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Consumption of aphid honeydew, a wheat yield reduction factor, by phyllosphere yeasts under field conditions

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Abstract

Aphid honeydew on wheat leaves can affect host physiology directly and can stimulate infection by necrotrophic pathogens. The role of naturally occurring saprophytes on wheat flag leaves in removing aphid honeydew was examined in five field experiments at different locations in the Netherlands in 1987 and 1988. Aphid populations, saprophyte populations and aphid honeydew were measured weekly. Diseases were monitored at 1-4 week intervals.

In the control treatment, sprayed with water, the naturally occurring saprophytes consumed the honeydew almost completely, except in one experiment in which the aphid population increased very rapidly. In the treatments in which the saprophytes were reduced by the broad-spectrum fungicide maneb in 1987 and with maneb alternated with anilazin in 1988, honeydew accumulated. The observed honeydew consumption by the naturally occurring saprophytic population is ascribed mainly to pink and white yeasts (*Sporobolomyces* spp. and *Cryptococcus* spp., respectively).

Additional treatments showed, that in the presence of the naturally occurring saprophytes the yield loss per aphid-infestation-day was lower than when the saprophytes were inhibited by fungicides, showing that yeasts can reduce the detrimental effect of aphid honeydew in wheat.

Additional keywords: Sporobolomyces, *Cryptococcus*, biological control, maneb, anilazin

Introduction

Rabbing *et al.* (1981) showed that a significant part of yield losses in wheat caused by aphids can be attributed to honeydew deposition on the leaves. Honeydew decreases CO_2-assimilation and increases leaf senescence. In a controlled environment, honeydew stimulated *Septoria nodorum* infection in the absence of saprophytes which

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Table 1. Data for wheat cultivation, plot sizes, treatments and spraying dates.

Experimental farm	Year	Winterwheat cultivar	Sowing date	Previous crop	N fertilization (date + rate)	Plot size (m^2)	Harvested (m^2)	Treatments	Spraying dates (month/day)
A.P. Minderhoudhoeve	1987	Obelisk	8-10-'86	potatoes	6-3-'87 54 kg ha^{-1} 25-5-'87 60 kg ha^{-1}	144	22.5	W M I	6/29, 7/7, 7/15 6/5, 6/12, 6/23, 6/29, 7/7, 7/15 6/29, 7/7, 7/15
Bouwing	1987	Granta	14-10-'86	potatoes	4-3-'87 115 kg ha^{-1} 9-5-'87 60 kg ha^{-1} 16-6-'87 40 kg ha^{-1}	90	9.0	W M I	6/10, 6/18, 6/25, 7/1, 7/9, 7/17 5/27, 6/10, 6/18, 6/25, 7/1, 7/9, 7/17 6/10, 6/18, 6/25, 7/1, 7/9, 7/17
Eest	1987	Urban	9-10-'86	potatoes	20-2-'87 60 kg ha^{-1} 25-5-'87 60 kg ha^{-1} 29-6-'87 55 kg ha^{-1}	108	13.5	W M I MI P PM	6/18, 6/29, 7/7, 7/14, 7/22 6/10, 6/18, 6/29, 7/7, 7/14, 7/22 6/29, 7/7, 7/14, 7/22 6/10(m), 6/18(m), 6/29(m + i), 7/7(m + i), 7/14(m + i), 7/22(m + i) 6/29 6/10(m), 6/18(m), 6/29(pr. + m), 7/7(m), 7/14(m), 7/22(m)
Bouwing	1988	Pagode	4-11-'87	potatoes	9-4-'88 130 kg ha^{-1} 10-6-'88 60 kg ha^{-1}	75	16.0	W MA hf MA lf I U	5/25, 6/8, 6/14, 6/21, 6/28, 7/6 5/25(a), 6/8(m), 6/14(a), 6/21(m), 6/28(a), 7/6(m) 5/25(a), 6/8(m), 6/21(m) 6/8, 6/14, 6/21, 6/28, 7/6
Eest	1988	Urban	26-10-'87	potatoes	9-3-'88 60 kg ha^{-1} 11-4-'88 60 kg ha^{-1} 25-5-'88 65 kg ha^{-1}	90	13.5	W MA hf MA lf I MAI	5/30, 6/13, 6/20, 6/30, 7/7, 7/15 5/30(m), 6/13(a), 6/20(m), 6/30(m), 7/7(a), 7/15(m) 5/30(m), 6/13(a), 6/30(m) 6/13, 6/20, 6/30, 7/7, 7/15 5/30(m), 6/13(a + i), 6/20(m + i), 6/30(m + i), 7/7(a + i), 7/15(m + i)

W = water; M = maneb; I = insecticide; MI = maneb + insecticide; MA hf = maneb/anilazin applied in a high frequency; MA lf = maneb/anilazin applied in a low frequency; MAI = maneb/anilazin + insecticide; U = untreated; P = prochloraz; PM = prochloraz + maneb; m = maneb; a = anilazin; i = insecticide; pr = prochloraz.

can compete for the honeydew (Fokkema *et al.*, 1983). Rabbinge *et al.* (1984) also showed a decrease in fungicide effectiveness in the presence of honeydew. This was confirmed under controlled conditions (Dik and Fokkema, 1988; Dik *et al.*, 1991).

Wheat leaves are colonized by saprophytes shortly after unfolding and saprophytic population densities can reach values of 10^5 Colony Forming Units (CFU) per cm^2leaf area. In temperate regions the saprophytic mycoflora mainly consists of pink and white yeasts (mainly *Sporobolomyces* spp. and *Cryptococcus* spp., respectively), *Aureobasidium pullulans* and *Cladosporium* spp. The yeasts are predominant (Fokkema *et al.*, 1975; Dickinson, 1976). Saprophytes, especially yeasts, can reduce the stimulating effect of honeydew on necrotrophic pathogens such as *Septoria nodorum* and *Cochliobolus sativus* by competing for honeydew as a nutrient source. Under controlled conditions, yeasts can effectively remove exogenous nutrients from wheat leaf surfaces (Fokkema *et al.*, 1983). In wheat cultivation in the Netherlands, however, yeasts are often reduced by the use of broad-spectrum fungicides, but they are insensitive to most selective fungicides (Fokkema, 1988; Fokkema and De Nooij, 1981).

The aim of the five field experiments presented in this paper was to determine whether naturally occurring yeasts and other saprophytes can prevent the accumulation of aphid honeydew under field conditions. The amount of honeydew was compared in plots in which the saprophytic populations were reduced by a broad-spectrum fungicide and plots in which the naturally occurring saprophytic populations were left intact. If the hypothesis, that saprophytes can remove nutrients from the leaves is correct, at the same aphid population density more honeydew will be recovered from plots with reduced saprophytic population densities.

Material and methods

Experimental fields. In 1987 and 1988, five field experiments were conducted at different locations in the Netherlands: both years on the experimental farms 'De Eest' (Noord-Oost Polder) and 'De Bouwing' (near Wageningen) and in 1987 also on the experimental farm 'De Ir. A.P. Minderhoudhoeve' (Flevoland).

All experiments were layed out as completely randomized blocks with eight replicates in 1987 and six in 1988. Details about the experiments are given in Table 1. All samples were taken just outside the net fields that were used to determine yields at the end of the season.

Treatments. Three treatments were applied to all experiments:

W: control, sprayed with water to minimize differences in run-off and microclimate between treatments

M: maneb treatment, sprayed with maneb (2 kg Maneb 80 (Akzo) ha^{-1}) te reduce yeast population densities.

I: insecticide treatment, sprayed with pirimicarb (0.25 Pirimor (I.C.I.) ha^{-1}) to eliminate aphids and thus prevent aphid honeydew deposition.

At 'De Eest' in both years a combination of maneb and insecticide (MI) was also applied to establish possible interference of maneb residues with the method used to measure honeydew.

In 1987, two more treatments were included at 'De Eest', viz. prochloraz (P) and prochloraz plus maneb (PM), to demonstrate the influence of honeydew on the effec-

tiveness of the fungicide prochloraz. In both treatments the prochloraz (1 l Sportak (Schering AAgrunol) ha^{-1}) was applied once at the beginning of flowering (DC 61, Decimal Code for crop development stage, Zadoks *et al.*, 1974), while in the PM-treatment maneb was applied at the same dates as in the M-treatment. It was expected that in combination with maneb, prochloraz would be less effective because of accumulation of honeydew. The effect of honeydew on the effectiveness of prochloraz can, in this experimental design, best be demonstrated for a pathogen that is not reduced by maneb.

