Synergistic Interaction of Fungicides in Mixtures

Ulrich Gisi

Agrobiological Research Station, SANDOZ AGRO Ltd., CH-4108 Witterswil and associate professor, Department of Botany, University of Basel, CH-4056 Basel, Switzerland.
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Fungicides are often combined in mixtures for three main reasons: (i) to widen the spectrum of antifungal activity to control several diseases occurring simultaneously in a crop; (ii) to exploit additive and synergistic interactions between fungicides, by which the overall activity is increased and the concentrations of the compounds can be reduced without loss of activity; and (iii) to delay the selection process of resistant individuals in a pathogen population to one component of the mixture. Many fungicide mixtures have been used for disease control for a long period of time without knowledge of the superior performance of the mixture compared with components used alone. The oldest examples are copper salt mixtures and mixtures containing different dithiocarbamates with a broad spectrum of activity. Modern fungicides are mostly single-site inhibitors. Mixtures containing such fungicides may provide better disease control than single components and, at the same time, reduce the risk of pathogens developing resistance.

Only recently, synergistic interaction between fungicides and its significance for performance (12–14,19,20) and resistance (11,14, 20,23) have been studied in detail under laboratory and field conditions. In this paper, examples describing synergistic interactions between compounds of different classes of antifungal agents tested under different conditions against a range of pathogens are presented. The general mechanisms involved in synergistic interactions are also elucidated. Examples of antagonistic interactions in certain fungicide mixtures (8,9) will not be discussed in this paper.

METHODS OF ANALYSIS

There are several methods of estimating the extent of interaction of fungicides in mixtures. They are described in detail by Gisi et al. (12), Kosman and Cohen (17), and Levy et al. (18). The two major methods used in this paper are those by Abbott (1,6) and Wadley (26,27). The expected efficacy of a mixture, expressed as percent control ($\%C_{exp}$), can be predicted by the Abbott formula (18):

$$\%C_{exp} = A + B - (AB/100)$$

in which A and B are the control levels given by the single fungicides. If the ratio between the experimentally observed efficacy of the mixture (C_{obs}) and the expected efficacy of the mixture (C_{exp}) is greater than 1, synergistic interactions are present in the mixture. The Abbott method is used without mathematical transformations to estimate the interaction for single concentrations of a mixture, provided the control levels of the single components are not higher than about 70%. Synergistic interactions always decrease rapidly with increasing control levels of the single components (18,19,22) and may be almost nil at high control levels. In the Wadley approach (12,18), dose-response curves of the single components, A and B, and the mixture, $A + B$, are constructed. With a logit-log (or more appropriately a probit-log) transformation, the dose-response curves are linearized by regression and then are used to calculate EC (effective concentration) values for different control levels, e.g., EC_{50} or EC_{90}. When a and b are the absolute amounts of the components in the mixture, the expected effective concentration (EC_{exp}) at any control level can be calculated (12) as

$$EC_{90exp} = (a + b)/[(a/EC_{90A}) + (b/EC_{90B})]$$

Synergistic interactions are present if the ratio of the expected and the observed EC values is greater than 1. The Wadley approach (12,18) can be used for estimation of interactions at any fungicide concentration, and its reliability is not dependent on the disease-control level. In single experiments, statistical procedures are available for dose-response relations but not for calculation of synergistic interactions. Synergy ratios calculated on the basis of EC_{50} and EC_{90} values in most cases are very similar to each other. Because statistical confidence levels of synergy ratios are missing, ranges rather than precisely fixed values may be defined to quantify antagonistic, additive, and synergistic interactions (Table 1), also taking into account the degree of variation in biological responses to antifungal compounds. More details on the Wadley approach are given by Kosman and Cohen (17).

TABLE 1. Suggested terminology for levels of interaction[a] in fungicide mixtures when the Wadley approach is used for quantification

Level of interaction	Mathematical definition[b]	Biological response[c]	Biological response[d]
<1.0	Antagonistic		
1.0	Additive		
>1.0	Synergistic		
<0.5		Antagonistic	
0.5–1.5		Additive	
>1.5		Synergistic	
<0.7			Antagonistic
0.7–1.3			Uncertain
>1.3–2.0			Weakly synergistic
>2.0			Strongly synergistic

[a] Ratio between expected and observed EC (effective concentration) values.
[b] According to Levy et al. (18) and De Waard and Gisi (8).
[c] According to Gisi et al. (12).
[d] This definition avoids the artificial case of additive interaction and is based on the examples given by Kosman and Cohen (17). The suggested interaction levels are arbitrary and are used in Table 2. The biological response definitions cannot be used for the Abbott approach.

