) gives a close approximation to a normal distribution, although a few outliers are present. As the LC parameter increases from 50 through 99.9, the distribution becomes somewhat flatter and broader, as might be expected, seeing that the error on the high LC values is greater than that on the LC_{50} . An essentially similar pattern is seen in the case of *R. e. evertsi* (Fig. 3). In both these species the distributions suggest that the populations are fairly homogeneous and that selection for a resistant population composed of a large number of strains has not yet taken place. In both, also, the normally distributed bulk, or susceptible portion, of the population does not shift above the recommended field strength for this pesticide, even at the higher LC values. The samples that showed LC values in excess of the field strength indicate ticks that are resistant, or are at least developing resistance, to the pesticides.

The data for *Boophilus* spp. shown in Fig. 4 are interesting, because a normal distribution of susceptible ticks, as seen in the other 2 species, is not as clearly evident. Either the distribution is much

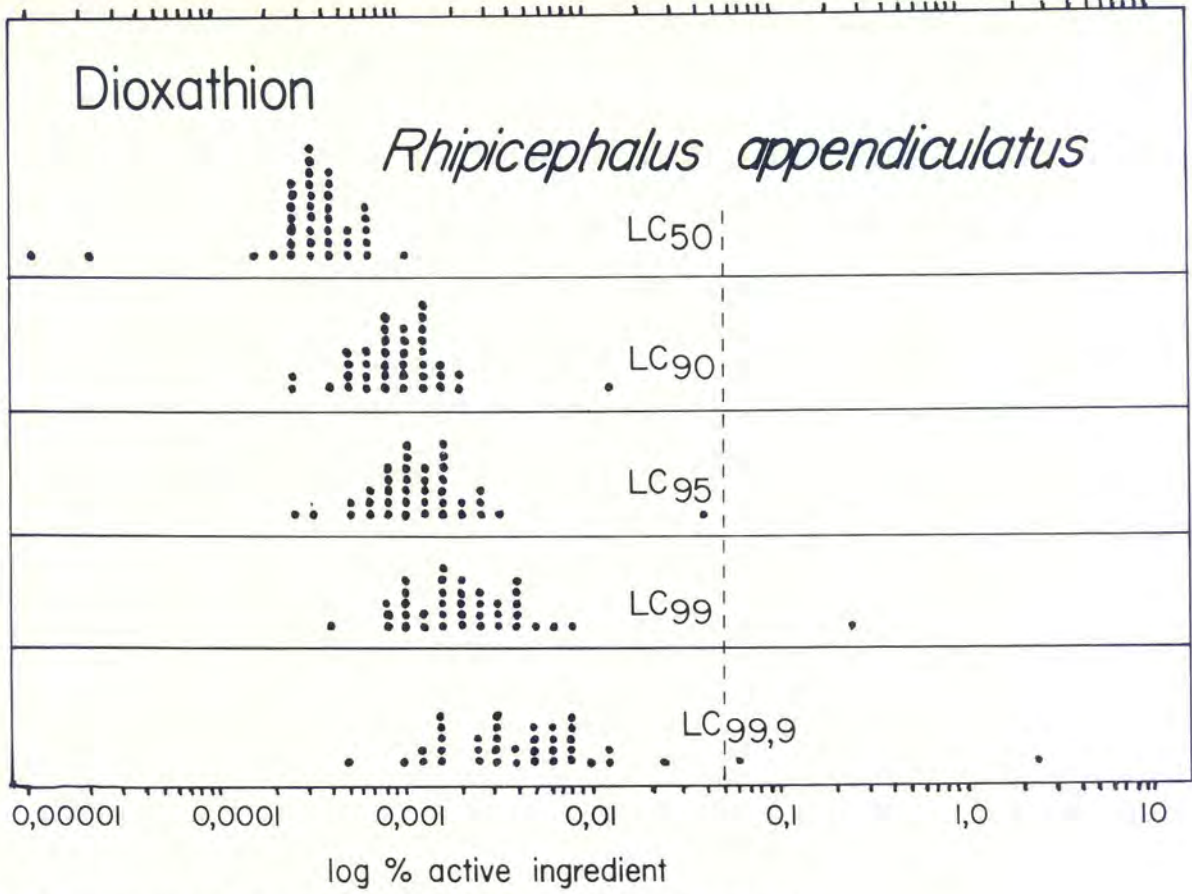


FIG. 2 Frequency distribution of *Rhipicephalus appendiculatus* assayed against dioxathion