The results of the 1987 experiments suggested an increase in maneb-resistant yeasts that had been selected for by spraying with maneb regularly. To prevent this in 1988 maneb was alternated with anilazin (4 l ha^{-1} Dyrene (Bayer)) and sprayed in two regimes, i.e. in a high frequency (MA hf), like the maneb treatment in 1987, and in a lower frequency (MA lf), which is more comparable to common practice. At 'De Bouwing' in 1988 an untreated control treatment was added.

Spraying dates are given in Table 1. All treatments were applied at a rate of 500 l ha^{-1}. When treatment days coincided with sampling days, the sampling was done first.

Weather data. On all farms daily rainfall was recorded. At 'De Bouwing' and 'De Minderhoudhoeve', temperature and relative humidity were recorded continuously. At 'De Eest', daily minimum and maximum temperatures were recorded.

Assessment of aphid populations. In 1987, aphid density was established by counting the aphids on 25 culms per plot, discriminating between the two species present *Sitobion avenae* and *Metopolophium dirhodum* and between larvae and aptere and alate adults. The regression equations of the relation between percentage infested culms and number of aphids per culm were not significantly different from the regression equation calculated by Ward *et al.* (1986) (Snedecor and Cochran, 1980; SAS Institute Inc., 1985). Therefore, in 1988 the percentage infested culms was established by recording the presence or absence of aphids on 100 culms per plot, without discriminating between species and developmental stages of the aphids. Counting of aphids was done on the same days as leaf samples were taken.

Analysis of the saprophytic microflora. Weekly, six flag leaves per plot were sampled. Dead leaf tips were cut off to avoid overestimation of *Cladosporium* spp. The total leaf area of the six leaves was measured with an automatic area meter (Hayashi Denkoh) and after cutting them into 3-4 cm lengths, they were put in 300 ml Erlenmeyer flasks containing 200 ml sterile Tween 80-solution (0.01%) and shaked vigorously in a Griffin Flask Shaker for 60 minutes. After appropriate dilution 100 μl aliquots of the leaf washings were plated on Basal Yeast Agar (BYA, containing 20 g glucose, 1 g yeast extract (Difco), 10 g proteose pepton (Difco), 15 g agar (Bitek), 10^6 I.U. streptomycinesulphate + 10^6 I.U. sodiumbenzylpenicillin (Pharmachemie B.V.) per liter) to determine population densities of yeasts and other saprophytic fungi, which all grow well on this medium, and 50 μl on Tryptic Soy Agar (TSA, containing 3 g Tryptic Soy Broth (Difco), 20 g agar (Bitek) + 100 mg cycloheximide (Sigma) per liter) to determine population densities of bacteria. All plating was done in triplicate. After incubation at 21°-23 °C for 3 days (TSA) or 5-7 days (BYA), colonies of all

saprophytes were counted separately to genus level and population densities expressed as CFU per cm^2 leaf area (both sides), were calculated.

Assessment of amount of honeydew on the leaves. Weekly, fifteen flag leaves per plot were sampled by cutting the stems at approximately 10 cm below the flag leaves. Ears, if present, were removed by cautiously pulling the leaf sheaths from the stems. The leaves were put together in 1 l Erlenmeyer flasks containing 500 or 1000 ml Tween 80 solution (0.01%) with the leaves under water and the sheaths above it to avoid leakage of carbohydrates from the cut edges. The Erlenmeyer flasks were washed with the detergent Extran (2%) before use. The flasks containing the leaves were put in an ultrasonic bath (Branson) for 5 minutes at 50 000 Hz. Control experiments showed that this way the most effective method to wash off honeydew with minimal leakage or carbohydrates from the leaves (A.J. Dik, unpublished results). A sample was taken from each Erlenmeyer flask and was filtered through a 0.2 μm membrane filter (Sartorius) to remove yeasts and other micro-organisms. The total amount of carbohydrates in the filtered solution was determined colorimetrically by using anthrone reagent (Hewitt, 1958). The total leaf area of the 15 leaves per plot was measured and the amount of sugars (expressed as glucose equivalents) per cm^2 leaf area (both sides) was calculated.

On one sampling day in the experiment at 'De Eest' in 1987 (July 13), the percentage leaf area covered with honeydew was estimated visually on the leaves before they were used for the colorimetrical assessment, to determine whether the expected difference in honeydew concentration was reflected in the percentage leaf area covered.

In 1987, the leaf washings of the control and the maneb treatment of July 8 at 'De Bouwing' and July 13 at 'De Eest' were filtered and freeze-dried. The dry samples were analysed with an Elementary Analyzer CHN (Heraeus) for total carbon and nitrogen content and the C/N-ratio was calculated.

Disease assessment. In the experiment at 'De Eest' in 1987, disease assessment took place weekly, but in all other experiments it was done on three occasions throughout the experimental period. On fifteen culms per plot the severity of all diseases present of all (partly) green leaves was estimated at the percentage leaf area covered with symptoms. Dead and chlorotic leaf areas were estimated separately. Ear diseases were recorded as the number of infected spikelets per ear.

Yields. At the end of the growing season the number of ears m^{-2} was calculated after counting the number of ears in a 2 m length of row. The centre part of each plot was harvested with an experimental plot combine and kernel yields, 1000-kernel weight and water content of the kernels were established. The exact harvested area of each plot was measured and yields (dry weight) were calculated as kg ha^{-1}.

Statistical analysis. Differences in treatment means for saprophytic populations, aphid infestation, amount of aphid honeydew and yield variables were analysed with a one-way ANOVA, if necessary after transformation of the data, followed by the t-test (Snedecor and Cochran, 1980; SAS Institute Inc., 1985).

The number of aphid-infestation-days (the percentage infested culms integrated over time), yeast-days (the yeast population density integrated over time) and

honeydew-days (the amount of honeydew integrated over time) was calculated for each plot for each period between two sampling dates by calculating the Area Under the Curves (AUC) using the formula:

$$AUC = (X_{min} + (0.5 * [X_2 - X_1])) * \delta t$$

in which X_1 = the value on the first of the two sampling dates, X_2 = the value on the second of the two sampling dates, X_{min} = the smallest value of X_1 and X_2, δt = the number of days between the two sampling dates

For aphid infestation, yeast population densities and honeydew, the AUC-values were added up for dry periods between three or more sampling dates. Total number of aphid-infestation-days and yeast-days were calculated by summation of all AUC-values for the experimental period. Honeydew was presumably washed off regularly by rain during the experiments. The total number of honeydew-days was not calculated, since it was assumed not to give an accurate estimate of the presence of honeydew on the leaves. Differences in aphid-infestation-days, yeast-days and honeydew-days were analyzed with a one way ANOVA, followed by a t-test. All statistical calculations were done with SAS (SAS Institute Inc., 1985).

Results

Crop development and weather. In 1987, crop development in spring was rather slow due to adverse weather conditions. May, June and July all had more than the average number of days with rain and temperatures were lower than normal, except for a two week period from the end of June until mid-July. Flowering started (DC 61) in the last week of June. Harvest took place on August 29 at 'De Bouwing', September 2 at 'De Eest' and September 9 at 'De Minderhoudhoeve'. Crop development was earlier at 'De Bouwing' than at 'De Eest' and 'De Minderhoudhoeve'. It is not clear what caused this difference.

The winter of 1987/1988 was mild and wet. In 1988, April was dry and May was dry and warmer than average, resulting in earlier crop development than in 1987. June and especially July were, as in 1987, very wet with temperatures below average. Flowering started (DC 61) in the second week of June. The fields were harvested on August 17 at 'De Bouwing' and August 15 at 'De Eest'. There was practically no difference in crop development between 'De Bouwing' and 'De Eest' in this year.

Daily temperatures and rainfall during the experimental period are shown in Figs. 1-5A.

Aphid populations. Aphid infestation, expressed as percentage infested culms, is shown in Figs. 1-5B. In 1987, aphids were present from flowering (DC 61) in all experiments. In 1988, the aphid populations built up before flowering. At 'De Bouwing', the aphid population grew more rapidly in both years than at the other farms and reached a peak density at the early milky-ripe stage (DC 71-75), whereas at the other farms the peak density was reached at the late milky-ripe or early dough stage (DC 77-83). The rapid decline of infested culms that occurred at 'De Bouwing' in both years was not observed as clearly in the other experiments. Peak densities, however, were comparable in all experiments in one year (70-80% infested culms in 1987 and

50-60% infested culms in 1988). Spraying with insecticide gave good control of aphids. In 1987, *Sitobion avenae* was the predominant aphid species.