Corresponding author: U. Gisi; E-mail address: ulrich.gisi@gwa.sandoz.com

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The Wadley approach can be used to define the optimum ratio of the components in the mixture to achieve the highest control levels (lowest EC values), i.e., minimum amounts of the components and maximum synergy level. For the mixture of oxadixyl + mancozeb tested against *Phytophthora infestans* on potato plants under greenhouse conditions, the synergy level was less than 3 for ratios below 1+4, reached a maximum of 3.6 for the ratio 1+7, and decreased again at ratios above 1+16 (13,18). Therefore, synergy levels are strongly dependent on the ratio of components in the mixture. By testing a range of ratios, isoboles can be constructed for two- or three-component fungicide mixtures. This approach was used for oxadixyl + mancozeb (18) and for oxadixyl + mancozeb + cymoxanil mixtures (14).

SYNERGISTIC INTERACTION IN MIXTURES OF DIFFERENT AND IDENTICAL MODES OF ACTION

Synergistic interactions are not restricted to a single chemical class of compounds. Examples will be given of components with different as well as identical modes of action tested against a range of pathogens at the enzyme, fungus, disease, and epidemic levels.

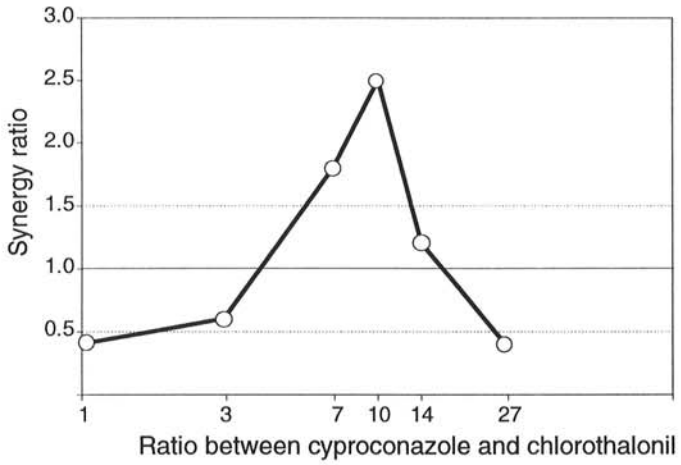

Fig. 1. Interaction (synergy ratio) between cyproconazole and chlorothalonil combined in mixture at different ratios (1+1 to 1+27) against *Mycosphaerella arachidis* on peanut (U. Hugelshofer and U. Gisi, *unpublished data*). Synergy ratio calculated according to Wadley (12,18). Logarithmic x-axis.

Different Modes of Action

Synergistic interactions are well documented for mixtures containing the systemic phenylamide fungicide oxadixyl, the contact fungicide mancozeb, as well as the systemic fungicide cymoxanil (all three with different modes of action) against *Phytophthora* and *Plasmopara* (12,14,21,22). Synergy ratios vary within a large range (e.g., 1.4 to 5.3). Mixtures of the triazole fungicide cyproconazole and the chlorophenyl fungicide chlorothalonil tested against *Mycosphaerella arachidis* on peanuts, produce strong synergistic interaction at ratios between 1+7 and 1+10, whereas at ratios below 1+2 and above 1+20 the mixtures may be additive or antagonistic (Fig. 1). Fungicides with different modes of action may affect the fungus at different biochemical sites and developmental stages, resulting in combined and synergistic activity of the mixture. The components preferably should be applied pre-

TABLE 2. Synergistic interaction[a] between different triazoles[b] tested under laboratory conditions[c]

Triazole in mixture	*Erysiphe graminis* on wheat	*Leptosphaeria nodorum* on wheat or in vitro	*Mycosphaerella graminicola* in vitro
With cyproconazole			
Flutriafol	+	++	++
Hexaconazole	(+)	(+)	(+)
Triadimenol	++	–	–
Tebuconazole	++	(+)	nt
Flusilazole	+	–	nt
Epoxiconazole	++	(+)	(+)
Propiconazole	++	+	++
With flutriafol			
Cyproconazole	+	++	++
Hexaconazole	++	+	(+)
Triadimenol	+	++	(+)
Tebuconazole	++	++	+
Flusilazole	+	+	–
Epoxiconazole	++	++	+
Propiconazole	+	++	(+)

[a] Level of interaction (according to the Wadley approach): ++ = strongly synergistic; + = weakly synergistic; (+) = uncertain; – = antagonistic; and nt = not tested.
[b] Ratio of components in mixture 1:1 to 1:4.
[c] Results by D. Hermann and U. Gisi (*unpublished data*).

