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ISOBOLES, A GRAPHIC REPRESENTATION OF SYNERGISM IN PESTICIDES[1]

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It is possible to give a graphic representation of the effect of two pesticides applied jointly or of the effect of pesticides to which substances have been added. Each of the components is expressed in a coordinate. In the graph a quantitatively defined effect, e.g. a mortality of 50%, is inserted. These values are obtained by interpolation. The line which connects the points is called an isobole after LOEWE & MUISCHNEK (1926) who used it for drugs. With an isobole the effect of different proportions of the components can be evaluated. The various types of isoboles are described and illustrated for some experiments with insecticides, herbicides and fungicides.

INTRODUCTION

The effect of a single pesticide can be drawn in a graphic representation, e.g. in a dosage response curve with two variables. When, however, the joint effect of various dosages of two pesticides is represented, there are three variables, viz. the dosages of the two components and the effect. Theoretically a three-dimensional system could be used with a coordinate for the substance A, for the substance B and for the effect. This, however, is very unpractical, since one has to construct curved planes instead of drawing curved lines (DE JONGH, 1961). These three variables can be reduced to two by selecting a quantitatively defined effect as criterion for a graphic representation of the joint action of the two components. For pesticides such a criterion can for instance be a mortality of 50% or 90%, or with herbicides a certain growth reduction. The results of experiments can be tabulated, the criterion estimated by interpolation and the obtained values inserted in a graph and connected by a line. LOEWE & MUISCHNEK (1926) in their studies on the joint action of drugs named such a line an isobole, a line of equal effects (bolos, from Greek = catch in a fishing net). All points of an isobole indicate the mixing values of both components at which a specific, quantitative effect is produced.

Various approaches are known to the assessment of joint action in pesticides, e.g. those of BLISS (1939), FINNEY (1947), DIMOND & HORSFALL (1945), GOWING (1959) and others. It seems, however, that the method of graphic representation, first used by LOEWE *et al.* (1926), though known in pharmacology, is not used or cited by pesticide toxicologists.

In the author's opinion the isobole deserves more attention in the study of the effect of mixing and formulating of pesticides, especially in the assessment of synergism. In his attempt to arouse more interest in the use of isoboles the author will restrict himself to experiments where the effect of pesticides on one biological species is studied. This article will not consider the mixing of pesticides performed to widen the spectrum of the biological action of the individual components.

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TERMINOLOGY

Some confusion exists as to the meaning of the term synergism (see VELDSTRA, 1956). In this paper the following terms and definitions will be used.

Synergism (synonym: super addition, potentiation): Cooperative action of two components of a mixture, such that the total effect is greater or more prolonged than the sum of effects of the two taken independently.

Addition (synonym: additive synergism): Cooperative action, such that the total effect is equal to the sum of the effects of the components taken independently. The components may be substituted for each other in amounts inversely proportional to their activity.

Independent effect (synonym: no influence, sub addition): The total effect is equal to the effect of the most active component alone.

Antagonism (synonym: sub addition): The total effect is smaller than the effect of the most active component alone.

The term *one sided effect* is used in this paper in relation to an isobole having only one point of intersection with the coordinates (Fig. 1). This occurs when a non-active component is used with an active one e.g. a wetting agent added to a pesticide.

Two sided effect is used in relation to an isobole having two points of intersection with the coordinates (Fig. 2). This is found when two active components are mixed.

TYPES OF ISOBOLES

The different types of isoboles are shown in Fig. 1 for a one sided effect and in Fig. 2 for a two sided one. In both cases a criterion of 50% mortality has been adopted (see also DE JONGH, 1961).

On the horizontal axis of Fig. 1 a point is given where the mortality is 50% for component A. When the added substance B has no influence at all, the isobole will run parallel to the ordinate (I). When, however, the added substance is synergistic, the amount of component A needed for the mixture to reach 50% mortality will be less when B is added (II). The reverse will be the antagonistic line III. These one sided effects are characteristic for non-poisonous additions to pesticides, usually made to improve the general effect.