Population density and composition of the saprophytic mycoflora. In all experiments, the saprophytic mycoflora consisted mainly of pink and white yeasts (*Sporobolomyces* spp. and *Cryptococcus* spp., respectively). Total yeast populations (pink + white yeasts) on the flag leaves in the control treatment increased steadily to $1\text{-}5 \times 10^4$ CFU cm^{-2} leaf area (Figs. 1-5C). Generally, the most rapid increase in population density coincided with the build up of the aphid populations.

In 1987, maneb gave good control of the yeasts in the first part of the experimental period, but later the differences between yeast population densities in the maneb-treated plots and the control plots decreased at 'De Minderhoudhoeve' and 'De Bouwing'. In 1988, spraying with maneb and anilazin gave good reduction of yeast populations and this reduction was consistent throughout the experimental period. The reduction was larger at 'De Bouwing' than at 'De Eest'. The effect of maneb/anilazin on yeasts was not affected by the frequency of spraying.

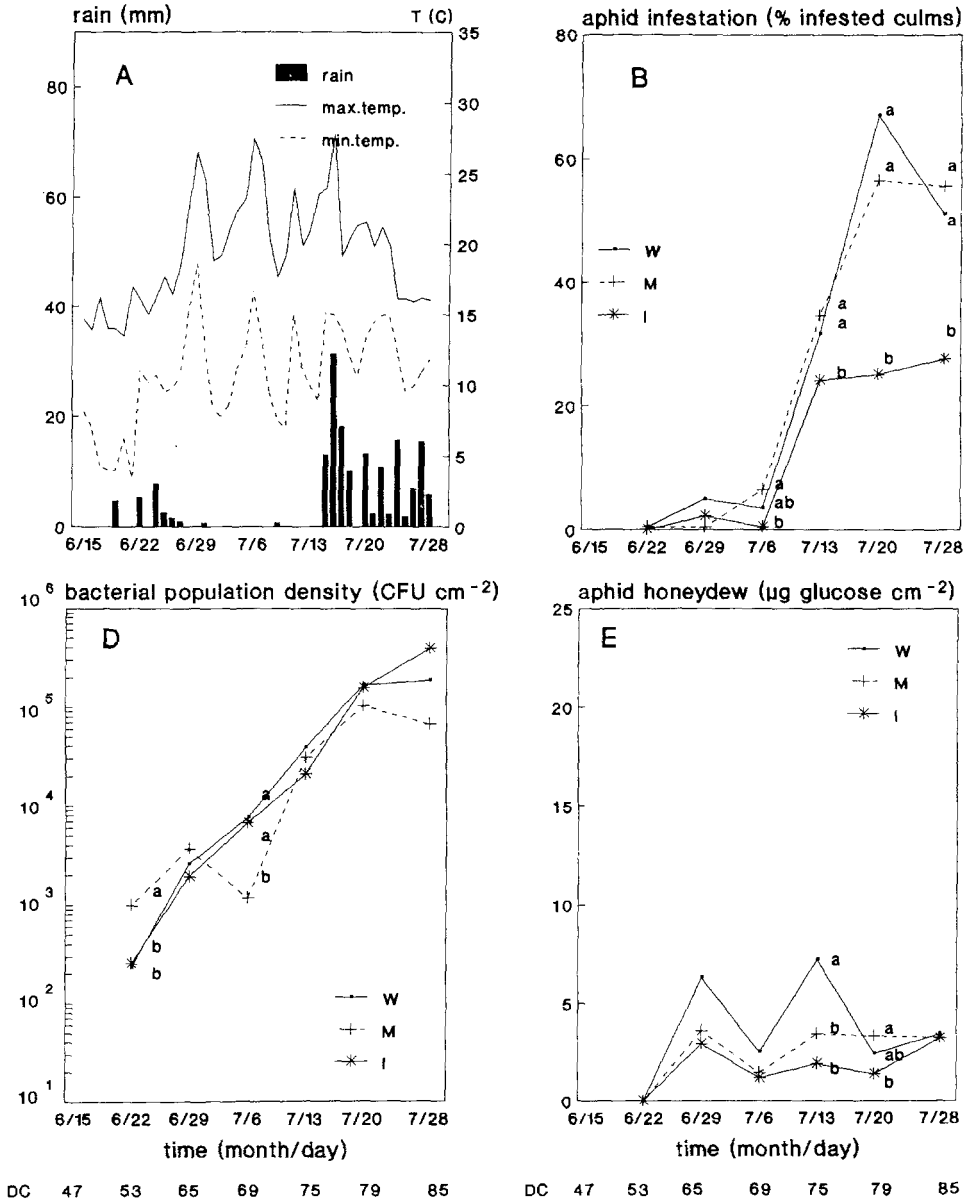
In the insecticide-treated plots, yeast populations initially developed in a similar manner as in the control plots, but the maximum population density was lower in most experiments.

At 'De Eest', the combination of maneb (1987) and maneb/anilazin (1988) with insecticide did not have significantly more effect than maneb or maneb/anilazin alone. The yeast population in the untreated control plots at 'De Bouwing' in 1988 did not differ from those in the water-treated control plots, except for July 6.

In 1987, the pink yeasts were predominant over the white yeasts in all experiments, whereas in 1988 pink and white yeasts were present in approximately equal densities. The relative occurrence of pink and white yeasts in the control treatment did not change between the onset of flowering and the late milky-ripe stage. Spraying with maneb in 1987 and with maneb/anilazin in 1988 caused a reduction in the total percentage yeasts at some early sampling dates, which was mainly due to reduction of the percentage pink yeasts. Insecticide had no effect on the composition of the mycoflora (Dik, 1990).

Bacterial population densities. Bacterial population densities on the flag leaves increased during the season (Figs. 1-5D). Maneb and maneb/anilazin caused significant reduction of the bacterial populations only on some sampling dates. At 'De Eest' in 1987, maneb alone sometimes reduced the bacterial population densities, whereas the combined treatment of maneb with insecticide had no effect. In 1988 at 'De Eest', both treatments had the same effect on bacteria.

Aphid honeydew. The amount of honeydew washed from the leaves was low in all experiments, most likely due to repeated washing off of the honeydew by heavy rainfall (Figs. 1-5E). There was either no difference in amount of honeydew or more honeydew was found in the maneb-treated plots, except for one sampling date at 'De Minderhoudhoeve' (July 13, 1987). In this experiment there was more infection by *S. tritici* in the control treatment than in the other treatments. Probably this resulted in increased leakage of carbohydrates which influenced the assessment of the carbohydrates in the leaf washings. Significantly higher levels of honeydew in the maneb treatment compared to the control treatment were observed in 1987 at 'De Eest' on three



sampling dates during dry periods and in 1988 at both 'De Eest' and 'De Bouwing' in the less intensively sprayed maneb/anilazin plots on one sampling date, which followed on a period of dry weather.