TABLE 3. Percent inhibition[a] of spore germination of *Botrytis cinerea* by combinations of different concentrations of gliotoxin (G) and endochitinase (E)

Concentration of G (ml/liter)	Concentration of E (mg/liter) 0	25	50	75	100	Synergy ratio of E + G (Wadley)[b]
0	...	**0**	**0**	**24**	**34**	
0.25	**0**	**0** 0 ...	**8** 0 ...	**32** 24 1.3	**42** 34 1.2	
0.50	**2**	**8** 2 4.0	**36** 2 18.0	**70** 26 2.7	**90** 35 2.6	
0.75	**2**	**54** 2 27.0	**76** 2 38.0	**96** 26 3.7	**100** 35 2.9	(100 + 0.75) SR = 1.1
1.00	**18**	**78** 18 4.3	**96** 18 5.3	**100** 38 2.6	**100** 46 2.2	(100 + 1.00) SR = 1.1
1.25	**48**	**92** 48 2.1	**100** 48 2.1	**100** 60 1.7	**100** 1.6	(100 + 1.25) SR = 1.2
1.50	**96**	**98** 96 1.0	**100** 96 1.0	**100** 97 1.0	**100** 97 1.0	(100 + 1.50) SR = 1.5

[a] Measured experimentally (according to Di Pietro et al. [10]; bold figures) or calculated according to Abbott (figures in the lower left corner of cells). Figures in the lower right corner of cells are synergy ratios calculated according to Abbott.
[b] For the Wadley approach, dose response relations of E + G mixtures at constant ratios of the components in the mixture are needed. Four E + G ratios with several dilution steps can be identified (indicated by arrows): 100 + 0.75 (three dilutions) and 100 + 1.00, 100 + 1.25, and 100 + 1.50 (each with four dilutions).

ventively and simultaneously for maximum expression of synergistic interactions.

Identical Modes of Action

All triazole fungicides have the same biochemical mode of action (inhibition of C14 demethylation in sterol biosynthesis) but differ significantly in their spectrum of activity and, therefore, are often combined in mixtures. Mixtures of cyproconazole or flutriafol and other triazoles were tested against three important pathogens of wheat: *Erysiphe graminis*, *Leptosphaeria nodorum*, and *Mycosphaerella graminicola* (Table 2). Surprisingly, synergistic interaction occurred in many fungicide combinations, whereas in others the biological response was uncertain. Generally, the level of synergistic interaction was not as pronounced as in mixtures of fungicides with different modes of action. Synergistic interactions were most pronounced against *E. graminis*. Obviously, there are important differences among triazole fungicides that allow synergistic interactions, including different systemicity and uptake into the fungus and plant as well as different physicochemical properties (e.g., water solubility and log *p*) and rates of metabolization.

Biocontrol Agents

Gliocladium virens is used for biological control of many diseases under controlled conditions, including *Botrytis cinerea*. The control mechanisms are probably not competition for nutrients or space nor are they hyperparasitism, but more likely toxins are produced. Recently, Di Pietro et al. (10) claimed enzyme activity is involved in the control mechanism. In experiments with spore germination of *B. cinerea* as the target process, they combined purified gliotoxin (G) and endochitinase (E) in different ratios and found increased activity with the mixture compared to the single components alone. When the results of Di Pietro et al. (10) are analyzed according to the Abbott approach, very strong synergistic interactions are found for certain G + E combinations, especially when control levels are low (Table 3). The mixture provides maximum synergy for G + E = 0.75 + 50 mg/liter (synergy ratio = 38.0). When the same results were analyzed according to Wadley, synergy ratios were less pronounced and were between 1.1 and 1.5 (Table 3). The synergistic interaction between the antifungal toxin and the enzyme was described for the pathogen in vitro. Whether the reported synergy levels are expressed at the disease level under greenhouse or field conditions is unknown. Synergy found experimentally for natural product combinations may represent an important principle of stability in coevolution of microbial communities in nature (discussed below).

TABLE 4. Fungicidal activities (effective concentration [EC_{90}]) of oxadixyl (o), mancozeb (ma), and cymoxanil (c) alone and in mixture and synergy ratio (SR) of mixtures against oxadixyl-sensitive (Sens.) and -resistant (Resis.) *Phytophthora infestans* on either tomato under growth-chamber conditions[a] (single strains) or potato under field conditions[b] (population)

			SR[c]	
Fungicide	Sens.	Resis.	Sens.	Resis.
EC_{90} (mg/liter) in growth chamber[d]				
o	34	>5,000	...	...
ma	776	495	...	...
c	48	23	...	...
o + ma = 1 + 7	69 (9 + 60)	406 (51 + 355)	3.0	1.4
ma + c = 7 + 0.4	134 (127 + 7)	51 (48 + 3)	3.2	4.6
o + c = 1 + 0.4	22 (16 + 6)	41 (29 + 12)	1.7	2.0
o + ma + c = 1 + 7 + 0.4	74 (9 + 62 + 4)	63 (8 + 53 + 3)	2.4	4.2
EC_{90} (g/ha) in field[d]				
o	60	>2,000	...	...
ma	760	1,130	...	...
c	350	310	...	...
o + ma = 1 + 7	100 (12 + 88)	550 (69 + 481)	3.2	2.3
ma + c = 7 + 2	220 (177 + 43)	260 (209 + 51)	2.8	2.9
o + ma + c = 1 + 7 + 2	110 (11 + 77 + 22)	150 (15 + 105 + 30)	2.8	5.3

[a] Data from Grabski and Gisi (14).
[b] Data from Samoucha and Cohen (21).
[c] SR calculated according to Wadley (12,18).
[d] Figures in parentheses are dosages of the individual components in the mixture. Recommended rate for *P. infestans* control in many countries is o + ma + c = 200 +1,400 + 80 g/ha.