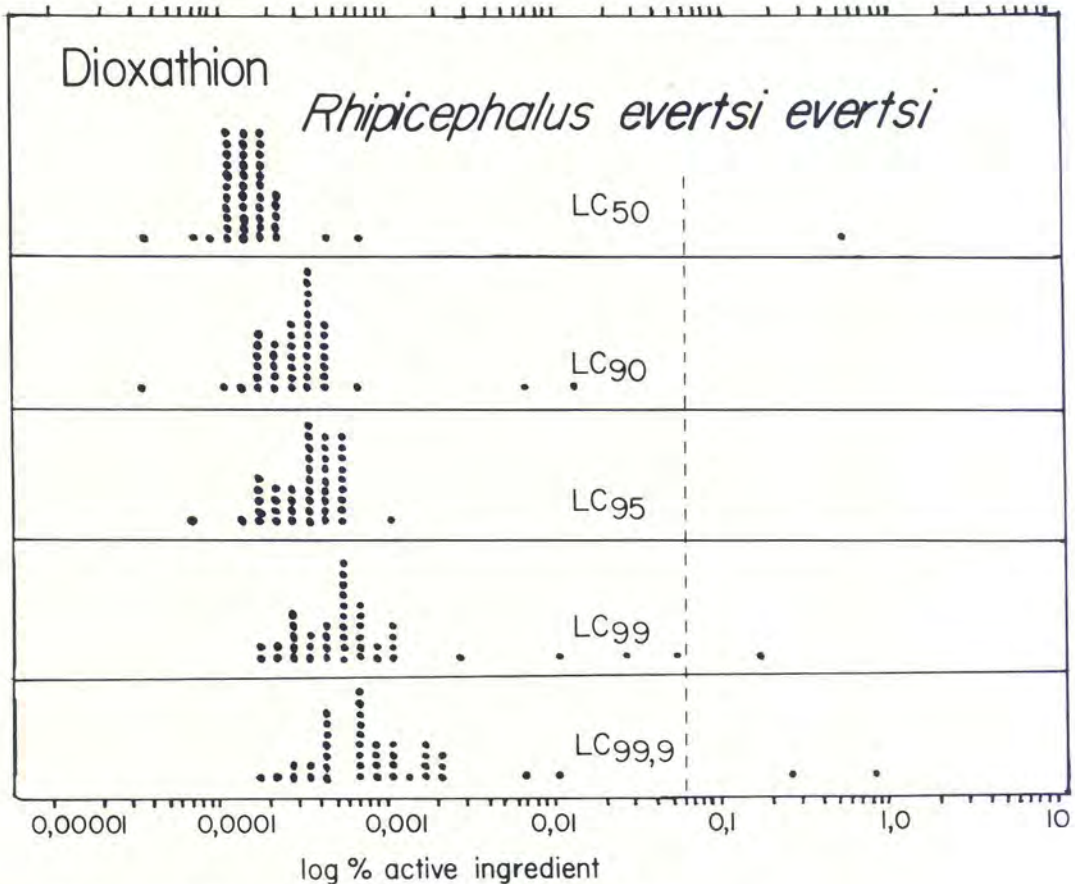


FIG. 3 Frequency distribution of *Rhipicephalus evertsi evertsi* against dioxathion