In 1987, peak honeydew levels at 'De Bouwing' in the dry period in July were much higher than in the other fields. At 'De Bouwing', honeydew accumulated in this period both in water-treated plots and in maneb-treated plots, while no accumulation was seen in the control treatment at 'De Eest' in the same period. At 'De Bouwing', the

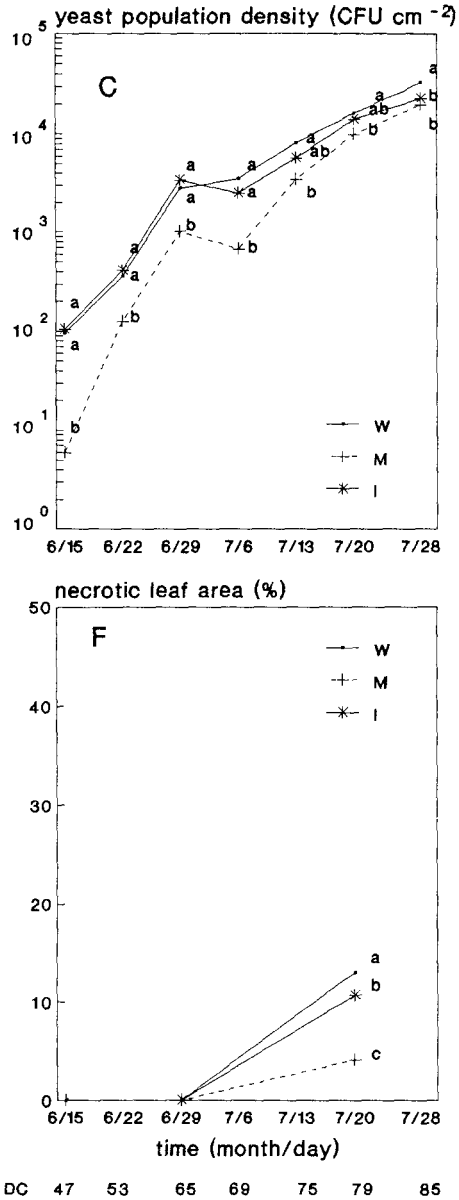
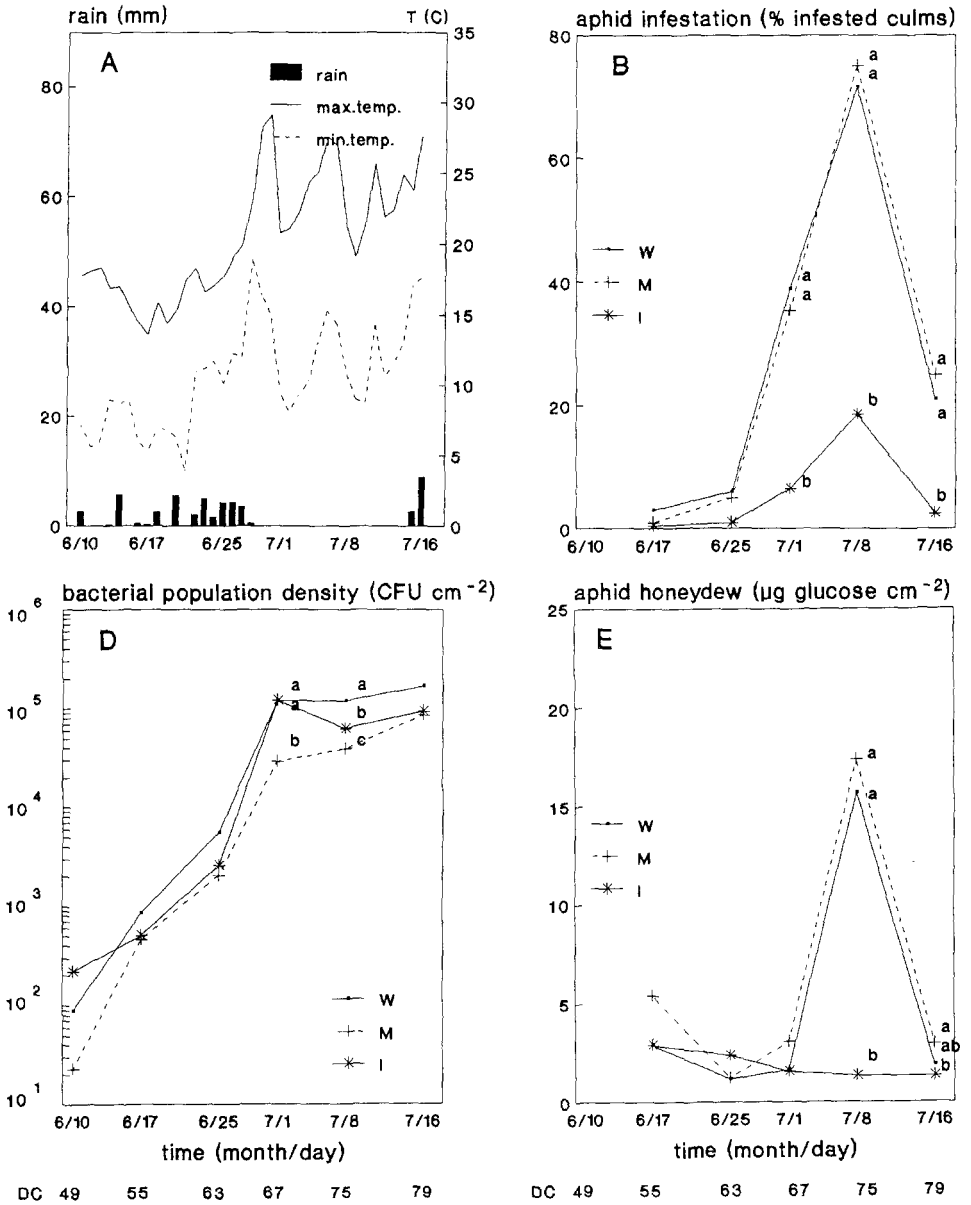


Fig. 1. Effect of different treatments on (B) aphid infestation, (C) yeast population density on the flag leaf, (D) bacterial population density on the flag leaf, (E) amount of aphid honeydew on the flag leaf, (F) necrotic area of the flag at 'De Ir. A.P. Minderhoudhoeve' in 1987. Weather data are shown in (A). On sampling dates with significant (P = 0.05) differences between treatment means, these means are followed by different letters. For treatments and spraying dates see Tabel 1.

dry period coincided with peak aphid densities after very rapid development of the aphid populations, whereas at 'De Eest', the aphid population developed more slowly. Comparison of aphid-infestation-days in the dry period shows a much larger aphid pressure at 'De Bouwing' than at 'De Eest' in this period (Table 2). At 'De Bouwing', the yeast population densities did not differ much between treatments in this period. The following decrease of honeydew in both treatments (July 16, 1987) can be caused by consumption by yeasts or by washing off by rain or both.



The C/N-ratio of the honeydew did not differ much between the maneb treatment and the control and between 'De Eest' and 'De Bouwing': on July 8, 1987 the C/N-ratio in the leaf washings of the water and the maneb treatment at 'De Bouwing' was 41.5 and 37.7 respectively, and on July 13, 1987 at 'De Eest' 44.4 in the water treatment and 49.1 in the maneb treatment.

Visual assessment of the amount of leaf surface covered with honeydew on July 13, 1987 at 'De Eest' showed that the percentage leaf area covered with aphid honeydew