In Fig. 2, showing isoboles with a two sided effect, there is both on the

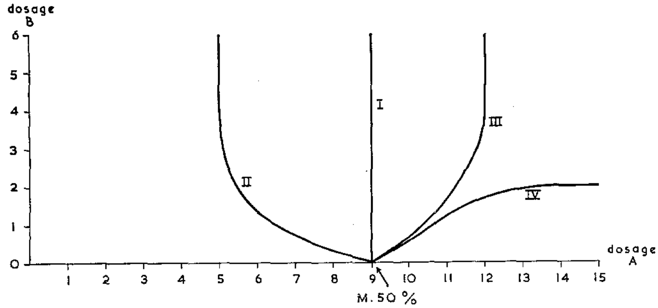
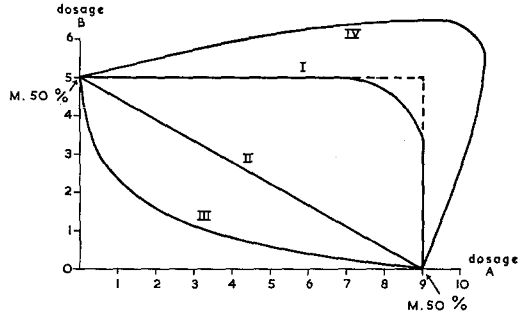


FIG. 1. Types of isoboles with a one sided effect.
I Independent effect.
II Synergism.
III Antagonism.
IV Blockade of A by B.

FIG. 2. Types of isoboles with two sided influence.
I Independent effect.
II Addition.
III Synergism.
IV Antagonism.



abscissa and the ordinate a point where 50 % mortality is reached. When both components act independently there will be two isoboles, parallel to abscissa and ordinate (I). They will intersect at a point where each, independently of the other, will kill 50 % of the population. When, however, one of the components takes 50 % of the population and the other takes 50 % of the remainder, the mortality will be 75 %. Near the point of intersection the right angle between the two lines will, therefore, be rounded off. Isobole II shows the situation of addition, where each component can be substituted by the other in a ratio determined by the distances of the mortality points on both axes. Isobole III represents synergism and IV antagonism.

DESIGN AND CALCULATION OF AN EXPERIMENT

As an example how an isobole is calculated an experiment is described using mixtures of two phosphorus insecticides, malathion and parathion. The tests were made with the bean weevil (*Acanthoscelides obtectus* Say). Each determination was made using 5 petri dishes with about 50 beetles each, sprayed with equal amounts of the mixture. The insects were quickly removed to clean dishes and the mortality was recorded after three days. The concentration of the components varied by a factor 2, and in all tests an excess of wetting agent was added. Concentrations are given in ppm in the spraying solution with 12 mg of solution per cm^2.

TABLE 1. Mortality of the bean weevil in % following spraying with mixtures of parathion and malathion.

Malathion ppm						
40	70	73	85	88	98	
20	36	53	74	84	96	
10	8	20	27	84	88	
5	2	5	19	58	90	
0	0	1	8	54	83	
	0	0.625	1.25	2.5	5	Parathion ppm

In practice it is convenient to make a preliminary experiment to determine the concentrations giving 50 % mortality for each component, so that out-of-range concentrations can be omitted. From the figures of Table 1 the values of 50 % mortality can be interpolated in rows and columns, in sofar as sufficient values are present in the neighbourhood of the 50 % mortality point. The interpolation can be carried out on log/probit paper, according to the well known method described by BLISS (1934). The interpolation values of Table 1 are as follows:

Rows ppm Malathion	ppm 50 % mortality Parathion	Columns ppm Parathion	ppm 50 % mortality Malathion
40	–	5	–
20	–	2.5	–
10	1.4	1.25	12.5
5	2.0	0.6	19
0	2.5	0	28

Figure 3 shows the interpolation on log/probit paper.

