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The joint action of fungicides in mixtures: comparison of two methods for synergy calculation

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Two methods for evaluating the joint action of fungicides in mixtures were analysed. In order to obtain a relatively rapid answer on the type of interaction between fungicides in a mixture (additivity, synergism or antagonism), one can apply the Abbott formula to data on fungus survival. Tests with this method will not be accurate at high effective dose values. A more accurate determination of the joint action of fungicides can be made by the Wadley method, applied to data on effective doses. This involves more experimental work than the Abbott method. Optimization of mixing ratios of fungicides required a set of experimental data on effective doses with several mixing ratios.

Introduction

The mixing of chemicals offers many possibilities in the search for broader and more potent uses of pesticides. In all cases when pesticide mixtures are applied, the biological effect may be equal, greater or smaller than might be expected from the sum of the activities of the components when administered separately. These phenomena are defined as additivity, synergism and antagonism, respectively. The term synergism was first used in pharmacology to represent the unusual and greater combined effect of drugs (Macht, 1929), and the phenomenon has since been extensively studied with herbicides (Morse, 1978) and insecticides (Busvine, 1971). The expression of synergism in fungicide mixtures was reviewed by Scardavi (1966).

Use of fungicide mixtures has increased significantly during the last decade due to the evolution of phytopathogenic fungal genotypes resistant to site-specific fungicides. Mathematical models, as well as experimental studies, have shown that mixtures of site-specific and broad-spectrum fungicides can delay the build-up of resistant genotypes of foliar fungal plant pathogens (Delp, 1980; Levy *et al.*, 1983; Staub & Sozzi, 1984).

Prepacked mixtures are extensively used by growers in spite of the uncertainty in the evaluation of the joint action of their components. Synergistic interaction between the fungicides oxadixyl and mancozeb for control of *Phytophthora infestans* in tomatoes and potatoes and *Plasmopora viticola* in grapevine was reported by Gisi *et al.* (1983, 1985) and between metalaxyl and mancozeb in controlling *Pseudoperonospora cubensis* in cucumbers by Samoucha & Cohen (1984). *In vitro* studies with the 'crossed paper strip bioassay' demonstrated synergism between fungicides against several fungal species (De Waard & Van Nistelrooy, 1982; De Waard, 1985; Katagiri & Uesugi, 1977). Differences in the evaluation of synergism have caused large variation in the results and interpretation of the joint action of pesticide mixtures (Sun & Johnson, 1960). There is voluminous literature on methodology for studying the joint action of insecticides and herbicides, but no attempt has been made to adapt and/or modify these methodologies to phytopathology. In this paper, we analyse the different concepts and calculation methods available for evaluating the interaction between components in mixtures, with the aim of making them easily available to plant pathologists. The interactions are measured as quantal response of the target populations, which means that either mortality or survival are the variables, or else effective doses to obtain the same effect on a target population.

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Concepts

Bliss (1939), and later Plackett & Hewlett (1952), put forward a biological classification of types of joint action, conceived originally for insecticides, which seems equally applicable for fungicides. Bliss (1939) defined three types of joint action: (a) independent joint action—the fungicides act independently and have different modes of action; (b) similar joint action—the fungicides produce similar but independent effects, so that one component can be substituted at a constant proportion for the other (Finney, 1971). The effectiveness of the mixture is then predictable directly from one of the constituents if their relative proportions are known; (c) synergistic or antagonistic action. One fungicide influences the biological activity of the other. In this case the effectiveness of a mixture cannot be assessed from that of the individual components. According to Bliss, a deviation from the expected efficacy of a mixture calculated from the activities of the individual components indicates synergism or antagonism, i.e. type (c) joint action.

Independent joint action: the Abbott method

If independent joint action of fungicides in mixture is assumed, the expected efficacy of a mixture can be predicted by the Abbott formula (Abbott, 1925) in terms of proportion of the population killed:

(1) $$E(exp) = a+(1-a)b = a+b-ab,$$

in which $E(exp)$ is the expected control efficacy of a mixture, and a and b represent the proportion of the population controlled by fungicides A and B, respectively. The value ab represents the proportion of the population killed by A and B together. The value $1-[(a+b)-ab]$ is therefore the proportion of the population which survived both fungicides. These survivors would contribute to synergism if it was operating. If efficacy is expressed in percent, equation 1 becomes:

(2) $$E(exp) = a+b-(ab/100).$$

For synergy calculation, the ratio (SF, synergy factor) between the observed experimental efficacy of the mixture $E(obs)$ and the expected efficacy of the mixture is computed:

(3) $$\mathrm{SF} = E(obs)/E(exp).$$

A ratio $E(obs)/E(exp)$ greater or smaller than 1 indicates a deviation from the hypothesis of independent action, which means that there is biological interaction between the fungicides. If $\mathrm{SF} > 1$, there is synergism; if $\mathrm{SF} < 1$, there is antagonism.

