

Interference of nutrients with fungicide activity against *Septoria nodorum* on wheat leaves

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The effect of nutrients on reduction of the germination and mycelial growth of *Septoria nodorum* by the fungicides propiconazole, fenpropimorph and prochloraz, and on the reduction of *S. nodorum* infection by prochloraz, was tested on wheat plants in a controlled environment. The nutrients tested were aphid honeydew, sucrose, a mixture of amino acids and combinations of sucrose with yeast extract and amino acids, all in different concentrations. All nutrients could stimulate *S. nodorum* infection in combination with the tested prochloraz concentrations (4.5–45 mg a.i./l), but amino acids did not significantly stimulate infection in the fungicide-free treatments. The stimulating effect of the nutrients was generally stronger with higher nutrient/fungicide ratios. Aphid honeydew had more effect than its main components sucrose and amino acids separately. Antagonism between nutrients and prochloraz was determined with the Colby method and from dose–response curves by comparing ED₅₀s and drawing antagonism isoboles. Aphid honeydew, sucrose and amino acids caused an up to 10-fold increase of the ED₅₀ and can be considered antagonistic to prochloraz.

INTRODUCTION

The effect of a fungicide on plant pathogens can be influenced by substances mixed with the chemical or naturally present in the environment of the pathogen. Such interaction is called synergism when the effect of the fungicide is enhanced and antagonism when it is decreased. When the toxicity, measured, for example as ED₅₀, is not changed, the effect of the chemicals is called additional (Tammes, 1964). The effect of substances that are commonly available to pathogens on the effectiveness of fungicides is not yet well documented.

Field experiments by Rabbinge *et al.* (1984) demonstrated that the control of phyllosphere fungi on wheat leaves by captafol was significantly reduced in the presence of aphid honeydew. As aphid honeydew has been shown to enhance spore germination and germ-tube growth of *Cochliobolus sativus* and lesion development of both *Septoria nodorum* and *Cochliobolus sativus* (Fokkema *et al.*, 1983), we became interested in the role of nutrients in the performance of fungicides. The aim of the work pre-

sented here was to investigate whether aphid honeydew is antagonistic to fungicides that are commonly used in cereals and, if so, to establish which of the main components of honeydew causes the effect.

Aphid honeydew produced by *Sitobion avenae* on wheat consists of carbohydrates (c. 98% of the dry matter) and of a mixture of many amino acids (c. 2%) (Rossing & Van de Wiel, 1990). The interaction of a range of amino acids and carbohydrates with the effect of different fungicides on various necrotrophic pathogens such as *Alternaria* spp., *Rhizoctonia* spp., *Fusarium* spp., *Aspergillus* spp. and *Pythium* spp. has been described. Both amino acids and carbohydrates can be either synergistic or antagonistic to fungicides (Grover & Kumar, 1965; Grover & Sidhu, 1965; Beynon & Brown, 1969; Chopra & Jhoo, 1974; Jhoo & Bains, 1976; Kataria & Grover, 1978). Richardson (1966), Beynon & Brown (1969), Dunn *et al.* (1971) and Chopra & Jhoo (1974) showed that leaf, root and spore exudates, containing both carbohydrates and amino acids, can all be antagonistic to a range of fungicides. Practically all these interactions, however, are

studied under *in vitro* conditions, measuring germination and mycelial growth. The effect on infection and the importance of these interactions in agricultural practice is unknown.

The experiments described here were conducted on spring wheat plants in a controlled environment with the necrotrophic fungus *Septoria nodorum* (teleomorph: *Leptosphaeria nodorum*), causal agent of glume blotch of wheat, as the test pathogen. The first experiments were carried out to assess the effect of a mixture of sucrose and yeast extract, which resembles the effect of aphid honeydew on necrotrophic pathogens (Fokkema *et al.*, 1983), on the activity of different fungicides. Subsequently, prochloraz was used as the test fungicide and the influence of composition and concentration of different nutrients, including aphid honeydew, on fungicide effectiveness was investigated.

MATERIAL AND METHODS

Plants

Spring wheat, *Triticum aestivum* cv. Minaret, was sown in potting soil (Triomf nr. 17, TRIO B.V.) in pots 20 × 20 cm (25 seeds per pot) and grown in the greenhouse at 18–25°C with additional light during autumn and winter. If necessary, mildew infection, caused by *Erysiphe graminis* f.sp. *tritici*, was suppressed by spraying with ethirimol (Milgo E, ICI) with avoidance of the flag leaves. Aphids, if present, were eliminated with pirimicarb (Pirimor, ICI), which does not affect *Septoria nodorum*. The flag leaves of the plants were used at growth stage DC 50–60 (Zadoks *et al.*, 1974).

Pathogen

Isolate WCS-F35 of the pathogen *S. nodorum* was grown on oatmeal agar plates at 18°C and under 12 h near-UV light per day. After incubation for 10–15 days, a conidial suspension was made in sterile Tween 80 solution (0.01%) by cautiously scraping the conidia from the plates. The suspension was filtered through glass wool to remove mycelium and pieces of agar and was diluted in sterile Tween 80 solution to a concentration of $1-5 \times 10^6$ spores per ml. The conidial suspension was not washed, as washing the suspension did not influence germination or germ-tube growth of the spores, so it is unlikely that nutrients from the agar or cirrhous extract interfered with the experiments (A. J. Dik, unpublished data).