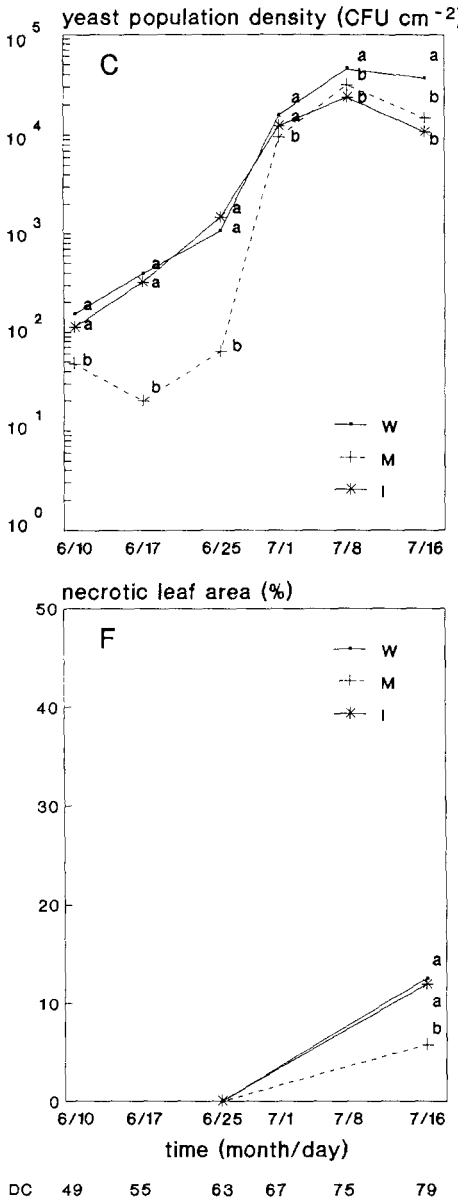
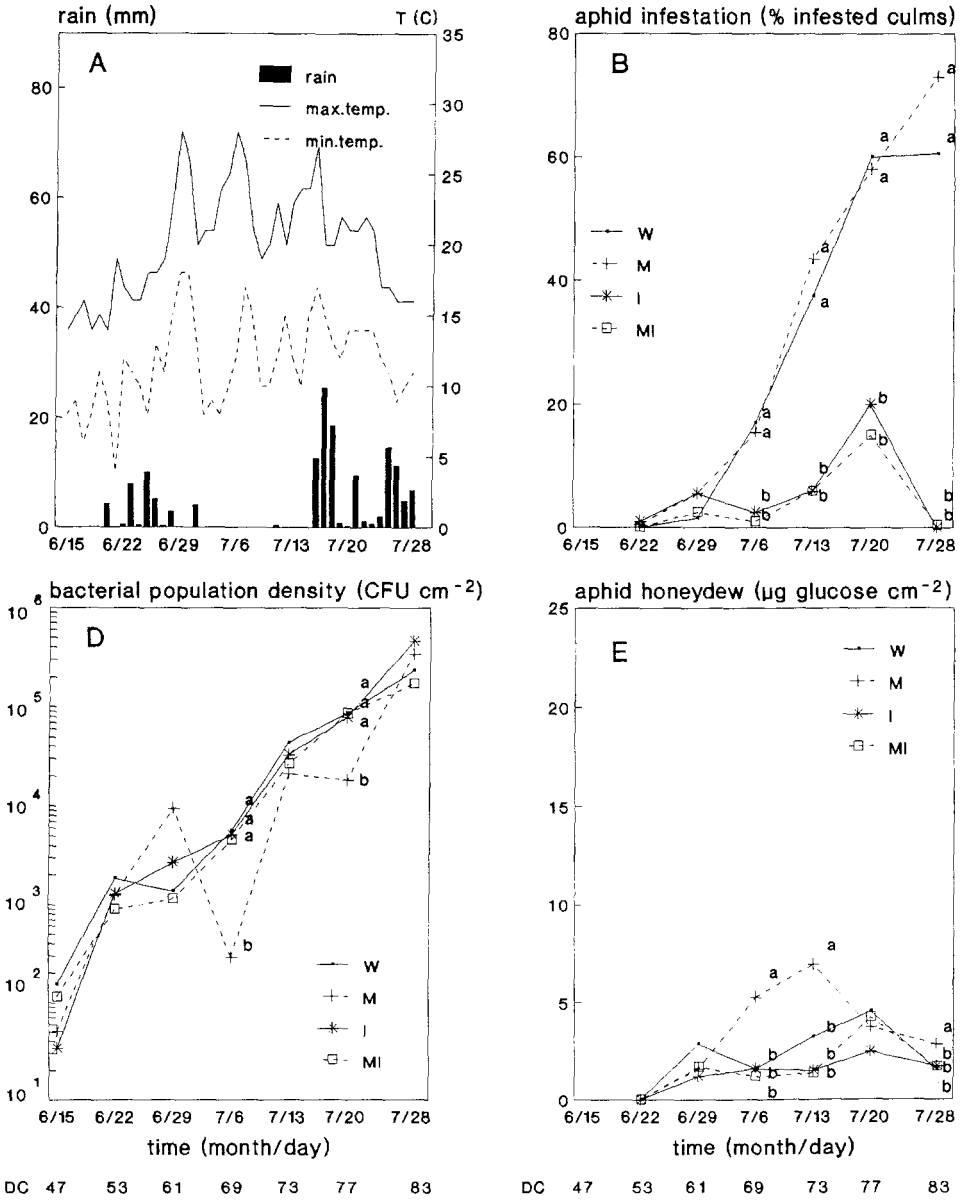


Fig. 2. Effect of different treatments on (B) aphid infestation, (C) yeast population density on the flag leaf, (D) bacterial population density on the flag leaf, (E) amount of aphid honeydew on the flag leaf, (F) necrotic area of the flag leaf at 'De Bouwing' in 1987. Weather data are shown in (A). On sampling dates with significant (P = 0.05) differences between treatment means, these means are followed by different letters. For treatments and spraying dates see Table 1.

was 5.9 ± 5.6 in the water-treatment and 12.5 ± 5.6 in the maneb-treatment. The percentage leaf area covered per μgram honeydew recovered from the leaves is the same for both treatments, viz. 1.81% leaf area (μgram honeydew $cm^{-2})^{-1}$.

At 'De Bouwing' in 1988 and 'De Eest' in 1987 and 1988, honeydew amounts in the water-treated plots were not significantly different from those in the insecticide-treated plots on any of the sampling dates. This demonstrates that the yeasts in these experiments could effectively remove aphid honeydew from the wheat leaves.



Diseases. Septoria tritici, causal agent of speckled leaf blotch on wheat, was the most important pathogen in all experiments. Because of the many days with rain, this fungus could spread easily through the crop. *S. tritici* infection was reduced by spraying with maneb or maneb/anilazin. Only at 'De Minderhoudhoeve' was infection by this pathogen lower in the insecticide treatment than in the control. At 'De Eest' in 1987, no *S. tritici* infection was observed on the flag leaf.

Mildew (*Erysiphe graminis* f.sp. *tritici*) and brown rust (*Puccinia recondita*) were only present in both experiments at 'De Bouwing'. Mildew infection was not in-

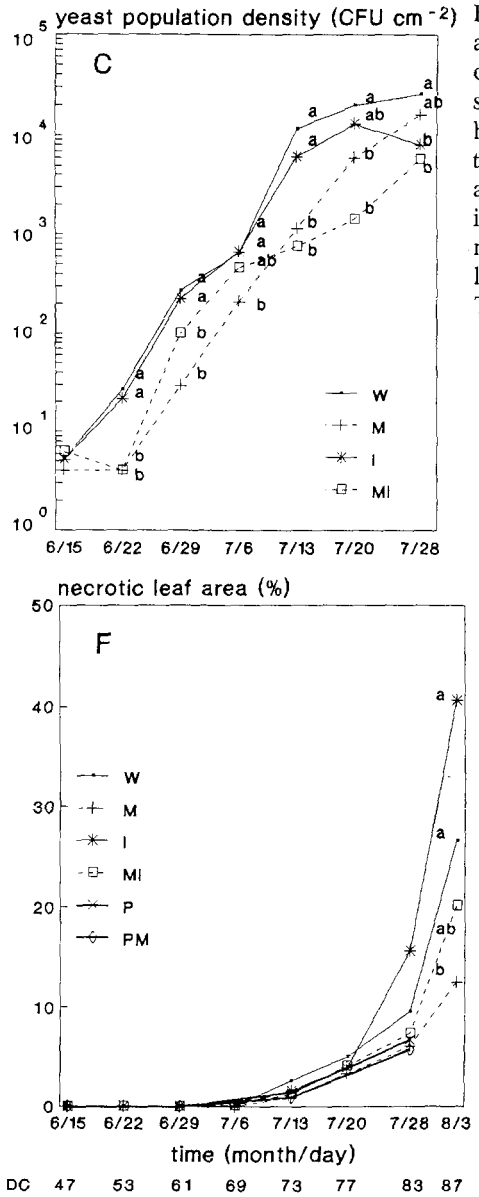
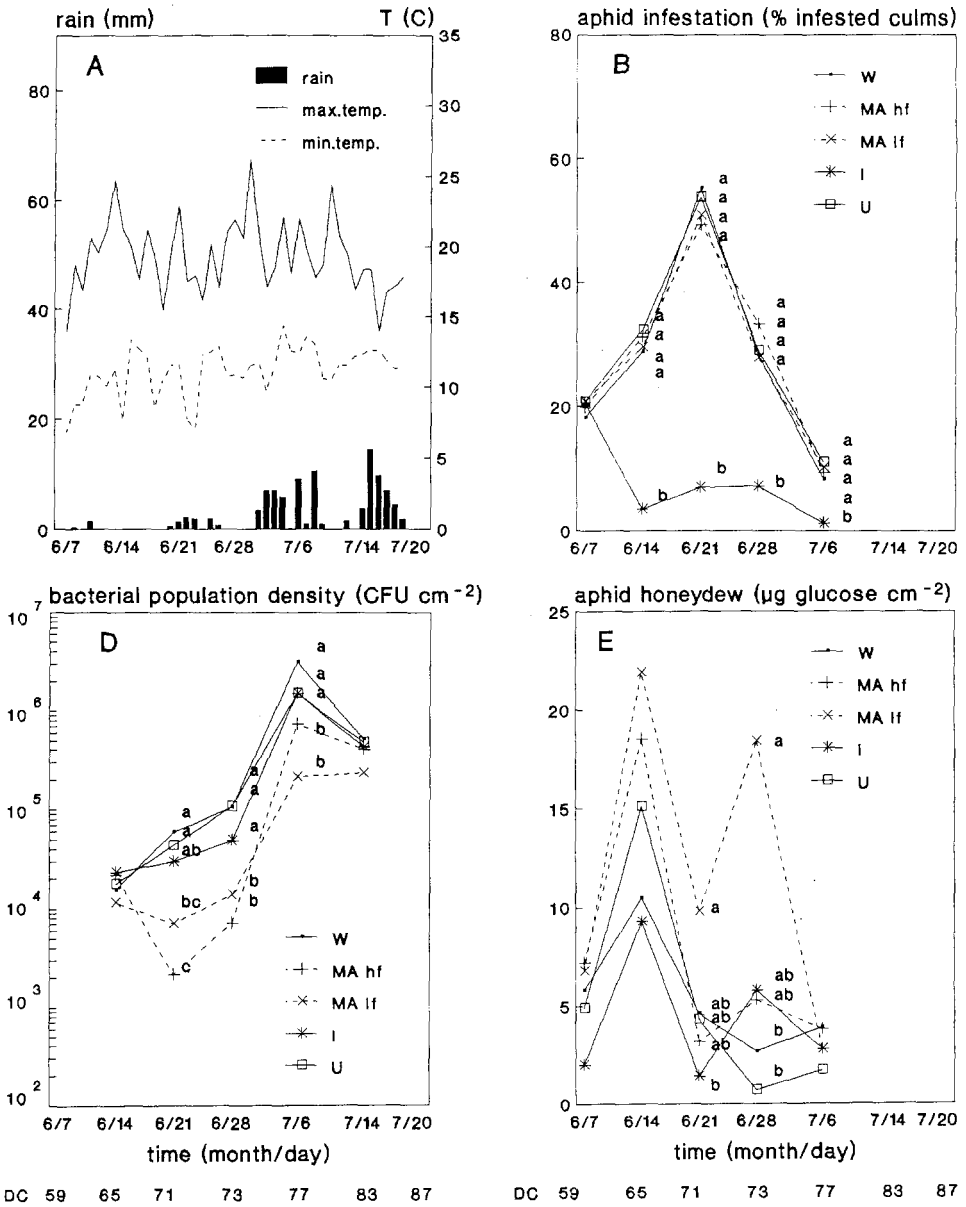