Synergistic Interaction in Mixtures at Individual and Population levels

For simplicity, most interaction experiments are performed under laboratory or greenhouse conditions (in vitro and in vivo) with a single isolate (strain). When oxadixyl, mancozeb, and cymoxanil were combined in mixtures against oxadixyl-sensitive or -resistant strains of *P. infestans,* the mixtures provided high synergy ratios for all combinations of the components (Table 4). When cymoxanil was included in the mixtures, synergy ratios were higher with the resistant than with the sensitive strain. The amount of fungicide necessary for 90% control (EC_{90}) was significantly reduced in the mixtures compared to single components. The most consistent EC_{90} values for both sensitive and resistant strains were observed with the three-way mixture.

Similar results were obtained in a field study, in which potatoes were artificially inoculated with a sensitive or a resistant isolate of *P. infestans* and sprayed with four fungicide treatments. The fungicidal activity was rated at the end of the season after the fungus underwent several disease cycles (Table 4). Synergistic interactions were observed for all mixtures; cymoxanil-containing mixtures showed especially strong or stronger synergy for resistant than for sensitive populations. The three-way mixture provided the most consistent EC_{90} values for both populations, and the amount of fungicide was considerably reduced in the mixtures. Both the high synergism of mixtures for resistant populations (higher than for sensitive ones) and the reduced rates of fungicides are important parameters for effectively reducing resistance development.

It is most important to use appropriate fungicide mixtures before resistant subpopulations become dominant, because the risk for control failures of populations consisting entirely of resistant isolates is high. Mixtures of oxadixyl + mancozeb and oxadixyl + mancozeb + cymoxanil delayed resistance build-up significantly compared to oxadixyl alone—the three-way mixture much more effectively than the two-way mixture (Fig. 2). The resistant sub-

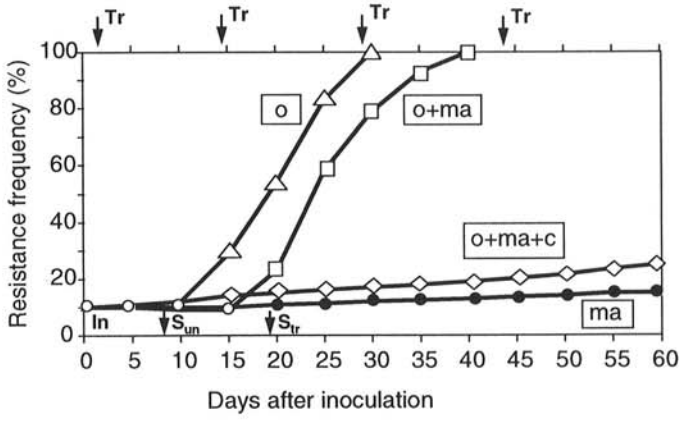

Fig. 2. Resistance frequency (percent) in a *Phytophthora infestans* population initially containing 10% oxadixyl-resistant sporangia developing on field-grown potatoes treated four times (Tr, arrows) with oxadixyl (o), mancozeb (ma), oxadixyl + mancozeb (o + ma), or oxadixyl + mancozeb + cymoxanil (o + ma + c). In = inoculation; S_{un} = first symptoms on untreated plants; and S_{tr} = first symptoms on treated plants (redrawn from Cohen and Samoucha [5])

population increased from 10 to ~20% after four applications of oxadixyl + mancozeb + cymoxanil, whereas a 100% resistant population was found after 40 and 30 days, respectively, for applications of oxadixyl + mancozeb or oxadixyl. The effectiveness of a mixture in delaying resistance build-up also depends on the initial amount of resistance in the population. For levels below 1% resistant sporangia in *P. infestans* populations, two to four sprays of oxadixyl + mancozeb may still give adequate control, whereas oxadixyl + mancozeb + cymoxanil also is effective on populations with higher proportions of resistant subpopulations (23).

In addition to reduced fungicide use in the mixture, synergistic interactions also provide longer residual activity compared to single components alone. This is because the initial activity of the mixture is higher than that of the single components. For 90% control of an oxadixyl-resistant population of *P. infestans* on potatoes, the duration of disease control was about twice as long (11 compared to 5 days) when the three-way mixture oxadixyl + mancozeb + cymoxanil was used (24). Synergistic interactions do not necessarily change during the period of several fungicide applications but may be responsible for consistent disease control after each application.