Fungicides

The fungicides propiconazole (Tilt 250 E.C., 250 g active ingredient/l, Ligtermoet Chemie B.V.), fenpropimorph (Corbel, 750 g active ingredient/l, BASF) and prochloraz (Sportak, 450 g active ingredient/l, Schering AAgrunol) were used, all belonging to the group of the ergosterol biosynthesis inhibitors (EBIs) and recommended for the control of various cereal-leaf diseases including glume blotch. Fungicide concentrations for all experiments are given in Table 1.

Nutrients

Sucrose (Merck) was used in different concentrations and was combined in the first experiments with yeast extract (Difco, 5 g/l), because the effect of this mixture on *S. nodorum* resembles that of aphid honeydew (Fokkema *et al.*, 1983).

In three experiments, a mixture of the following amino acids was used: alanine (0.12 g/l), asparagine (0.25 g/l), glutamine (0.20 g/l), glycine (0.07 g/l), histidine (0.31 g/l), isoleucine (0.14 g/l), leucine (0.23 g/l), lysine (0.08 g/l), proline (0.25 g/l), phenylalanine (0.20 g/l), threonine (0.84 g/l), tyrosine (0.23 g/l) and valine (0.25 g/l) (BDH, Merck, Sigma). This composition is the same as determined by HPLC in honeydew of the aphid *Sitobion avenae* reared for 3 days in a controlled environment on field-grown winter wheat plants cv. Arminda (Rossing & Van de Wiel, 1990), based on 15% dry matter. This mixture contains 3.17 g amino acids/l and was also used in higher and lower concentrations.

In three experiments aphid honeydew was used. It was collected on glass slides in a growth cabinet in which *S. avenae* was reared on spring wheat plants cv. Minaret. After 3–4 days of exposure in the growth cabinet the glass slides were washed in ultrapure water in an ultrasonic waterbath (Branson) and the washings were freeze dried. The dry weight was assessed and the honeydew was resuspended in ultrapure water and diluted to concentrations of 10, 20 and 50 mg honeydew/ml. Actual concentration of the sugars in the honeydew was established by anthrone reagent, which measures the total amount of carbohydrates (Hewitt, 1958).

Nutrient concentrations for all experiments are given in Table 1. Control leaves were sprayed with nutrients without addition of *S. nodorum* spores to assess a possible effect of the nutrients on the leaves.

Table 1. Fungicide and nutrient concentrations in all experiments

Experiment	Fungicide	Fungicide concentration (mg a.i./l)	Nutrients
1	Propiconazole	0, 12.5, 25, 50	20 g sucrose/l + 5 g YE/l
2	Fenpropimorph	0, 75, 150, 300	20 g sucrose/l + 5 g YE/l
3	Prochloraz	0, 4.5, 11.3, 22.5	20 g sucrose/l + 5 g YE/l
4	Prochloraz	0, 22.5, 45	20 g sucrose/l + 5 g YE/l
5	Prochloraz	0, 45, 135	20 g sucrose/l + 5 g YE/l
6	Prochloraz	0, 4.5, 11.3, 22.5, 45, 135, 225	10, 20, 50, 100, 150 g S/l + 5 g YE/l
7	Prochloraz	0, 4.5, 11.3, 22.5, 45	1.1, 3.2, 10.6 g amino acids/l; 8.5, 19.0, 47.0 g honeydew/l
8	Prochloraz	0, 4.5, 11.3, 22.5, 45	10, 20, 50, 100 g sucrose/l; 3.8, 7.5, 18.8 g honeydew/l
9	Prochloraz	0, 4.5, 11.3, 22.5, 45	1.1, 10.6 g amino acids/l; 20, 50 g sucrose/l; 1.1 g AA/l + 20 g S/l, 1.1 g AA/l + 50 g S/l, 10.6 g AA/l + 20 g S/l, 10.6 g AA/l + 50 g S/l, 18.8 g honeydew/l

YE, yeast extract; S, sucrose; AA, amino acids; a.i., active ingredient.

Application of treatments and climatic conditions

The flag leaves of the plants were guided through humidity boxes described by Ubels (1979). Each box contained six leaves, of which three received the same treatment to avoid a differential effect of the boxes. When the leaves in one part of the box were sprayed, other leaves in the box were covered and neighbouring boxes were closed. Suspensions of conidia, nutrients and fungicide were made in triple concentrations and mixed before application. Treatments were applied with a glass reagent sprayer (Desaga) at the upper surface of the leaf until the leaves were covered with a fine mist. The plants were placed in a growth chamber at 18–21°C, 12–15 h light per day, 85% r.h. during the light period and 95% r.h. during the dark period. The humidity boxes were kept closed until the end of the experiment (max. 15 days) to guarantee a constant high humidity, which stimulates infection by *S. nodorum* (Ubels, 1979).

Assessment of percentage germination, germ-tube length and necrotic leaf area

After 2 days, six leaves per treatment in Experiments 1–6 were used to make leaf imprints on Sellotape which were stained with lactophenol

cotton blue (0.1%). The germ-tube length of 50 spores per leaf was measured with a microscope, drawing tube and semi-automatic image analyser (Morphomat 10, Zeiss). The percentage germinated spores and the average germ-tube length per spore, based on both ungerminated and germinated spores, were calculated for each leaf.