Fig. 3. Effect of different treatments on (B) aphid infestation, (C) yeast population density on the flag leaf, (D) bacterial population density on the flag leaf, (E) amount of aphid honeydew on the flag leaf, (F) necrotic area of the flag leaf at 'De Eest' in 1987. Weather data are shown in (A). On sampling dates with significant (P = 0.05) differences between treatment means, these means are followed by different letters. For treatments and spraying dates see Table 1.

fluenced by the maneb-treatments, while brown rust was decreased by the treatments with maneb and maneb/anilazin. Some yellow rust (*Puccinia striiformis*) was seen at 'De Eest' in 1988 late in the season. *Gerlachia nivalis* was present on leaves in all experiments, but only in very low densities. *Fusarium* spp. in the ear were found in all experiments at low severities and were not influenced by any of the treatments. *Septoria nodorum* in the ear was only present at the experiments at 'De Bouwing' with a maximum average severity of 0.5 spikelet ear^{-1}.



The average percentage not-green leaf area, i.e. total diseased, chlorotic and dead leaf area, of the flag leaf is shown in Figs. 1-5F. Spraying with maneb in 1987 and with maneb/anilazin in 1988 reduced the percentage not-green leaf area due to reduction of *S. tritici* infection.

The treatments at 'De Eest' in 1987 with prochloraz and prochloraz + maneb were meant to establish the influence of honeydew on the effectiveness of prochloraz. However, the pathogen *S. tritici*, which was the only necrotrophic pathogen present

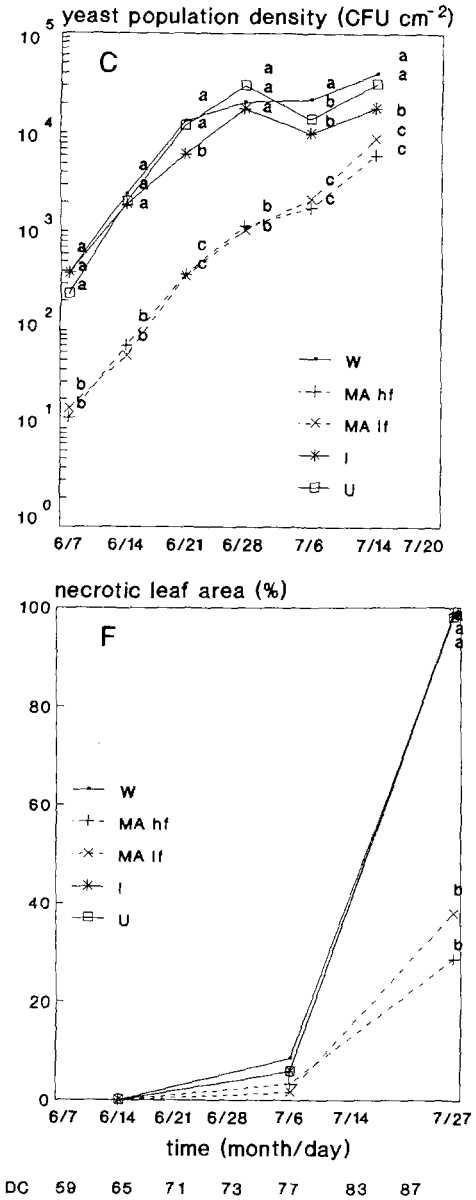
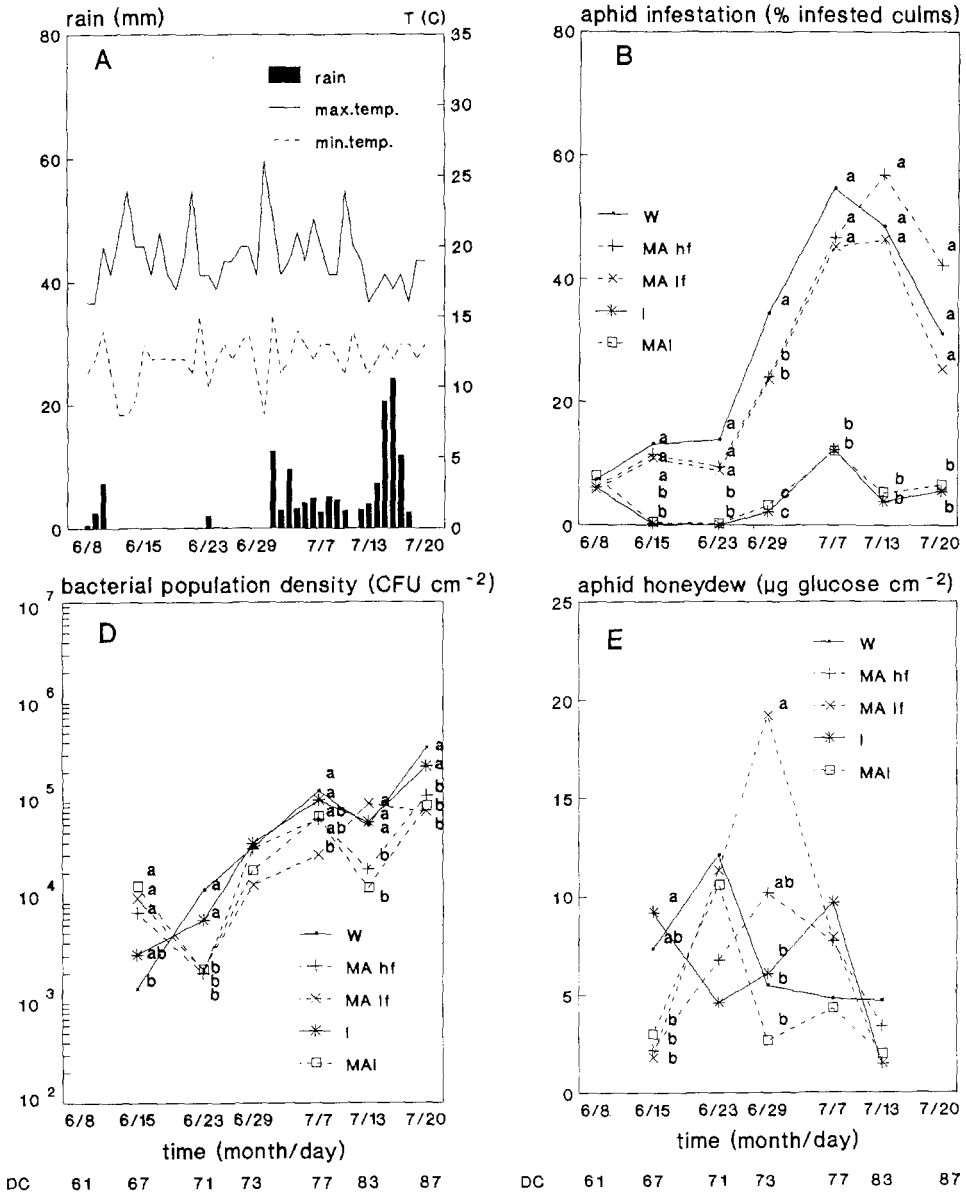