The most important advantage of this method is that it can evaluate, with no mathematical treatment, an interaction between two fungicides with only three test elements, i.e. the fungicides A and B alone and the mixture A+B. However, the accuracy of this method is doubtful at relatively high response levels of the components due to the rapid decrease of the maximal SF towards 1.0 with increasing response levels. Table 1 shows the maximal values of synergism that can be measured with the aid of the Abbott formula. It shows that the higher the response levels of the components, the smaller the synergy value that can be measured. Equation 3 calculates, therefore, the efficacy of a given mixture relative to the summed efficacies of its components.

Similar joint action: the Wadley method

The Wadley method (Wadley, 1945, 1967) is based on the second hypothesis of Bliss, which assumes that one component can substitute at a constant proportion for the other. The expected

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Table 1. Maximal synergy factor SF that can be computed with the aid of the Abbott formula at various response levels of fungicide mixtures
Valeur maximale du coefficient de synergie (SF) qu'il est possible d'établir par utilisation de la formule d'Abbott en fonction du niveau d'efficacité de deux fongicides et de leur mélange

Response level fungicide A	Response level fungicide B	Expected response level of the mixture*	Maximal SF‡
0.01	0.01	0.0199	50.2512
0.05	0.05	0.0975	10.0256
0.10	0.10	0.1900	5.2631
0.15	0.15	0.2775	3.6036
0.20	0.20	0.3600	2.7777
0.25	0.25	0.4375	2.2988
0.30	0.30	0.5100	1.9607
0.35	0.35	0.5775	1.7316
0.40	0.40	0.6400	1.5625
0.45	0.45	0.6975	1.4336
0.50	0.50	0.7500	1.3333
0.55	0.55	0.7975	1.2539
0.60	0.60	0.8400	1.1904
0.65	0.65	0.8775	1.1396
0.70	0.70	0.9100	1.0989
0.75	0.75	0.9375	1.0666
0.80	0.80	0.9600	1.0416
0.85	0.85	0.9775	1.0230
0.90	0.90	0.9900	1.0101
0.95	0.95	0.9975	1.0025
1.0	1.0	1.0000	1.0000

* Calculated from equation (1).
‡ Calculated from equation (3), assuming that $E(obs) = 1$.

their relative proportions are known. Wadley developed a short-cut graphic procedure to estimate the expected effectiveness of the mixture. Dose–response curves, obtained experimentally, are plotted on log-probit paper (Finney, 1971) for each ingredient and eye-fitted lines are drawn. A hypothetical dose–response curve is then drawn for the mixture based on the assumption that one ingredient can be substituted for the other. The experimental dose–response curve of the given mixture is also plotted and the ratio between corresponding equally effective doses (ED) of the observed and expected lines of the mixture is calculated. In addition to the graphic method, ED values can also be calculated by probit or logit analysis (Finney, 1971). Based on Sun & Johnson (1960), the formula used for the calculation of the hypothetical values (leading to equations 7 and 8) can be established as follows.

The relative efficacy of two fungicides A and B is expressible by a single figure, the ratio R of equally effective doses, ED_A and ED_B, respectively. The efficacy of the second component (B) relative to the first (A) is given by:

$$R_B = ED_A/ED_B \quad (4)$$

Since in case of similar joint action in a mixture each component can be substituted at a constant proportion for the other, the relative efficacy Re of a mixture can be expressed as:

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Table 2. Summary of differences between the Abbott and the Wadley methods for calculating interactions between fungicides in mixtures
Résumé des différences entre les méthodes d'Abbott et de Wadley pour calculer les interactions entre fongicides utilisés en mélange

	Abbott method	Wadley method
Basic hypothesis*	independent joint action	similar joint action
Variable measured	% mortality (control)	concentration
Dose–response curves	not required	required
Synergism factor	control efficacy observed / control efficacy expected	expected effective dose‡ / observed effective dose
Reliability	dependent on response level	not dependent on response level
Optimization of mixing ratio	not possible	possible, isoboles

* According to Bliss (1939).
‡ Usually 90%.

where ED(exp) is the expected equally effective dose, *a* and *b* are the proportions of fungicides A and B in the mixture, and $a+b=1$.