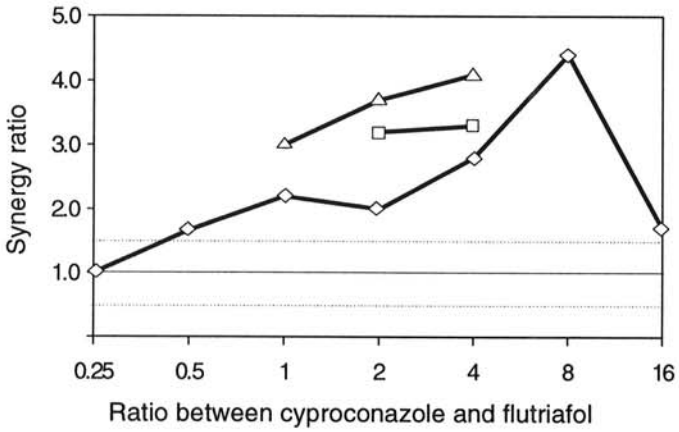

Fig. 3. Synergistic interaction (synergy ratio) between cyproconazole and flutriafol combined in mixture at different ratios (0.25+1 to 1+16) against *Mycosphaerella graminicola* in vitro (◇) and *Leptosphaeria nodorum* in vitro (□) and on wheat (△) (D. Hermann and U. Gisi, *unpublished data*). Fungicides were used as technical grade in *M. graminicola* tests (seven values) and as formulated products in *L. nodorum* tests (two and three values, respectively). Synergy ratio calculated according to Wadley (12,18). Logarithmic x-axis.

A MECHANISTIC APPROACH TO SYNERGISTIC INTERACTION

In Vitro and In Vivo Activity of Formulated and Technical Grade Fungicides

It is sometimes claimed that formulation ingredients, such as surfactants, solvents, and salts (normally considered nonfungicidal), are responsible for producing synergistic interactions when combined with active ingredients of antifungal compounds. A combination of different ratios of technical grade cyproconazole and flutriafol tested against *M. graminicola* in vitro (growth of yeast form on agar plates) resulted in synergistic interactions over a wide range of ratios (1+0.5 to 1+16) with a maximum at 1+8 (Fig. 3). Tests with formulated cyproconazole and flutriafol in mixture against *L. nodorum* revealed significant synergistic interactions both in vitro (mycelial growth on agar plates) and in vivo (disease symptoms on wheat leaves). Therefore, formulation ingredients cannot be the major reason for the production of synergistic interactions of the two fungicides, but they may improve their efficacy through better distribution and uptake of fungicides. More important is the question of whether synergy is expressed equally strongly at the fungus and disease levels and whether synergy occurs under field conditions. There also are well-known examples of synergistic interactions between nontoxic compounds (piperonyl butoxide) and insecticides; they are probably due to reduced detoxification of insecticides.

Activity of Mixtures at Disease, Fungus, and Enzyme Levels

Cyproconazole is an antifungal compound with two chiralic C atoms in the molecule resulting in four stereoisomers. The four isomers, A^+, A^-, B^+, and B^-, are present in mixture AB (cyproconazole) in the ratio 1+1+1+1. The fungicidal activity of the single isomers were compared with mixture AB against a range of pathogens (biotrophic and necrotrophic), and synergy ratios were calculated according to Wadley (12,18). All four isomers showed fungicidal activity, with B^- and A^- showing the strongest effects, about as strong as AB. Also, B^+ and A^+ provided some activity, especially against biotrophic pathogens (Table 5). All isomers, including the least active ones, are obviously responsible for the overall fungicidal activity of mixture AB, because in all cases (except *Botrytis* on agar and enzyme) synergistic interactions were observed. For the necrotrophic pathogens, synergy was observed both at the disease and mycelial growth levels (Table 5). In the case of *Botrytis*, slight synergy was observed at the disease level, whereas at the fungus and especially at the enzyme levels (sterol C14 demethylase), additive interactions were found. Therefore, synergy

TABLE 5. Fungicidal activity (effective concentration [EC_{90}]) of cyproconazole (AB) and its four isomers against fungi in vitro and in vivo and the synergy ratio (SR)[a]