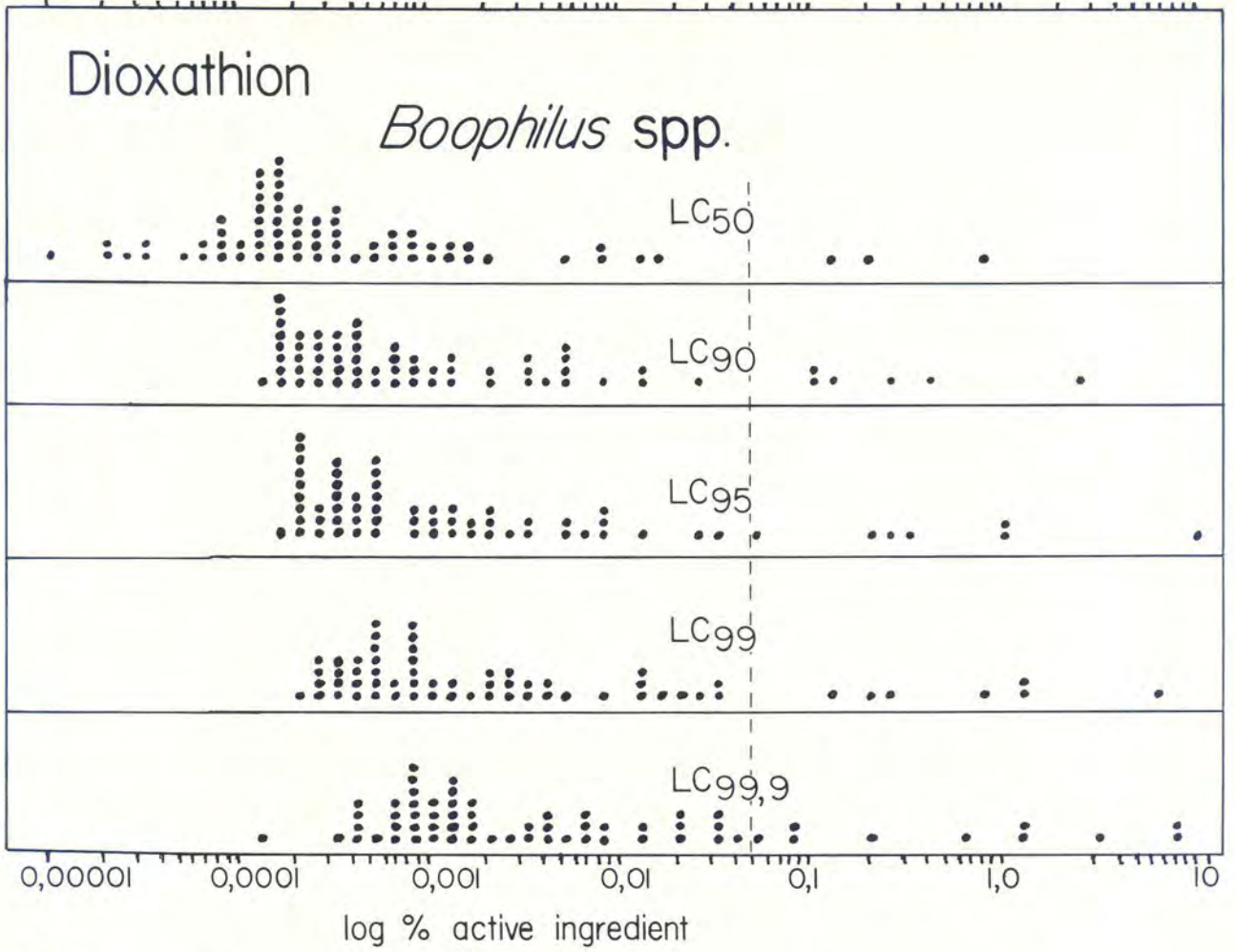


FIG. 4 Frequency distribution of *Boophilus* spp. against dioxathion

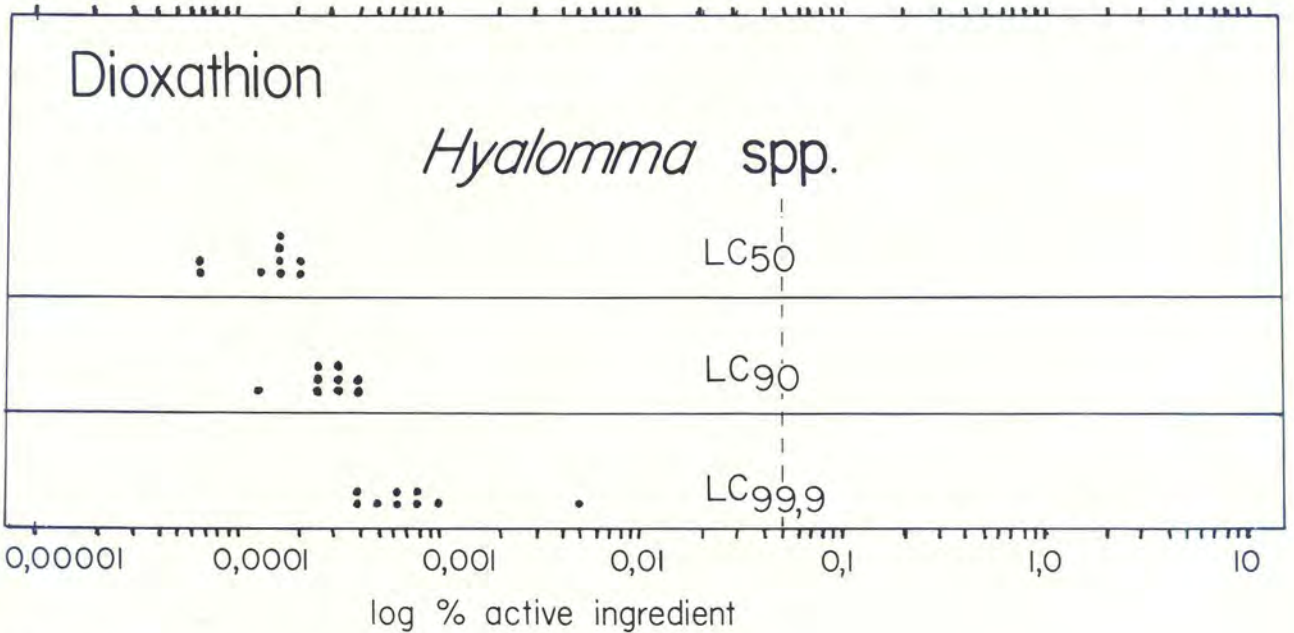