Except for Experiments 1–3, diseased leaf area was assessed on six leaves per treatment after 11 days (Experiments 4 and 5) or 15 days (Experiments 6–9), dependent on disease development. After cutting the leaves at the edges of the box, total area and necrotic leaf area of each leaf were measured with a semi-automatic image analyser (Morphomat 10, Zeiss). The percentage necrotic leaf area (severity) was calculated per leaf and used as a variate representing infection.

Statistical analysis and calculation of antagonism isoboles

Treatment means were compared only within a fungicide concentration by analysis of variance followed by a *t*-test after logarithmic or arcsine- $\sqrt{x}$ -transformation (Snedecor & Cochran, 1980).

In addition to comparing treatment means, the data in Experiments 6–9 can be used to calculate antagonistic interaction. Different methods of

calculating antagonism and synergism have been developed, all for combinations of poisonous chemicals (Levy *et al.*, 1986). The Abbott formula (Abbott, 1925), which has been simplified by Colby (1967), was developed for a combination of herbicides, but it can be adapted for combinations of nutrients and fungicide. The underlying assumption is that the chemicals have independent joint action (Levy *et al.*, 1986). The severities in an experiment are all expressed as a percentage of the severity in the treatment without any additions (control) and the expected effect is calculated according to formula (1) (Colby, 1967):

$$E_{1\text{exp}} = \frac{X_1 \times Y_1}{100} \quad (1)$$

in which X_1 is the severity as a percentage of control with chemical A at concentration x , Y_1 is the severity as a percentage of control with chemical B at concentration y , and $E_{1\text{exp}}$ is the expected severity as a percentage of control with chemicals A+B at concentrations $x+y$. In Experiments 6–9, X_1 is the effect of the nutrients only and Y_1 the effect of the fungicide only. X_1 will then be more than 100 in most cases, but this should not make any difference for the calculation. $E_{1\text{exp}}$ is then compared to the observed severity, expressed as percentage of control ($E_{1\text{obs}}$). The level of interaction can be expressed in an antagonism factor (in synergy calculations between two fungicides called the synergy factor):

$$\text{Antagonism factor (AF)} = \frac{E_{1\text{obs}}}{E_{1\text{exp}}} \quad (2)$$

If AF is greater than 1, the nutrients are antagonistic to the fungicide and if AF is smaller than 1, the nutrients and fungicide are synergistic.

The second commonly used method to calculate interaction is the Wadley formula (Wadley, 1945). This formula is based on dose-response curves and compares ED_{50} s, but as it assumes similar joint action of two chemicals and uses ED_{50} s for both chemicals it can not be used for combinations of fungicides and nutrients. However, the design of Experiments 6–9 allows calculation of dose-response curves, comparison of ED_{50} -values and the drawing of isoboles (lines of the same effect) as described by Tamme (1964) and De Waard (1985).

In Experiments 6–9 separate fungicide dose-response curves for each nutrient concentration were calculated as follows. Each severity was expressed as the percentage reduction compared

to the severity in the treatment with no fungicide (and the same nutrient level):

% reduction =

$$100 - \left(\frac{\text{severity}}{\text{severity (no fungicide)}} \times 100 \right) \quad (3)$$

The reduction percentages were probit transformed. Linear regression of these probits against log-transformed fungicide doses yielded dose-response curves for each nutrient concentration in an experiment (Finney, 1947):

$$\text{probit (\% reduction)} = c + a \times \log(\text{fungicide dose}) \quad (4)$$

where c is a constant, and a is the slope of the regression line.

Antagonism can then be calculated in two ways, depending on the definition of ED_{50} .

1. $ED_{50(N)}$ is the fungicide dose needed to obtain 50% reduction in severity within a certain nutrient concentration, as compared to the fungicide-free treatment at that nutrient level. $ED_{50(N)}$ is calculated by entering 50 as percentage reduction in formula (4).
2. $ED_{50(c)}$ is the fungicide concentration that reduces the severity to 50% of that in the treatment without fungicide and without nutrients, as compared to the control. Entering the percentage reduction needed to obtain half of the severity found without nutrients and fungicides in the regression comparisons (formula (4)) gives $ED_{50(c)}$ values for each nutrient concentration.

Plotting of $ED_{50}/ED_{50 \text{ no nutrients}}$ to nutrient concentrations yields antagonism isoboles. All statistical calculations were done with the statistical software SAS (SAS Institute Inc., 1985).

RESULTS

Influence of sucrose + yeast extract on the effect of different fungicides

In Experiments 1–5 the percentage germination was high in all treatments (80–100%). The mixture of 20 g sucrose/l and 5 g yeast extract/l stimulated germ-tube growth significantly in the presence of the three fungicides propiconazole, fenpropimorph and prochloraz in all concentrations tested (Fig. 1, Table 2). In the case of fenpropimorph and prochloraz this resulted in longer germ tubes than when neither fungicide nor nutrients were added. Increasing the fungi-