From equation 5, ED(exp) can be expressed as follows:

$$\text{ED(exp)} = \text{ED}_A/(a\,\text{R}_A + b\,\text{R}_B) \quad (6)$$

or, after arithmetic transformation,

$$\text{ED(exp)} = 1/(a/\text{ED}_A + b/\text{ED}_B). \quad (7)$$

In fact, ED(exp) is the harmonic mean of ED_A and ED_B weighted by their respective proportions *a* and *b* in the mixture. When *a* and *b* are not relative values but absolute amounts of the components in a mixture, equation 7 becomes:

$$\text{ED(exp)} = (a+b)/(a/\text{ED}_A + b/\text{ED}_B). \quad (8)$$

ED(exp) is compared with ED(obs) (the equally effective dose observed in experiment) in order to test the hypothesis of similar joint action. The measure for deviation from the hypothesis is again the synergy factor (SF):

$$\text{SF} = \text{ED(exp)}/\text{ED(obs)}. \quad (9)$$

If $\text{SF} = 1$, the hypothesis of similar joint action (or additivity) can be accepted; otherwise it will be rejected. If SF is greater than 1, there is synergistic action, while, if SF is smaller than 1, there is antagonistic interaction between the fungicides.

Unlike the method based on the Abbott formula, the method of Wadley allows determination of synergy at any fungicide concentration. A summary of the differences between the two methods described is given in Table 2.

Optimization of mixing ratios of fungicides by isobolograms

One of the main objectives of studying synergy is to maximize the cost/benefit ratio of fungicides

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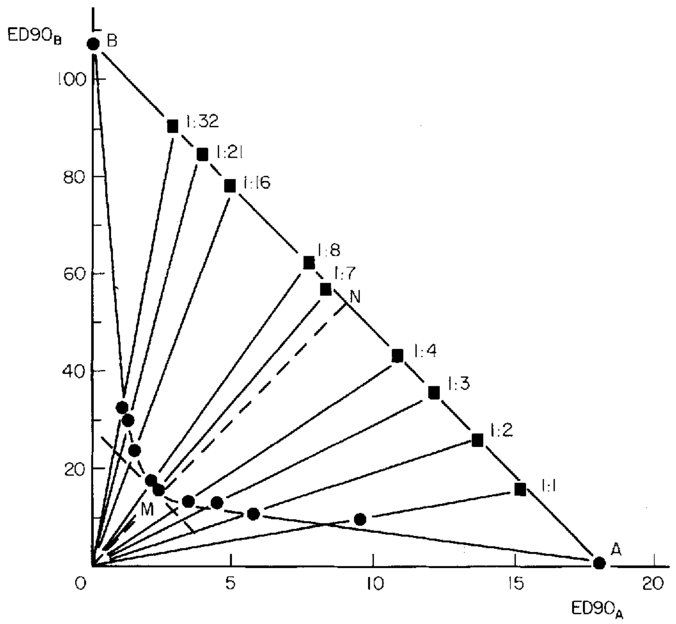

Fig. 1. Isobologram for the two fungicides A (oxadixyl) and B (mancozeb) active against *Phytophthora infestans* on potato plants, giving a theoretical (ANB) and an experimental (AMB) isobole for A+B mixtures with ratios between 1+1 and 1+32. The level of synergy can be measured by the ratio R=ON/OM which is maximal for the mixture A+B=1+7.

Isobologramme des deux fongicides oxadixyl (A) et mancozèbe (B) pour leur activité contre *Phytophthora infestans* sur pomme de terre. L'isobole ANB représente la courbe théorique en l'absence de synergie; l'isobole AMB donne les résultats d'un essai utilisant différents mélanges (1:1 jusqu'à 1:32). Le rapport R=ON/OM sert d. nesure du coefficient de synergie; il est maximal pour le mélange 1:7 de A et B.

The Wadley method is applied using mixture of components at different ratios in order to obtain a fixed control efficacy for each mixture, e.g. ED90. A so-called isobole is then plotted along the dose pairs of points producing these control efficacies. A theoretical isobole (straight line between A and B) is plotted according to the Wadley method for each mixture. Various possible synergistic interactions are represented by experimental isoboles falling within triangle ABO (Fig. 1).

The ratio ON/OM serves as a measure of SF (Fig. 1). This ratio is maximal where the distance between the points M and N is largest, representing the highest level of synergistic interactions of the components in the mixture; thus, the ratio of the components in the mixture is optimal (De Waard, 1985; Plackett & Hewlett, 1948; Tammes, 1964). For the highest value of ON/OM, the point M is given by the intersection of the experimental isobole with the tangent parallel to the straight line between A and B. If ED_A and ED_B are plotted on the axis using the same scale, the isobologram is in most cases asymmetric, making the plotting difficult. In practice, the distances OA and OB should be made equally long (symmetric isobologram, as in Fig. 1) so that triangle ABO is bisected by the line ON into two symmetric triangles ANO and BNO. In case of synergistic interactions of the components A and B, any dose pair of points for a single mixture is in one of those areas; it is then easy to decide in what direction one has to optimize the mixing

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