FIG. 5 Frequency distribution of *Hyalomma* spp. against dioxathion

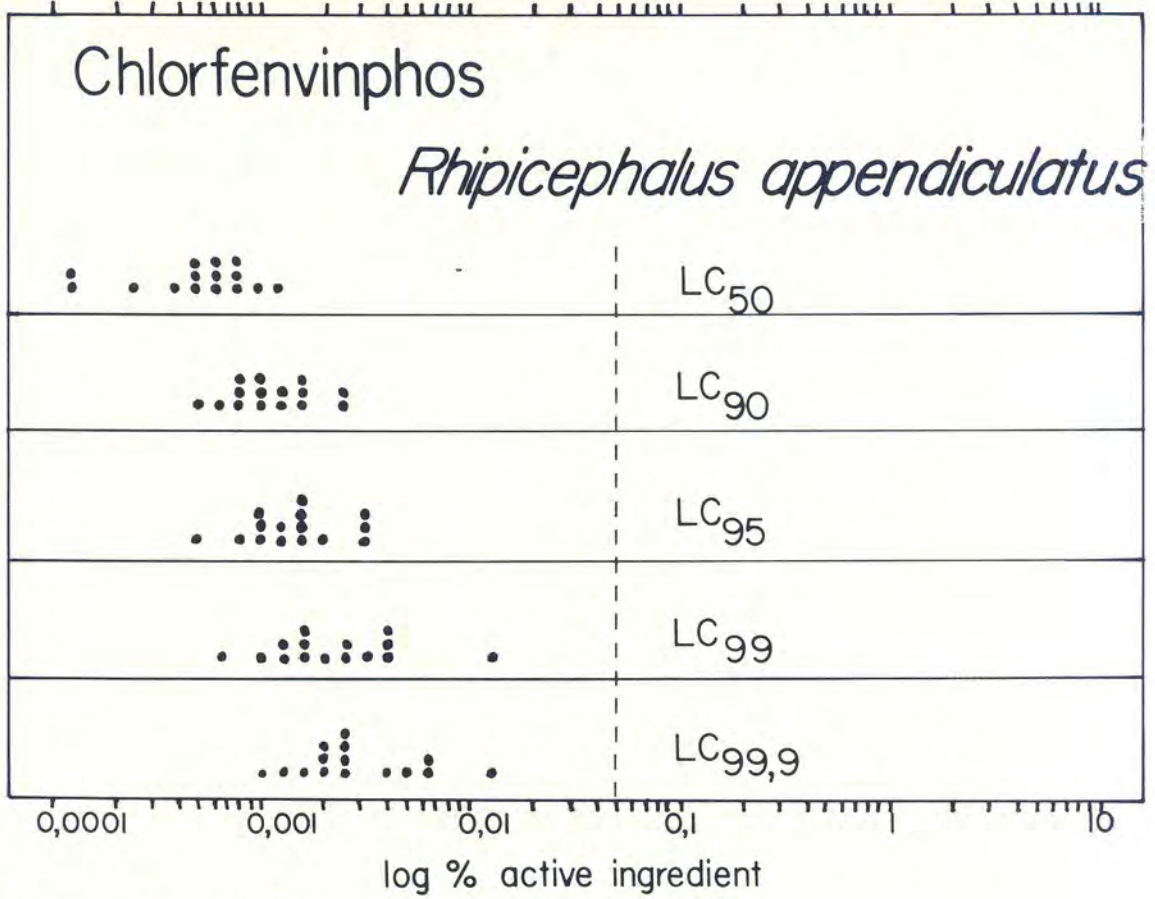


FIG. 6 Frequency distribution of *Rhipicephalus appendiculatus* against chlorfenvinphos