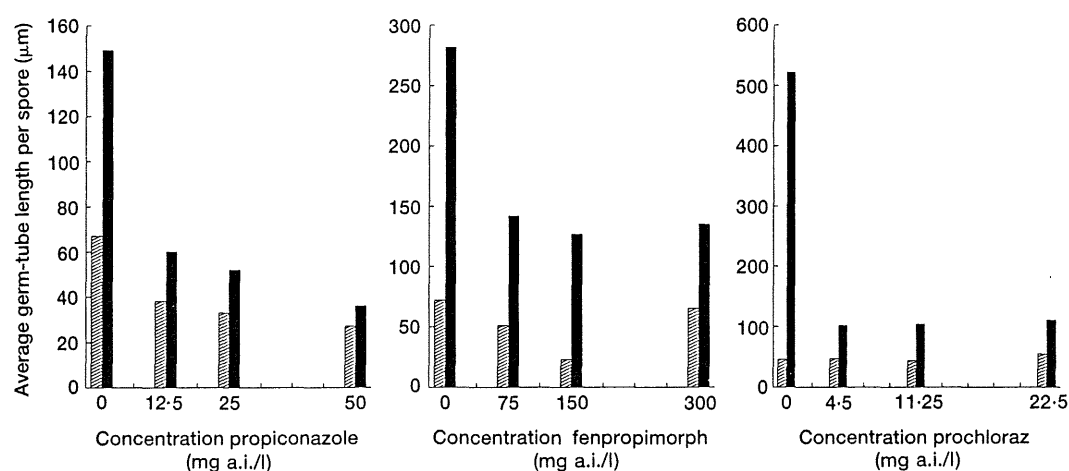


Fig. 1. Influence of 20 g sucrose (S)/l and 5 g yeast extract (YE)/l on inhibition of germ-tube growth by propiconazole, fenpropimorph and prochloraz (Experiments 1–3). ▨, no nutrients; ■ 20 g S/l + 5 g YE/l.

Table 2. Influence of sucrose (S) and yeast extract (YE) on inhibition of germ-tube growth and necrotic leaf area by prochloraz (Experiments 4 + 5). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Average germ-tube length per <i>S. nodorum</i> spore (μm) on day 2		Percentage necrotic leaf area on day 11	
	No nutrients	20 g S/l + 5 g YE/l	No nutrients	20 g S/l + 5 g YE/l
Experiment 4				
0	76 (6.3)	358* (39.5)	8.7 (6.2)	27.9* (11.1)
22.5	35 (8.5)	105* (6.8)	5.1 (3.9)	11.6* (2.3)
45.0	44 (10.9)	105* (12.6)	0.7 (0.7)	1.3 (1.2)
Experiment 5				
0	68 (11.2)	367* (21.6)	14.4 (7.5)	80.8* (30.3)
45.0	32 (4.8)	97* (8.2)	0.4 (0.4)	6.7* (4.9)
135.0	48 (8.8)	81* (8.6)	0.0 (0.0)	1.0* (0.5)

* Significantly different at $P=0.05$ from treatment without nutrients and the same fungicide concentration.

cide dose did not increase inhibition of germ-tube growth in these experiments. The number of branches per germ tube increased linearly with the average germ-tube length per spore ($r^2=0.99$, data not shown).

The sucrose/yeast extract mixture also gave significant ($P=0.05$) stimulation of diseased leaf area at different prochloraz concentrations (Table 2), although at higher fungicide concentrations there was a strong reduction of diseased leaf area.

Influence of composition and concentration of nutrients on infection in the presence of prochloraz

Application of nutrients without *S. nodorum* spores did not cause yellowing or necrosis of leaves in any of the experiments.

In Experiment 6 different sucrose concentrations, combined with yeast extract (5 g/l), all demonstrated approximately the same stimulation of germ-tube length (Table 3). This stimu-

Table 3. Influence of sucrose concentration in combination with yeast extract on inhibition of germ-tube growth by prochloraz (Experiment 6). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Average germ-tube length per spore (μ m), day 2					
	No nutrients	10 g S/l + 5 g YE/l	20 g S/l + 5 g YE/l	50 g S/l + 5 g YE/l	100 g S/l + 5 g YE/l	150 g S/l + 5 g YE/l
0.0	31.1 (5.7)	76.5* (15.9)	82.3* (23.9)	103.4* (28.8)	94.4* (49.3)	113.4* (24.5)
11.3	29.2 (9.0)	61.0* (10.3)	59.7* (4.6)	63.2* (14.7)	70.4* (11.6)	56.3* (4.7)
22.5	25.1 (8.2)	50.7* (11.9)	52.9* (6.3)	51.9* (4.1)	50.5* (9.1)	51.7* (5.9)
45.0	26.5 (5.9)	ND	49.3* (17.0)	ND	ND	ND

S, sucrose; YE, yeast extract; ND, not determined.

* Significantly different at $P=0.05$ from treatment without nutrients and the same fungicide concentration.