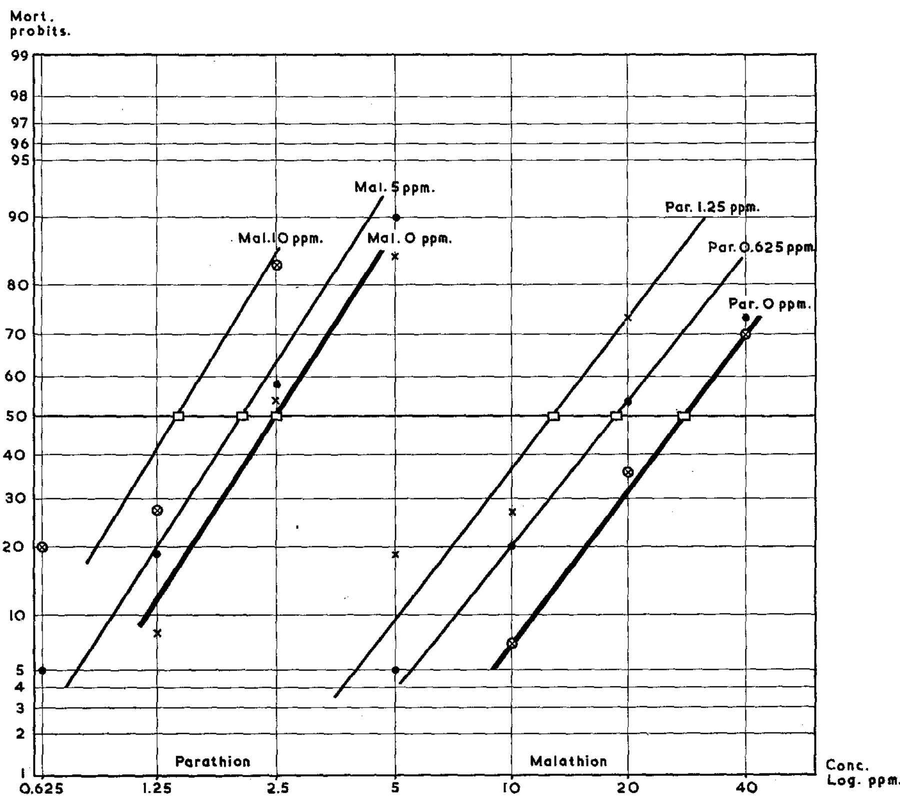


FIG. 3. Interpolation on log/probit paper of mortality data from an experiment with malathion-parathion mixtures.

The values can now be inserted in a graphic representation and the isobole is given in Fig. 4.

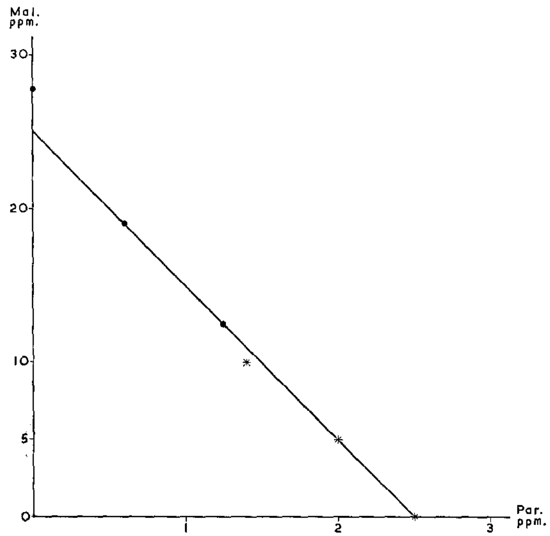


FIG. 4. Isobole for mixing values of parathion and malathion sprayed on bean weevils to yield 50% mortality.

It is evident that in order to obtain the same effect on weevils about ten times as much malathion is needed as parathion. In this ratio the two components can be substituted for each other. The effect was 'additional' and no indication of synergism was observed.