Fig. 4. Effect of different treatments on (B) aphid infestation, (C) yeast population density on the flag leaf, (D) bacterial population density on the flag leaf, (E) amount of aphid honeydew on the flag leaf, (F) necrotic area of the flag leaf at 'De Bouwing' in 1988. Weather data are shown in (A). On sampling dates with significant (P = 0.05) differences between treatment means, these means are followed by different letters. For treatments and spraying dates see Table 1.

in considerable amounts, was inhibited by maneb, so the expected stimulating effect of honeydew on infection was counteracted by the reducing effect of the maneb. The effect of honeydew on maneb activity can be calculated by comparing severities in the treatments M-MI in 1987 and MA-MAI in 1988 at 'De Eest'. No significant differences in necrotic leaf area occurred between the M and MI treatments in 1987 nor between the MA and MAI treatments in 1988.



Yields. Kernel yields and 1000-kernel-weight are shown in Table 3, together with total aphid-infestation-days. The treatment with maneb in 1987 and both treatments with maneb/anilazin in 1988 caused a significant increase in yield and 1000-kernel-weight in all experiments. Insecticide alone significantly increased yield and 1000-kernel-weight only at 'De Bouwing' in 1987. In combination with maneb insecticide had an additional effect on yield at 'De Eest' in 1987. The effect on kernel yields can be largely ascribed to an effect on 1000-kernel-weight, since both variables are influenced in the

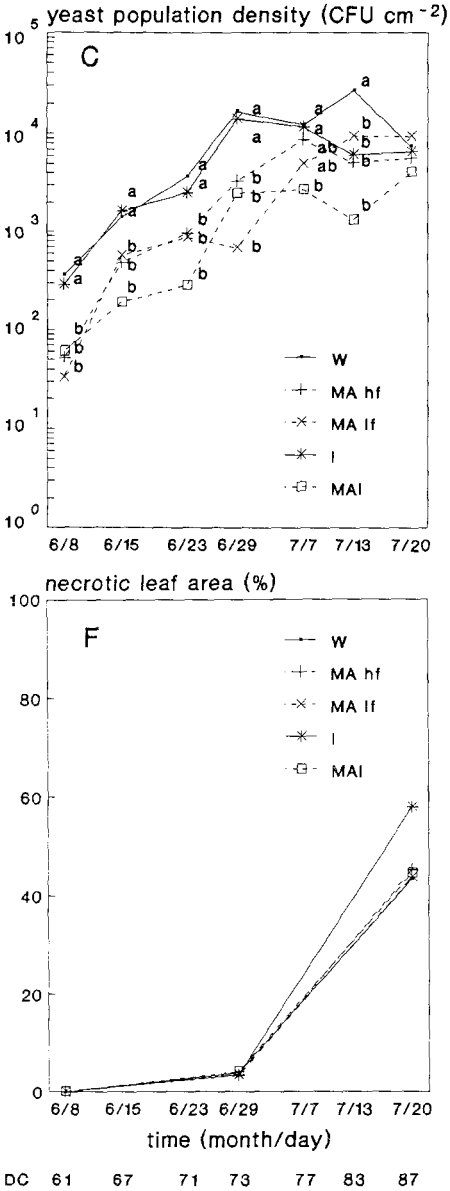


Fig. 5. Effect of different treatments on (B) aphid infestation, (C) yeast population density on the flag leaf, (D) bacterial population density on the flag leaf, (E) amount of aphid honeydew on the flag leaf, (F) necrotic area of the flag leaf at 'De Eest' in 1988. Weather data are shown in (A). On sampling dates with significant (P = 0.05) differences between treatment means, these means are followed by different letters. For treatments and spraying dates see Table 1.

same way and number of ears m^{-2} (not shown) was not influenced.

For both experiments at 'De Eest' the yield loss per aphid-infestation-day in plots with high yeast densities and with reduced yeast densities was calculated by comparing the differences in yields and aphid-infestation-days between the water treatment and the insecticide treatment and between the maneb or maneb/anilazin treatment and the maneb or maneb/anilazin + insecticide treatment. In 1987, the yield loss (dry weight) per aphid-infestation-day was 0.11 kg ha^{-1} aphid-infestation-day^{-1} when the saprop-

Tabel 2. Area Under the Curve-values for aphid-infestation, yeasts and honey-dew during dry periods.

Experiment	Period (month/day)	Treatment	Aphid-infestation-days	Yeastdays (× 1000)	Honeydew-days
Minderhoud-hoeve 1987	6/29-7/13	W	152.3 ab	66.1 a	66.4 a
		M	168.0 a	20.4 b	34.5 a
		I	95.4 b	49.5 a	25.0 b
Bouwing 1987	6/25-7/8	W	521.8 a	267.0 a	69.3 a
		M	508.3 a	177.0 b	84.7 a
		I	110.0 b	168.7 b	22.2 b
Eest 1987	6/29-7/13	W	255.5 a	45.6 a	40.7 b
		M	280.0 a	5.5 c	76.7 a
		I	57.8 b	26.5 b	20.7 c
		MI	36.8 b	6.2 c	19.2 c
Bouwing 1988	6/7-6/21	W	459.1 a	64.1 a	138.1 a
		MA hf	460.3 a	1.8 c	165.5 a
		MA lf	457.3 a	1.7 c	211.5 a
		I	121.3 b	35.6 b	76.7 a
		U	487.7 a	56.3 a	137.8 a
Eest 1988	6/8-6/29	W	324.3 a	86.0 a	155.4 a
		MAhf	246.3 b	20.1 b	94.7 a
		MAlf	234.0 b	12.7 b	150.3 a
		I	30.6 c	72.0 a	156.2 a
		MAI	43.6 c	11.0 b	104.8 a

For explanation of treatments and calculation of aphid-infestation-days, yeastdays and honeydewdays see text. Means followed by the same letter are not significantly different ($P = 0.05$).

hytes were reduced and 0.08 kg ha^{-1} aphid-infestation-day^{-1} in the presence of the naturally occurring saprophytic population. In 1988, no yield loss could be ascribed to aphids in the presence of high saprophyte population densities. In the maneb/anilazin treatment, the yield loss was 0.21-0.25 kg ha^{-1} aphid-infestation-day^{-1}. Since the aphid population developed the same in the control and the maneb treatment, the influence of the crop development stage on aphid damage was not taken into account in this calculation.

Discussion

The experiments show that naturally occurring saprophytes on wheat leaves can effectively remove aphid honeydew under field conditions and that reduction of the saprophytic population by a broad-spectrum fungicide leads to accumulation of aphid honeydew on wheat leaves in dry periods. The prevention of accumulation of

Table 3. The effect of different treatments on yield variables and total number of aphid-infestation-days.