Table 4. Influence of sucrose concentration in combination with yeast extract on inhibition of disease development by prochloraz (Experiment 6). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Percentage necrotic leaf area, day 15					
	No nutrients	10 g S/l + 5 g YE/l	20 g S/l + 5 g YE/l	50 g S/l + 5 g YE/l	100 g S/l + 5 g YE/l	150 g S/l + 5 g YE/l
0.0	6.3 (2.9)	89.7* (10.5)	90.0* (5.2)	92.7* (4.5)	90.9* (8.7)	75.6* (22.8)
4.5	5.2 (4.1)	45.4* (21.6)	22.2 (12.9)	37.3* (28.0)	26.3* (11.5)	28.6* (9.1)
11.3	1.5 (0.6)	1.7 (1.3)	11.4* (10.3)	11.2* (9.6)	19.5* (8.6)	9.3* (6.6)
22.5	1.0 (1.5)	3.2 (4.2)	4.1 (2.3)	7.8* (6.6)	6.6 (5.2)	9.9* (11.5)
45.0	0.4 (0.8)	5.6 (6.0)	1.9 (2.6)	3.6 (3.9)	3.5 (5.1)	29.2* (34.4)
135	0.5 (0.5)	ND	1.3 (0.5)	2.5* (2.2)	1.4 (1.1)	2.1* (1.3)
225	0.0 (0.0)	ND	0.5 (0.2)	1.0 (0.7)	0.6 (0.3)	15.5* (11.4)
r^2	0.81	0.43	0.96	0.92	0.98	0.17
ED ₅₀ (N)	8.8	1.8	0.8	1.4	1.6	0.1
ED ₅₀ (c)	8.8	31.4	42.8	74.5	59.6	3450

S, sucrose; YE, yeast extract; ND, not determined.

* Significantly different at $P=0.05$ from treatment without nutrients and the same fungicide concentration.

r^2 , coefficient of determination of dose-response curves.

ED₅₀(N), ED₅₀(c), see text.

lation occurred both in the absence and presence of fungicide.

All sucrose concentrations in Experiment 6 gave strong stimulation of necrotic leaf area in the treatments without fungicide. With increasing fungicide concentrations generally more sucrose was needed to obtain significant stimulation compared to the treatment without nutrients (Table 4).

The mixture of amino acids did not significantly stimulate germination and germ-tube growth either with or without fungicide (data not shown). In Experiment 7 there was no significant

stimulation of necrotic leaf area by the amino-acid mixture in the fungicide-free treatments, but there was stimulation in combination with some prochloraz concentrations (Table 5). In the same experiment aphid honeydew stimulated necrosis both in the treatments with and without fungicide, especially at the higher honeydew concentrations. Again, as in Experiment 6, at higher fungicide concentrations more honeydew was needed to increase severity significantly.

When only sucrose was supplied (Experiment 8) in concentrations of 20 g/l or more, stimulation of disease development occurred both with and

Table 5. Influence of amino-acid concentration and honeydew concentration on inhibition of disease development by prochloraz (Experiment 7). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Percentage necrotic leaf area, day 15						
	No nutrients	1.1 g amino acids/l	3.2 g amino acids/l	10.6 g amino acids/l	8.5 g honeydew/l	19.0 g honeydew/l	47.0 g honeydew/l
0.0	40.3 (29.1)	42.9 (24.1)	48.5 (40.6)	64.9 (27.6)	64.2 (26.1)	75.3* (21.1)	76.9* (14.9)
4.5	24.5 (12.8)	44.0 (18.3)	24.0 (15.3)	46.4 (5.3)	51.3* (23.5)	37.7 (17.0)	80.3* (16.8)
11.3	14.0 (8.7)	20.1 (20.1)	30.6 (35.3)	44.4* (23.9)	22.8 (21.8)	16.1 (13.0)	52.1* (20.2)
22.5	8.9 (8.7)	9.7 (13.7)	28.5* (20.3)	39.3* (21.8)	10.0 (7.7)	8.3 (4.3)	45.6* (21.5)
45.0	6.5 (4.6)	4.6 (1.7)	19.1* (26.3)	12.8 (15.8)	ND	12.9 (10.9)	7.6 (2.4)
r^2	0.97	0.99	0.13	0.72	0.99	0.70	0.85
ED ₅₀ (N)	6.5	9.9	30.2	18.5	8.9	2.9	19.3
ED ₅₀ (c)	6.5	10.8	264.4	44.1	13.6	11.5	31.9

ND, not determined.

* Significantly different at $P=0.05$ from treatment without nutrients and the same fungicide concentration. r^2 , coefficient of determination of dose-response curves.ED₅₀ (N), ED₅₀ (c), see text.

without fungicide, whereas in the concentration of 10 g/l there was no significant stimulation (Table 6). In this experiment aphid honeydew caused somewhat stronger stimulation than sucrose alone (Table 6).

The effect of two amino acid concentrations and two sucrose concentrations separately and combined and the effect of aphid honeydew was compared in Experiment 9 (Table 7). In the mixture of 1.1 g amino acids/l and 50 g sucrose/l the ratio between amino acids and carbohydrates was the same as in the honeydew analysed by Rossing & Van de Wiel (1990). The effect of amino acids on infection in Experiment 9 was similar to the effect found in Experiment 7. The effect of sucrose alone, however, was smaller than that in Experiment 8. Adding sucrose to the lowest amino acid concentration gave additional stimulation of infection, which did not seem to be dependent on sucrose concentration. At the higher amino acid level addition of sucrose only in some treatments further stimulated infection. When the treatments with 20 g sucrose/l are compared to those with 20 g sucrose/l plus different concentrations of amino acids, the *Septoria* infection was further stimulated by the addition of amino acids, especially in the highest concentration. Adding amino acids to 50 g sucrose/l also increased disease intensity. In this experiment the effect of aphid honeydew was stronger than that of only sucrose at approximately the same con-

centration (20 g/l) and comparable to the effect of this sucrose concentration in combination with amino acids (Table 7).