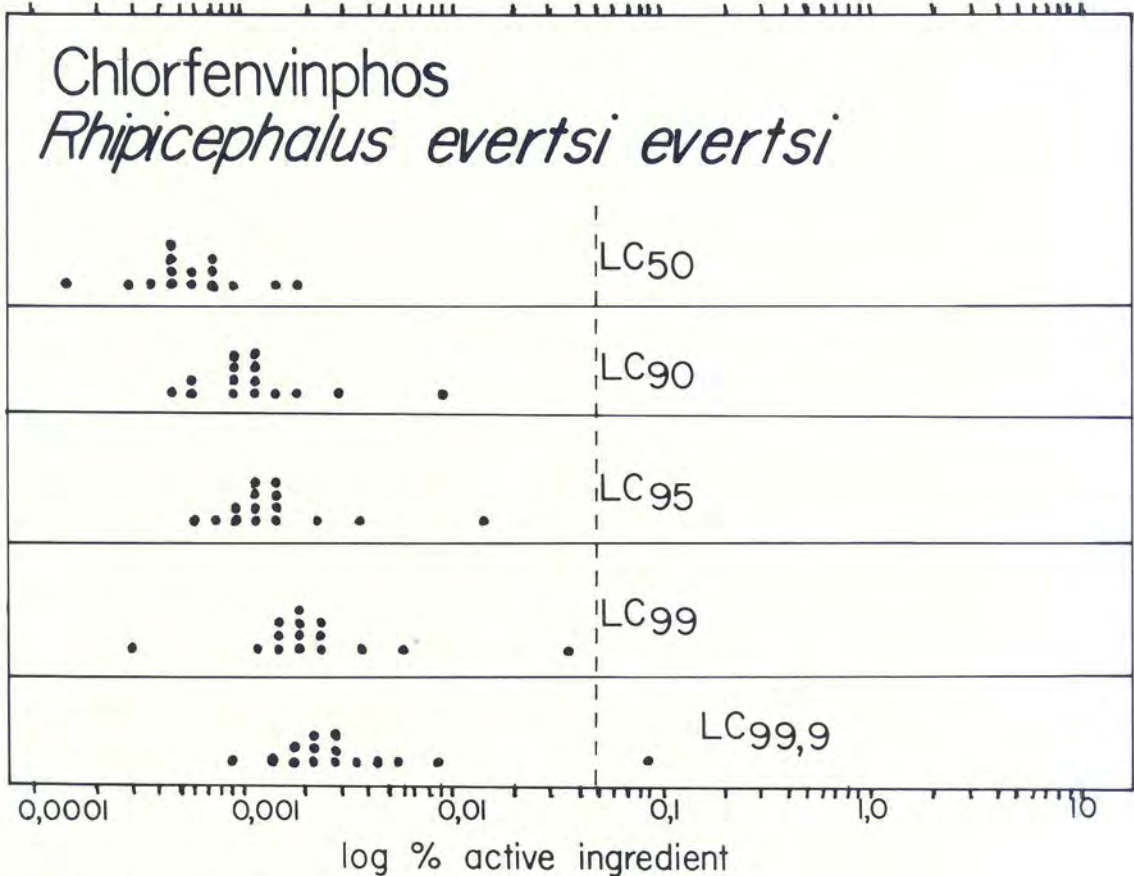


FIG. 7 Frequency distribution of *Rhipicephalus evertsi evertsi* against chlorfenvinphos