Fungus/host or medium	Fungicidal activity (EC_{90}, mg/liter)					SR[b]
	AB	B^-	A^-	A^+	B^+	
Sphaerotheca fuliginea/cucumber	1	1	1	4	6	1.9
Erysiphe graminis/wheat	2	2	2	7	9	1.6
Erysiphe graminis/barley	4	3	3	21	16	1.3
Uncinula necator/grape	2	2	2	11	9	1.7
Puccinia recondita/wheat	2	5	7	7	18	3.7
Cercospora beticola/sugar beet	36	36	97	650	390	2.6
Leptosphaeria nodorum/wheat	10	12	20	670	190	2.8
Leptosphaeria nodorum/agar	10	14	16	>900	380	2.9
Cochliobolus sativus/barley	2	1	3	47	69	1.5
Cochliobolus sativus/agar	6	9	11	85	120	3.0
Rhizoctonia cerealis/agar	1	1	3	84	44	2.9
Rhizoctonia solani/agar	6	9	8	>200	>200	2.7
Botrytis cinerea/bean	142	83	165	590	>900	1.4
Botrytis cinerea/agar	13	3	17	>200	>200	0.6 (0.8)[c]
Botrytis cinerea/enzyme	...	...	...	...	...	(0.5)[c]

[a] According to Grabski and Gisi (15).
[b] SR calculated according to Wadley (12,18).
[c] Values calculated on the basis of EC_{50} and IC_{50}, respectively; results by Stehmann and De Waard (25).

between isomers may not be related to the biochemical site of fungicide action but to more complex phenomena (such as expression of disease symptoms) or to a combination of effects. Although all four isomers are expected to have an identical and primary mode of action at the P450 enzyme level (binding to heme complex), they may differ in binding to secondary sites, such as other P450 enzymes.

Different Application Types

An important question is whether the components in a mixture have to be applied together (type and timing of the treatment) to provide synergistic interactions. To protect foliage of tomato plants against *P. infestans,* the systemic fungicide oxadixyl was applied as a soil drench; after 1 day the plants were spray-treated with the contact fungicide mancozeb (22). The fungicides, when applied alone, provided control levels of 5 and 7%, respectively, whereas the combination of the two application types resulted in 75% control, representing a synergy ratio of 6.4 (Abbott method). Thus, fungicides can be separated by space and still provide synergy as long as they meet again at the site of infection for combined inhibition of the fungus and if the application interval is not too long.

In another approach, spray application of the fungicides oxadixyl, mancozeb, and cymoxanil was made either simultaneously as a preventive measure (oxadixyl + mancozeb or oxadixyl + cymoxanil) or as a split (first mancozeb and then oxadixyl) as a curative measure 48 or 72 h after the first treatment (22). Control levels of *P. infestans* on tomato plants were generally lower after split applications compared to simultaneous treatment. Synergy ratios decreased over time: for oxadixyl + mancozeb from 5.2 to 2.3 (48 h) to 1.2 (72 h) and for oxadixyl + cymoxanil from 2.9 to 1.7 (48 h) to 1.0 (72 h) (22). Thus, the longer the interval between applications, the less pronounced are the synergistic interactions between components. Synergistic interaction may be stronger if mixtures are applied as a preventive rather than as a curative measure, because more stages of pathogen development are affected.

TABLE 6. Fungicidal activity (effective concentration [EC_{90}]) and synergy (SR)[a] between cyproconazole and different herbicides against *Rhizoctonia cerealis* in vitro and on wheat[b]

Cyproconazole plus	EC_{90} (mg/liter) in vitro[c]			EC_{90} (mg/liter) on wheat[d]		
	Exp.[e]	Obs.[e]	SR	Exp.	Obs.	SR
Dicamba						
1 + 1	3.9	0.8 (0.4 + 0.4)	4.8	42	20 (10 + 10)	2.1
1 + 0.5	2.9	1.2 (0.8 + 0.4)	2.4	36	15 (10 + 5)	2.4
Bromoxynil						
1 + 5	7.6	6.6 (1.1 + 5.5)	1.2	138	30 (5 + 25)	4.6
1 + 2.5	5.4	5.2 (1.5 + 3.7)	1.0	87	35 (10 + 25)	2.5

[a] SR calculated according to Wadley (12,18).
[b] Kataria and Gisi (16).
[c] EC_{90} values of cyproconazole, dicamba, and bromoxynil when used alone were 1, >1,000, and 18 mg/liter, respectively.
[d] EC_{90} values of cyproconazole, dicamba, and bromoxynil when used alone were 27, 92, and 770 mg/liter, respectively.
[e] Exp. = expected; Obs. = observed.

TABLE 7. Pretreatment effect of different herbicides[a] on subsequent radial growth rate and sensitivity to cyproconazole (effective concentration [EC_{90}]) of *Pseudocercosporella herpotrichoides*[b]

Inoculum from medium	Radial growth rate (mm/day)	Sensitivity to cyproconazole (EC_{90}, mg/liter)[c]
No herbicide	2.0	4.1
Dicamba	2.0	0.8
Bromoxynil	1.8	1.6

[a] Concentration 10 mg/liter.
[b] Kataria and Gisi (16).
[c] EC_{90} values of cyproconazole, dicamba, and bromoxynil when used alone were 4, >1,000, and 85 mg/liter, respectively.