Interaction between nutrients and fungicide effectiveness

Calculation of interaction according to Colby (1967) resulted in antagonism factor (AF) values smaller than 1 in Experiment 6, suggesting synergism. In Experiments 7–9 the AF values were larger than 1 in almost all combinations of nutrients and fungicide, so in these experiments the nutrients were antagonistic to prochloraz. AF values for aphid honeydew in Experiments 7–9 are shown in Table 8.

The ED₅₀ for prochloraz against disease development of *S. nodorum* in the absence of nutrients ranged from 2.2 to 8.8 mg a.i./l. Most dose-response curves have high coefficients of determination (r^2). Values of r^2 , ED₅₀ (N) and ED₅₀ (c) are given in Tables 4–7. The values of the ED₅₀ (N) were lower in Experiment 6 and higher in Experiments 7–9 in the presence of nutrients compared with the treatment without nutrients. The ED₅₀ (c) was higher in all experiments in the presence of nutrients. The indication for synergism in Experiment 6, which was found in both the Colby method and in comparing ED₅₀ (N), is probably due to the very large stimulation of severity by the nutrients in the fungicide-free

Table 6. Influence of sucrose concentration and honeydew concentration on inhibition of disease development by prochloraz (Experiment 8). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Percentage necrotic leaf area, day 15									
	No nutrients	10 g sucrose/l	20 g sucrose/l	50 g sucrose/l	100 g sucrose/l	3.8 g honeydew/l	7.5 g honeydew/l	18.8 g honeydew/l		
0.0	66.4 (22.0)	82.3 (11.1)	96.9* (3.3)	84.0* (15.4)	96.9* (5.8)	92.3* (4.6)	90.6* (8.3)	96.7* (4.2)		
4.5	44.5 (13.5)	59.0 (9.7)	62.4 (22.1)	73.2* (8.8)	82.4* (18.8)	55.1 (10.5)	69.4* (25.0)	75.0* (22.5)		
11.3	27.9 (16.7)	25.3 (14.8)	51.2* (17.9)	73.3* (15.2)	46.8 (15.2)	42.2 (17.7)	44.1 (20.0)	39.2 (23.9)		
22.5	12.0 (6.3)	14.0 (11.6)	48.1* (8.2)	48.8* (10.9)	62.6* (10.6)	26.8* (15.3)	24.7 (15.4)	31.2* (20.5)		
45.0	4.2 (2.6)	ND	42.0* (8.9)	19.6* (7.3)	39.5* (17.4)	ND	10.2 (6.3)	15.4* (13.5)		
r^2	0.99	0.98	0.97	0.84	0.66	0.98	1.00	0.96		
ED _{50(N)}	8.0	7.7	20.0	25.4	27.8	7.9	10.8	10.9		
ED _{50(c)}	8.0	9.9	119.9	35.1	66.6	16.4	16.2	19.0		

ND, not determined.

* Significantly different at $P = 0.05$ from treatment without nutrients and the same fungicide concentration.

r^2 coefficient of determination of dose-response curves.

ED_{50(N)} ED_{50(c)}, see text.

Table 7. Influence of composition and concentration of nutrients on inhibition of disease development by prochloraz (Experiment 9). Standard deviations of mean values are given in parentheses

Concentration prochloraz (mg a.i./l)	Percentage necrotic leaf area, day 15									
	No nutrients	1.1 g amino acids/l	10.6 g amino acids/l	20 g sucrose/l	50 g sucrose/l	1.1 g AA/l + 20 g S/l	1.1 g AA/l + 50 g S/l	10.6 g AA/l + 20 g S/l	10.6 g AA/l + 50 g S/l	18.8 g honeydew/l
0.0	65.4 (10.2)	53.3 (22.2)	71.5 (22.4)	40.6 (33.3)	85.1 (12.9)	86.0* (18.1)	96.8* (3.1)	81.5 (12.7)	92.8* (8.8)	82.6 (11.5)
4.5	22.9 (24.0)	31.9 (18.0)	36.5 (17.7)	38.5 (21.9)	33.2 (5.2)	67.5* (16.9)	49.8* (32.0)	83.6* (11.8)	79.0* (14.0)	46.8* (24.1)
11.3	14.0 (14.3)	21.3 (20.4)	51.0* (20.3)	17.2 (10.7)	21.9 (13.3)	47.2* (28.6)	38.4* (22.6)	72.2* (21.6)	21.4 (12.1)	38.9* (30.3)
22.5	20.5 (24.4)	21.7 (15.0)	19.2 (11.8)	15.1 (13.9)	18.1 (14.6)	38.4 (28.4)	36.1 (23.5)	28.3 (21.0)	13.4 (10.6)	18.7 (11.9)
45.0	3.7 (3.3)	19.4 (8.1)	27.0* (26.2)	2.4 (2.6)	23.6 (24.2)	21.5 (16.7)	22.2 (17.9)	56.8* (37.6)	16.9 (14.7)	ND
r^2	0.60	0.79	0.28	0.93	0.17	0.99	0.93	0.73	0.73	0.88
ED _{50(N)}	2.2	8.2	11.0	13.8	<0.1	15.7	5.4	32.4	9.2	7.0
ED _{50(G)}	2.2	2.5	16.2	7.1	2.3	25.5	20.4	36.6	14.4	11.3

AA, amino acids; S, sucrose. ND, not determined.