Another experiment is given in Fig. 5 and exemplifies synergism in the addition of amitrole to a commercial formulation of the herbicide atrazine applied to quack-grass (*Agropyron repens*). It was designed for a mortality of 90%. There were about 50 plants per pot, each plant from a separate portion of rhizome, and the plants were sprayed when about 15 cm high. All parts

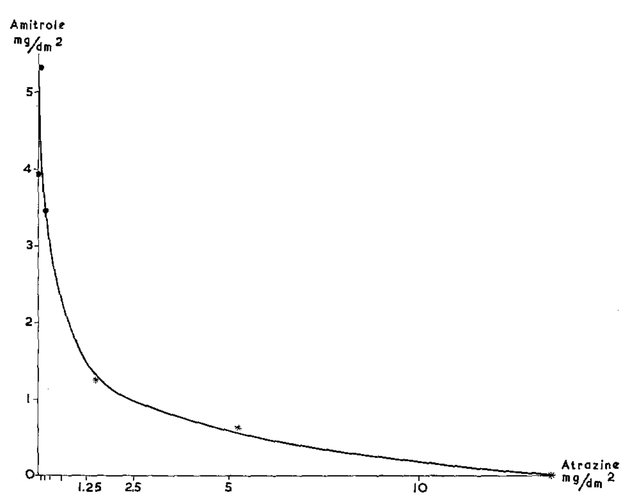


FIG. 5. Isobole of amitrole and atrazine mixture on quack-grass. Mortality 90% (Synergism). Interpolation log/probit herbicides expressed in active substance/dm².

above the soil were killed and after six weeks the percentage of rhizomes giving new growth was recorded.

The effect of an addition of amitrole to a commercial formulation of diuron, using barley (*Hordeum vulgare*) as a test plant, is shown in Fig. 6. Young seedlings with one leaf were sprayed with the mixture. Calculation in terms of grams per ha. Results recorded ten days after application.

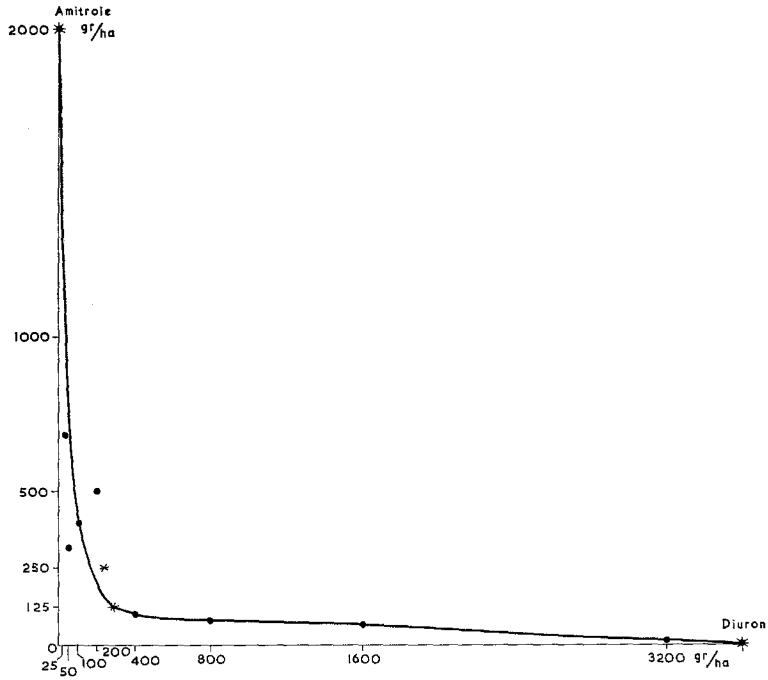


FIG. 6. Synergism of amitrole and diuron on barley seedlings. Isobole for 50% growth reduction. Interpolation linear. Calculation in terms of grams of substance per ha.