Fungicide-Herbicide Mixtures

Mixtures of fungicides and herbicides may be needed if both diseases and weeds are to be controlled at the same time. Some fungicides may have slight effects on the physiology of plants, and some herbicides may have slight fungicidal activity. The herbicide dicamba, which shows auxin-like activity in broad-leaf weeds, did not show any fungicidal activity against *Rhizoctonia cerealis* and *Pseudocercosporella herpotrichoides* in vitro but had some activity against sharp eyespot on wheat caused by *R. cerealis* (Table 6, footnote). In contrast, bromoxynil, an ATP uncoupler, had no effect against sharp eyespot, but inhibited *R. cerealis* and *Pseudocercosporella herpotrichoides* in vitro to a certain degree. Both herbicides, however, were much less active against the pathogens than cyproconazole (Tables 6 and 7, footnote).

When mixtures of cyproconazole and herbicides were used to control *R. cerealis* in vitro as well as on wheat, significant synergistic interactions were observed with cyproconazole + dicamba and cyproconazole + bromoxynil mixtures (Table 6). To elucidate the mechanisms of synergy in the fungicide + herbicide mixtures, the pathogen (*Pseudocercosporella herpotrichoides)* was pretreated with the herbicides and transferred to the fungicide-amended agar.

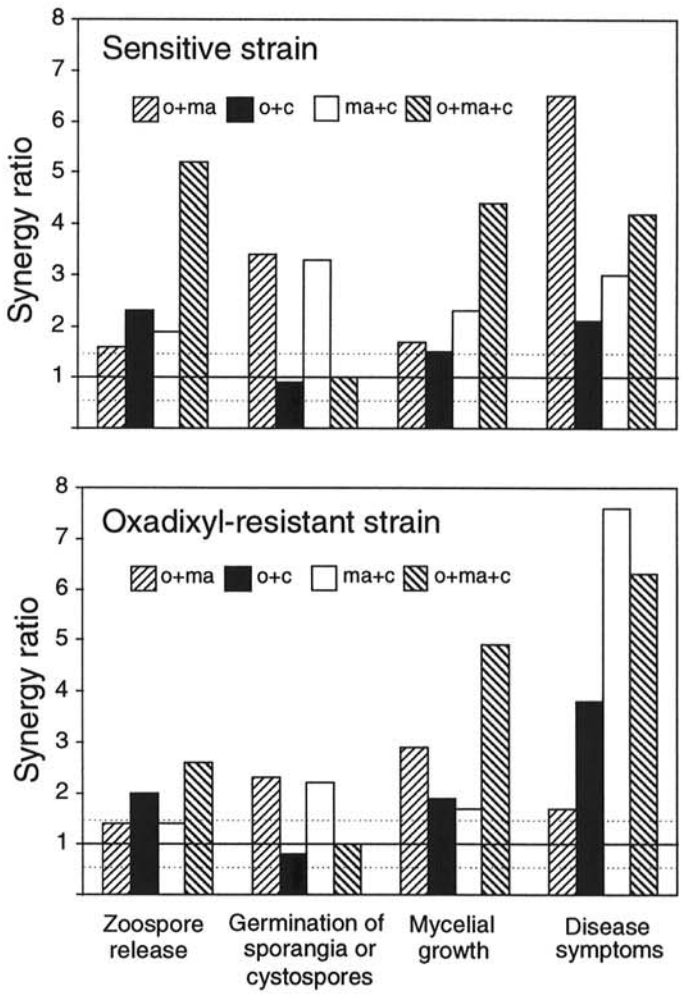

Fig. 4. Synergistic interaction (synergy ratio) in mixtures of oxadixyl (o), mancozeb (ma), and cymoxanil (c) against a sensitive strain and an oxadixyl-resistant strain of *Phytophthora infestans* in different stages of the disease cycle (I. Muntwiler and U. Gisi, *unpublished data*). Synergy ratio calculated according to Wadley (12,18).

There was no difference in radial growth rate of the pathogen with or without pretreatment with the herbicide, but sensitivity to cyproconazole significantly increased after pretreatment (lower EC values; Table 7).

Similar pretreatment studies were done by Bashan et al. (3) using mancozeb first and then oxadixyl against *P. infestans*, resulting in a more sensitive response of the pretreated sporangia compared with the untreated. Therefore, synergistic interactions may be based on sublethal effects induced by the first component (often not measurable) followed by a detrimental effect (of normally sublethal concentrations) by the second component. This hypothesis may result in decreased aggressiveness of the pathogen (11) and in synergistic interaction of the fungicides in the mixture. This explanation of synergy is mainly based on the observation that the first treatment (herbicide in the first example and mancozeb in the second) affects the mitochondrial respiration of the pathogen, weakening its metabolic activity. Therefore, treatment of the pathogen in an early stage of its development may result in the strongest synergistic interactions.