* Significantly different at $P=0.05$ from treatment without nutrients and the same fungicide concentration. r^2 , coefficient of determination of dose-response curves.ED_{50(N)}, ED_{50(G)}, see text.

Table 8. Antagonism factor ($E_{1\text{ obs}}/E_{1\text{ exp}}$) for aphid honeydew, calculated according to the Colby method

Concentration prochloraz (mg a.i./l)	Experiment 7			Experiment 8			Experiment 9
	8.5 g HD/l	19.0 g HD/l	47.0 g HD/l	3.8 g HD/l	7.5 g HD/l	18.8 g HD/l	18.8 g HD/l
4.5	1.3	0.8	1.7	0.9	1.1	1.2	1.6
11.3	2.9	2.9	2.9	1.1	1.2	1.0	2.2
22.5	0.7	0.5	2.7	1.6	1.5	1.8	0.7
45.0	ND	1.1	0.6	ND	1.8	2.5	ND

ND, not determined; HD, aphid honeydew.

treatments. The net effect of combinations of nutrients with fungicide however, indicated by $ED_{50(c)}$, is antagonistic in all experiments.

Antagonism isoboles of $ED_{50(c)}$ for different nutrients are shown in Fig. 2. Only data from dose-response curves with coefficients of determination higher than 0.70 were used. Contrary to De Waard (1985) and Tammes (1964) nutrient concentrations are on the abscissa and relative ED_{50} s on the ordinate. The isoboles show that ED_{50} s increase up to ten times with increasing nutrient concentrations.

DISCUSSION

Aphid honeydew and its components sucrose and amino acids can stimulate infection of wheat leaves by *S. nodorum* in the presence of the fungicide prochloraz. This effect on infection could not be adequately estimated by assessing percentage germination and germ-tube length. Stimulation of germ-tube growth is accompanied by stimulation of necrosis, but the converse is not necessarily true. Amino acids, for example, do not stimulate germ-tube growth but can enhance necrosis in the presence of prochloraz. Furthermore, ergosterol biosynthesis-inhibiting fungicides generally have little effect on germination and germ-tube growth (Siegel, 1981), so the effect of these fungicides and antagonism against them can best be studied in *in-vivo* experiments, measuring infection.

Infection by *S. nodorum* is strongly stimulated by sucrose combined with yeast extract, whereas the effect of sucrose alone is variable. Fokkema *et al.* (1983) also found that stimulation of necrosis by *Cochliobolus sativus* was smaller in the presence of sucrose than in the presence of sucrose + yeast extract or aphid honeydew.

The influence of the mixture of amino acids on severity is similar in Experiments 7 and 9. A synergistic effect of some of the amino acids that were used has been reported, but in a mixture this effect can be nullified by the antagonistic effect of the other amino acids (Chopra & Jhoo, 1974). *Cirrhus* extract of *S. nodorum* also contains a mixture of amino acids (Rapilly & Skajennikoff, 1974) and might also stimulate infection of *S. nodorum* in the presence of fungicides.

Aphid honeydew stimulated infection more strongly than its components sucrose and amino acids separately. In the analysis of the antagonism caused by leaf exudates similar results were reported by Beynon & Brown (1969), who found that the compounds glucose and fructose in the exudates were the most important antagonistic factor but that the effect could not be completely stimulated by these carbohydrates alone.

The stimulation of infection by all nutrient sources is generally larger when the ratio of nutrients/fungicide is larger, but because of the differences in severity between experiments in the treatment without fungicide and without nutrients, it is not possible to estimate severity as a function of nutrient concentration and fungicide concentration. Due to large variation of the necrotic leaf area within the treatments in an experiment, differences between percentages of diseased leaf area are not always significant at the 5% level. However, in accordance with Finney (1947), increasing the number of replicates to reduce variation at the expense of the number of treatments was rejected, because it would have decreased the number of values on which dose-response curves are based.

The method described of calculating dose-response curves generally gives high coefficients of determination. Both methods of calculating

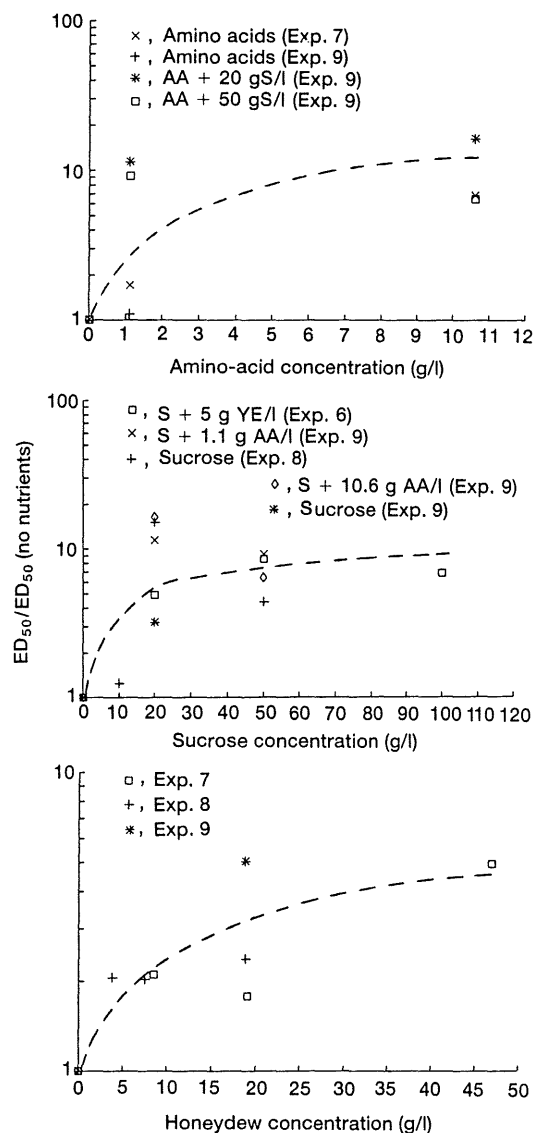


Fig. 2. Antagonism isoboles for amino acids (AA), sucrose (S) and aphid honeydew against prochloraz.