Sometimes it is possible to draw an isobole from tabulated results given by others. DIMOND & HORSFALL (1945) during the last war tried to substitute copper in fungicides. From their study isoboles can be calculated. An example is given in Fig. 7, relating to the interaction between copper and sulfur.

An example of the use of isoboles in studying effects of a wetting agent is given in Fig. 8. The wetting agent was added to a commercial formulation of parathion sprayed on bean weevils, in the same way as was done in the experiment on malathion/parathion cited in the first example. It can be seen that at first there was some synergistic action, but that a limit was soon reached and the effect then became independent of further additions of the wetting agent.

THE USE OF ISOBOLES IN COMMERCIAL PRACTICE

An isobole can be transposed on a scale giving on the axes the monetary value of each chemical (e.g. in florins per hectare). In the case of synergism a

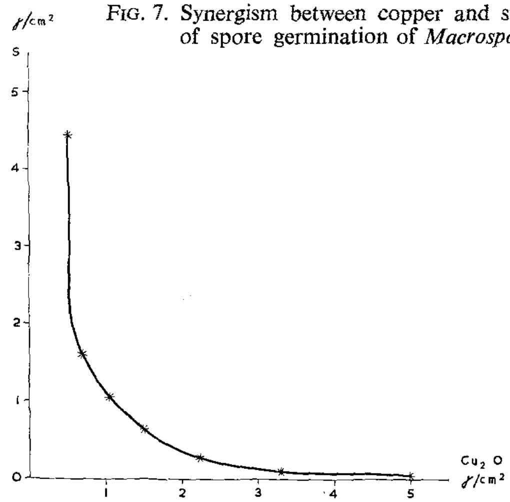


FIG. 7. Synergism between copper and sulfur, determined from 50% inhibition of spore germination of *Macrosporium sarcinaeforme*.

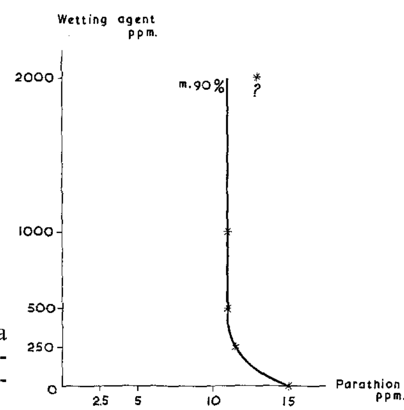


FIG. 8. The effect of adding wetting agent to a commercial formulation of parathion. Interpolation, in rows only, log/probit. Mortality of 90%.

tangent at 45° with the axes can be drawn, touching the isobole at the point where for the smallest amount of money the desired effect can be obtained.

SIGNIFICANCE OF RESULTS

For the interpolated values an estimate of errors is possible according to methods given by BLISS (1934) and FINNEY (1947). A computer programme for such a calculation is available for IBM at the Mathematical Centre for Agriculture at Wageningen.

It is reasonable to assume that the error of any point on the isobole will not exceed the error of the interpolated values. Isoboles can be based only on tests with fairly large numbers of individuals. With small numbers of test plants it would be difficult to obtain fair average values with which to interpolate.

INTERPOLATION

Most mortality dosage response curves are sigmoid and such curves are also obtained in experiments with herbicides, using their effect on dry matter production as criterion of their effectiveness (SAMPFORD, 1952; VAN DER ZWEEP, 1958). With a sigmoid distribution the interpolation of the criterion on log/probit paper is possible (Fig. 3). Interpolation is possible in rows or columns or in both. In Figs. 4 and 5 the interpolations are in rows and in columns. In Fig. 7 it was only possible in the rows, because the line is almost vertical. In special cases, e.g. when there are many values between zero and first step only a linear interpolation can be applied (Fig. 6). In the case of abnormal dosage/response curves, e.g. in a curve with two maxima, the interpolation may fail.

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