Activity Against Different Stages of the Disease Cycle

Different stages in the disease cycle of a pathogen, such as zoospore release, germination of sporangia and cystospores, mycelial growth, and symptoms caused by *P. infestans*, often show different sensitivities to fungicides. Thus, synergistic interactions of fungicide mixtures also may vary for different developmental stages. With few exceptions, synergistic interactions of all combinations of oxadixyl, mancozeb, and cymoxanil were most pronounced for symptom development followed by mycelial growth, whereas zoospore release and germination showed lower and more variable synergy ratios depending on the mixture and the sensitivity of the isolate to oxadixyl (Fig. 4). With a few exceptions, the three-way mixture oxadixyl + mancozeb + cymoxanil gave the highest synergy ratios (not for germination however). The two-way mixture oxadixyl + mancozeb provided especially high synergy ratios for germination and disease symptoms but only for the sensitive isolate. Synergistic interaction may be enhanced when several developmental stages of the pathogen and several fungicidal components are involved in the interaction and may be based on the sum of several events rather than on single specific effects.

Uptake, Efflux, and Degradation of Components in Mixture

Recently, Bashan et al. (2) observed a decreased uptake of phenylamides into a phenylamide-resistant isolate of *P. infestans*. As in other classes of fungicides, a mixture of components with different modes of action may inhibit the energy-dependent efflux of single fungicidal components that consequently result in a higher concentration of the fungicide within the cell (7). Whether an accelerated efflux proposed by De Waard (7) for isolates of several fungi resistant to demethylation inhibitors (DMI) is related to changes in P-glycoprotein activity and also for other types of fungicide resistance remains to be investigated. When applied to the surface of tomato plants, enhanced uptake and translocation and accelerated degradation of cymoxanil were observed in plants treated with a mixture of oxadixyl and cymoxanil compared to plants treated with cymoxanil alone (4). More studies are necessary to elucidate the mechanisms of synergistic interaction between fungicides, but changes in translocation of one fungicide as affected by a second component may be one of the most important effects in fungicide interactions.

SYNERGISTIC INTERACTIONS IN AREAS OUTSIDE PLANT PATHOLOGY

The more we try to attribute synergistic interactions of fungicide mixtures to a single specific effect in the target pathogen, the less conclusive are the explanations. It seems that synergy is a phenomenon best described as the sum of several simultaneous events. This theory may be supported by examples from other areas. (i) The multiple-factor syndrome in medicine maintains that the severity of symptoms of a sick person cannot always be attributed to single events found in the diagnosis but only to the sum of more minor symptoms when they occur separately from each other. (ii) The well-known die-back of forests in many European countries can rarely be attributed to a single stress factor, such as ozone damage, acid rain, unbalanced nitrogen nutrition, or toxic concentrations of aluminum ions in the soil, but the sum of all these factors is detrimental. (iii) Often during purification of antifungal microbial broths, the chemical "principle" that should be responsible for the antifungal activity is much less or even lost in the single fractions compared with the original broth, suggesting that several active principles are involved in the overall (synergistic) activity. (iv) Symbiosis is often defined as synergistic interaction between two organisms that is beneficial for both and is the opposite of an antagonistic interaction in antibiosis. In evolution, synergistic (symbiotic) interactions between species are very common and important for the stability of ecosystems. Most synergistic interactions are time dependent, i.e., they are not permanent but transient in the development of organisms; they depend on many factors, whether they persist over a long time period. The term synergy is often misused (e.g., instead of complementarity). It should be restricted to those cases in which synergistic interactions can be measured as a stronger response of the combination compared to the sum of the individual effects.

CONCLUSIONS

Synergy is a frequent phenomenon in fungicide mixtures. Its magnitude depends on the ratio of the components in the mixture and their modes and mechanisms of action. It is affected by the sensitivity of individuals to fungicides and the composition of populations. Synergy may reduce the selection process of resistant subpopulations and allows a longer duration of fungicidal activity. Rates of fungicides can be reduced using synergistic mixtures without loosing efficacy. Synergistic interaction is most pronounced when components are applied simultaneously; it decreases when the components are used in split applications. Synergy may occur between antifungal compounds of different natures and sources (including natural products) and between fungicides, herbicides, and insecticides with different or identical modes of action and in different formulations. Synergy may occur at the enzyme, fungus, disease, and epidemic levels, depending on the type and timing of fungicide application. Synergy may be expressed at different intensities during different stages of the disease cycle. The mechanisms of synergy are speculative and may be due to a combination of effects rather than to a single specific effect; decreased aggressiveness of the pathogen and increased concentration of components at the target site may be major effects. The term "synergy" has a well-defined meaning in biology but is often misused in the nonscientific world. Therefore, it should be used with great caution.

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