ED_{50} s indicate antagonism in Experiments 7–9, but in Experiment 6 the results seem contradictory. The $ED_{50(N)}$ gives information about the behaviour of a fungicide at a certain nutrient level, whereas the $ED_{50(c)}$ gives information about the net effect of the combined presence of nutrients and fungicide. In synergy calculations the ED_{50} is usually defined as the dose that reduces severity to 50% of that in the treatment without any additions, which is the same as the

$ED_{50(c)}$ in this report. Dunn *et al.* (1971) calculated the effect of exudates on ED_{50} by measuring percentage germination, but as there was close to 100% germination in the treatment without fungicide and without exudates, the exudates could not strongly stimulate germination in the fungicide-free treatment and therefore $ED_{50(N)}$ and $ED_{50(c)}$ are approximately the same. Antagonism calculations between a stimulating and a toxic component have to our knowledge not yet been described for experiments in which infection was measured. In our opinion, using the $ED_{50(c)}$ in the calculations is preferable, because the net effect is most important. Using the $ED_{50(N)}$, however, can give additional information about the way the net effect is reached. Comparison of ED_{50} is preferred to the Colby method (Colby, 1967), because that method does not allow for generalization of the effect of a certain nutrient concentration on fungicide effectiveness, but only gives information about single combinations of nutrients and fungicide. The Colby method, however, can be used when the number of treatments is too small for calculation of dose-response curves. It can be concluded from the antagonism calculations that aphid honeydew and its main components are antagonistic to the fungicide prochloraz and that this effect is partly the result of an additional effect of stimulation and inhibition, partly caused by detoxification of the fungicide.

Antagonism against fungicides can be based on a physico-chemical interaction with the fungicide outside the pathogen or on a physiological interaction with the metabolism of the pathogen (De Waard, 1985). In the work presented here, chemical interaction outside the pathogen does not seem very likely, because the antagonism was found with different kinds of nutrients and sucrose + yeast extract also showed an antagonistic effect on other kinds of fungicides (Dik & Fokkema, 1988). Control experiments, in which nutrients were left on leaves for 3 days and then washed off before the pathogen was applied, did not show stimulation of either germ-tube growth or diseased-leaf area of *S. nodorum*, so it also seems unlikely that the effect is caused by a change in the physiology of the plants (A. J. Dik, unpublished data). In our opinion the antagonistic effect is caused by stimulation of the metabolism of the pathogen. The carbohydrates might cause an increase of the energy-dependent efflux of the fungicide, while the amino acids might stimulate the production of detoxifying enzymes.

The antagonistic effect of aphid honeydew on

fungicide activity still has to be demonstrated in field experiments. It might very well be of practical importance, because in wheat crops aphids and therefore honeydew are normally present from May to August. Furthermore, the antagonistic effect of nutrients on fungicide activity was also found when *C. sativus* was used as a test pathogen (Dik & Fokkema, 1988). Because of the non-specificity of the effect, it seems highly likely that other necrotrophic pathogens, e.g. *Septoria tritici*, will be stimulated by honeydew in the same way. There is probably little effect on biotrophic pathogens such as rusts and mildew, because these fungi do not use nutrients from the leaf surface before penetration. Although the fungicide concentrations used in the experiments are much lower than spraying concentrations in the field, the actual fungicide concentration on field-grown wheat leaves can be approximately the same because the concentration decreases steadily after spraying due to, for example, washing-off by rain. Most selective fungicides are sprayed only once, so there is no repeated increase in fungicide concentration.

Possibly other nutrient sources in the phyllosphere beside aphid honeydew, such as pollen, leaf exudates and cirrus extract, also reduce fungicide activity. Pollen, for example, stimulates infection of *S. nodorum* and *C. sativus* (Fokkema, 1971) and might well be also antagonistic to fungicides. However, the naturally occurring microflora on wheat leaves, consisting mainly of yeasts, can use both pollen and honeydew as a nutrient source. It has been demonstrated that, in a controlled environment, yeasts can effectively remove nutrients from wheat leaves (Fokkema *et al.*, 1983). In wheat fields, however, the naturally occurring microflora is often reduced by non-selective fungicides. In such cases, aphid honeydew and pollen can accumulate on the leaves. Removal of aphid honeydew by yeasts has been demonstrated under field conditions and will be described elsewhere (A. J. Dik, unpublished data). This can imply that leaving the naturally occurring microflora intact by using only selective fungicides can prevent the antagonistic effect of aphid honeydew and other nutrients on fungicide activity. In this light the often-applied combination of EBI-fungicides with broad-spectrum fungicides is not to be recommended.

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