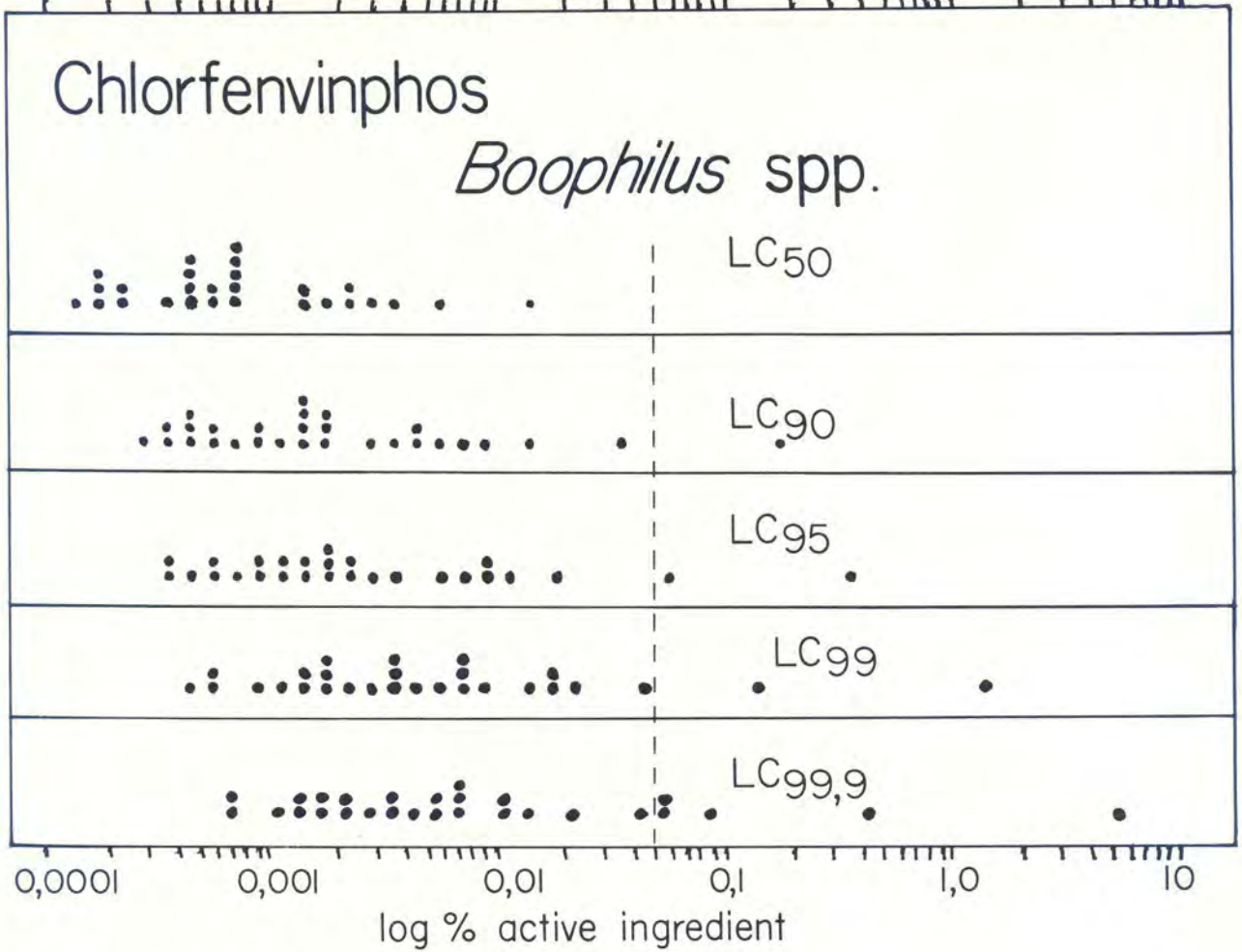


FIG. 8 Frequency distribution of *Boophilus* spp. against chlorfenvinphos

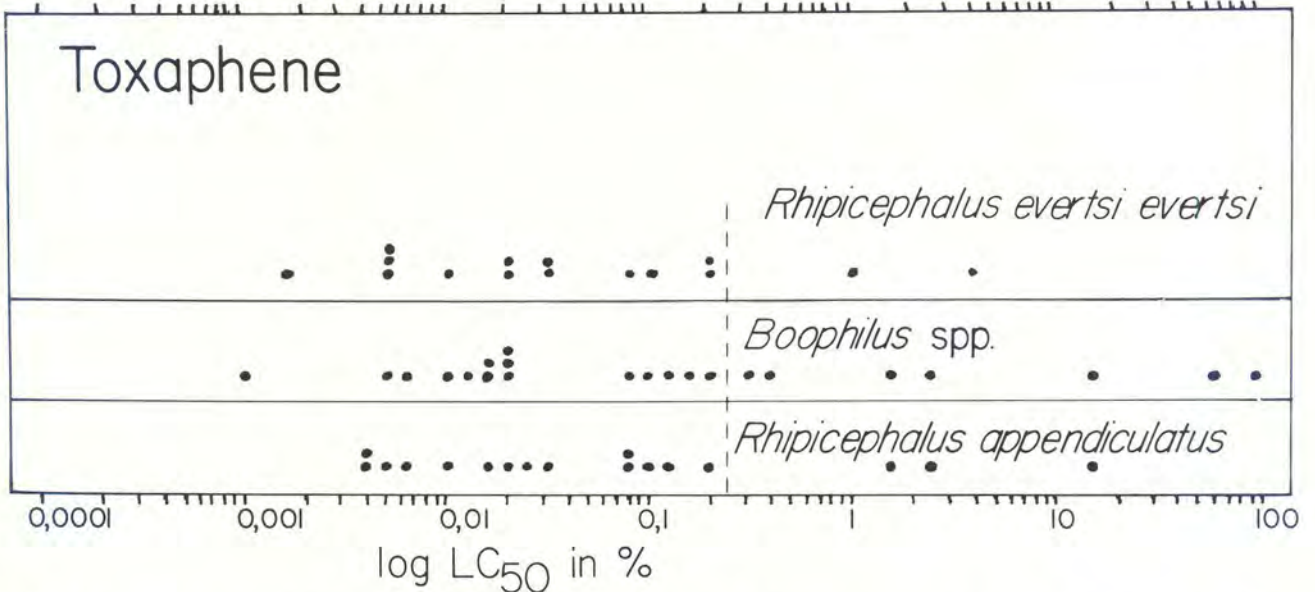


FIG. 9 Frequency distribution of the LC₅₀s of *Rhipicephalus evertsi evertsi*, *R. appendiculatus* and *Boophilus* spp. against toxaphene

broader or it consists of 2 overlapping distributions. Coupled with this, the number of outliers is larger than in either of the other 2 species. The fact that some of these outliers even exceed the recommended field strength at the LC_{50} level suggests very high levels of resistance. The large proportion of samples lying above the field strength indicates a greater frequency of resistance in these species, a finding that is confirmed by field observations. Those ticks with $LC_{99.9}$ values between field strength and the susceptible portion of the population could be regarded as showing developing resistance and could, in theory, be controlled by close attention to the recommended concentration of the acaricide. Although the data are based on samples of both *B. decoloratus* and *Boophilus microplus*, no unidentified samples of *B. microplus* were found outside the susceptible portion of the population, suggesting that at present resistance is possibly confined to *B. decoloratus*.

The results for the *Hyalomma* spp. (Fig. 5) are based on a rather small number of samples and therefore cannot be considered to be representative of the whole population. However, the close grouping, compared with the results obtained from the other multi-host ticks, suggests that there is little resistance in the *Hyalomma* spp. at present.

A similar trend was noted in the case of chlorfenvinphos. Both *R. appendiculatus* and *R. e. evertsi* (Fig. 6 & 7, respectively) showed little indication of widespread resistance. *Boophilus* spp. (Fig. 8) again showed indications of more widespread resistance, and this is confirmed by field observations.

In the case of toxaphene some of the higher LC values gave concentrations in excess of 100% and, even in the case of the LC_{50} (Fig. 9), some values were so high as to be unobtainable in practice in the dip tank. These very high values are most probably not a true reflection of the resistance situation in the field but rather an artifact of the bioassay technique. Laboratory observations suggest that the action of toxaphene is slow and possibly the incubation times used in the larval bioassay were too short. For this reason frequency distributions of the higher LC values were not plotted.

A number of LC values were included in this analysis in an attempt to determine if any one of them would be more useful than the others as a criterion of field resistance. In most cases the distribution patterns did not vary much from one LC value to the next except for a shift to higher concentration and a slight broadening of the distribution. From a practical point of view, it is logical to use a higher LC value as a criterion of resistance. Clearly, for adequate control, almost all the ticks on the host should be killed. Also, the practical observation that one is often dealing with populations of differing heterogeneity, and thus with different slopes of the dose-mortality line, suggests that a high value such as the $LC_{99.9}$ would be the most useful criterion for determining resistance. It is suggested, therefore, that, when the $LC_{99.9}$ of a sample of ticks falls within the bounds of the susceptible portion of the population, the sample should be regarded as being susceptible. When it lies above the recommended field concentration, the ticks should be considered as coming from a resistant population. When the $LC_{99.9}$ lies between these 2 limits, the ticks should be considered as coming from a population in which resistance is developing, i.e. they can most probably be controlled by close attention to dipping practice and field strength, but may undergo selection quite rapidly.

Obviously, these criteria depend on the construction of a frequency distribution based on an adequately large sample of tick strains (probably at least 50 or more) and this has not as yet been possible for all species and acaricides.

The limited availability of ticks from the field and the problems associated with the culture of a second generation of adults from a field sample preclude the use of adults in bioassays. This calls in question the validity of the larval bioassay as an indicator of adult resistance. Laboratory observations have shown that adult ticks are less susceptible than larvae of the same strain, but a correlation between LC values of these two stages has not yet been shown. This is probably less important in the case of the single-host ticks, where, in a well-managed dipping programme, the larvae should be at the controlled stage of the life cycle, but it could be of major importance in the case of the multi-host ticks where some larvae may never in fact be exposed to pesticides. Since so little work has been done on the correlation in susceptibility between the larval, nymphal and adult stages, it can only be assumed that ticks that are susceptible during the larval stage will develop into susceptible adults, and *vice versa*. Although it is unlikely that the larval and adult stages will both show the same LC values towards a particular pesticide, all stages of a particular tick probably belong to the same group in the population, i.e. either susceptible or non-susceptible.

It is further suggested that the frequency distribution method for determining resistance criteria in ticks is the most practical method available, since it gives a true reflection of the field situation in a particular area and for a particular tick species. Use of this system would also enable future trends to be predicted, following observations on shifts in the distribution of the susceptible and non-susceptible populations with the passage of time. Its main disadvantage is the large numbers of data required for this type of analysis and also the length of time required to collect these data. Despite these disadvantages, this method for determining the criteria of resistance in a pest population has a high potential for future use, not only for tick control but also for any other organisms that are exposed to pesticides and in which selection can lead to resistance.

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