Experiment	Treatment	Kernel yield (dw, × 1000 kg ha^{-1})	1000-Kernel-weight (dw, g)	Aphid-infestation-days
Minderhoud-hoeve 1987	W	6.12 a	31.8 a	969 a
	M	7.09 b	35.4 b	935 a
	I	6.21 a	32.4 a	477 b
Bouwing 1987	W	5.96 a	33.2 a	938 a
	M	7.06 c	37.9 c	958 a
	I	6.55 b	35.2 b	198 b
East 1987	W	6.34 a	30.1 ab	1372 a
	M	6.74 b	30.8 ab	1529 a
	I	6.43 a	29.3 a	255 b
	MI	6.89 c	31.5 b	183 b
	P	6.69 b	27.9 ab	n.d.
	PM	7.00 c	31.3 b	n.d.
Bouwing 1988	W	6.70 a	34.1 a	917 a
	MA hf	8.04 b	38.6 b	958 a
	MA lf	7.69 b	38.9 b	925 a
	I	6.82 a	35.3 a	209 b
	U	6.67 a	34.4 a	982 a
Eest 1988	W	6.66 a	35.1 a	1018 a
	MA hf	6.93 b	36.2 b	865 a
	MA lf	6.92 b	36.1 b	809 a
	I	6.59 a	34.9 a	192 b
	MAI	7.08 b	35.7 b	163 b

Treatment means followed by the same letter are not significantly different ($P = 0.05$). For explanation of treatments, see text.

honeydew by saprophytes in the field is consistent with the rapid removal of exogenous nutrients from wheat leaves by yeasts in a controlled environment (Fokkema *et al.*, 1983). However, at 'De Bouwing' in 1987, honeydew accumulated almost as much on control leaves as on the maneb-treated leaves during the dry period in July. It is unlikely that the composition of the honeydew caused decreased honeydew consumption by the yeasts at 'De Bouwing', since the C/N-ratio was approximately the same as at 'De Eest' and control experiments showed that yeasts can consume all the main carbohydrates normally present in honeydew (A.J. Dik, unpublished results). The accumulation of honeydew in the control treatment at 'De Bouwing' was probably due to the more rapid growth of the aphid population in this experiment than in the other experiments. The number of aphid-infestation-days during the dry period (Table 2) was much higher at 'De Bouwing', which means that a higher amount of honeydew was produced per day. Furthermore, in the first week of July 1987 the growth rate of

the yeast population was slightly inhibited in all experiments. This coincided with short periods of relative humidity above 90% during the night. Rodger and Blakeman (1984) found accumulation of carbohydrates on sycamore leaves due to honeydew deposition by large numbers of aphids when the saprophytic population density was still low, so in that case the saprophytes also could not completely prevent accumulation of honeydew. Clearly, the extent to which saprophytes can prevent accumulation of honeydew depends on the balance between honeydew production rate and the population growth of the saprophytes.

It has been argued by Hirano and Upper (1986), that sampling microbial populations with intervals of one week does not give a good impression of the population dynamics of bacteria, since population densities show diurnal fluctuations and are influenced by weather and may therefore vary considerably during this period. However, fluctuations in population densities during the day are smaller for yeasts than for bacteria (Hirano and Upper, 1986; A.J. Dik, unpublished results) and by sampling at the same time of day on each sampling date, the influence of these fluctuations has been minimalized. Furthermore, the treatments in the field experiments were usually applied shortly after sampling and thus the observed effect of the treatments reflects the minimum rather than the maximum effect of the treatments.

The antagonistic effect of yeasts on necrotrophic pathogens is assumed to be based on nutrient competition (Fokkema, 1976, 1981; Blakeman and Fokkema, 1982). Although the antagonistic role of naturally occurring saprophytes against necrotrophic pathogens is difficult to demonstrate, because means to reduce the saprophytes (viz. fungicides) will also affect the pathogens (Fokkema *et al.*, 1987), this role has now been confirmed by determination of the reduction of available nutrients. Interesting in this respect is the experiment at 'De Minderhoudhoeve'. This experimental field was situated next to an experiment which was artificially inoculated with *S. tritici* spores, which may have increased the inoculum pressure of this pathogen in our experiment and thus created a competitive advantage for this pathogen compared to the saprophytes. The fact that only in this experiment *S. tritici* infection was reduced by insecticide indicates that in the other experiments with natural inoculum pressure the saprophytes competed succesfully for the honeydew. This is in accordance with previous findings under controlled conditions (Fokkema *et al.*, 1983).

Both weather and nutrient availability influence colonization of the leaves by saprophytes. In experiments by Dickinson and Wallace (1976), the pink yeasts were predominant early in the season and the white yeasts at the end of the season. The relative occurrence of pink and white yeasts was in our experiments more dependent on year than on location and did not change during the season (Dik, 1990). Magan and Lacey (1986) also found a difference in relative occurrence of pink and white yeasts between years. It is most likely that this depends on weather. The fact that the white yeasts were predominant in the year with the higher temperatures during the spring and the increase in white yeasts during the season found by Dickinson and Wallace (1976) both indicate that the white yeasts may have a higher optimum temperature for growth than the pink yeasts.

The maximum population densities of yeasts in the experiments reported here were lower than found by Fokkema *et al.* (1975, 1979, 1987), Rabbinge *et al.* (1981) and Dickinson and Wallace (1976), probably because nutrients were repeatedly washed off the leaf by the extreme amount of rain during all experiments. This also explains why

honeydew levels were lower than expected from calculations of honeydew production based on Vereijken (1979) and Rabbinge *et al.* (1981). The relation between maximum yeast population density and nutrient level is confirmed by the fact that the maximum yeast population densities were lower in the insecticide treatment than in the control. The use of maneb and maneb/anilazin to create differences in yeast population densities was generally successful. However, this difference decreased in 1987 during the experimental period. Probably two factors caused this decrease. It has been shown that the effect of fungicides can be reduced in the presence of nutrients (Dunn *et al.*, 1971; Rabbinge *et al.*, 1984; Dik and Fokkema, 1988; Dik *et al.*, 1991). This might also apply to maneb activity against yeasts in the presence of honeydew. An other explanation is a decreased sensitivity of yeasts to maneb, which is supported by the results of plating leaf washings on maneb-containing agar (A.J. Dik, unpublished results). Fokkema *et al.* (1987) also had difficulties in creating different yeast population densities with carbendazim, because the yeasts showed resistance to this fungicide. The phenomenon of decreased sensitivity to maneb in yeasts, although interesting in itself, has in our opinion no practical implications, since in normal wheat cultivation practice in the Netherlands maneb is in general not applied more than twice (Daamen, 1990). Indeed, the strong reduction of the yeast population density in the less intensively sprayed maneb/anilazin treatments in 1988, which resemble the frequency of spraying of broad-spectrum fungicides in practice, confirms that in common wheat cultivation the yeast population will often be much lower than it would be if only selective chemicals were used. Furthermore, from the composition of the saprophytic mycoflora we can conclude that spraying with maneb or anilazin has more effect on the yeasts than on *Cladosporium* and *Aureobasidium* spp., since the relative occurrence of the yeasts is decreased. This means that a competitive advantage for the black moulds is created by spraying with these broad-spectrum fungicides. This is undesirable since high yeast densities have no detrimental effect (Fokkema *et al.*, 1987), but the black moulds may reduce grain quality and decrease yields by intercepting light and by triggering energy demanding defense reactions in the plants (Smedegaard-Petersen and Tolstrup, 1986).

Apart from the yeasts and black moulds, bacteria were also present on the leaves and were sometimes reduced by the broad-spectrum fungicides. Generally, no antagonistic effect of bacteria on leaf surfaces can be expected at densities below 10^6 CFU cm^{-2} (Sleesman and Leben, 1976; A.J.Dik, unpublished results). Furthermore, bacterial population densities normally decline rapidly during the day due to desiccation (Hirano and Upper, 1986), whereas yeast populations are less variable during one day (Blakeman and Fokkema, 1982) and the bacterial populations were not reduced consistently. Therefore, the observed removal of honeydew is ascribed mainly to the yeasts.

The effect of the different treatments on kernel yields show that with the exception of 'De Bouwing' in 1987, insecticide had no effect on yield. This corresponds with the fact that only at 'De Bouwing' in 1987, honeydew levels in the control treatment were significantly higher than in the insecticide treatment. From the experiments at 'De Eest' it becomes clear that when the naturally occurring saprophytic microflora is preserved, the yield loss per aphid-infestation-day is reduced considerably in comparison with the situation where the saprophytes are reduced. The detrimental effect of aphid honeydew is partly caused by interference with the photosynthesis of the leaves

(Rabbinge *et al.*, 1981). The fact that the percentage leaf area covered with honeydew per microgram honeydew recovered from the leaves was the same in the control and the maneb treatment at 'De Eest' in 1987, indicates that the yeasts do not just reduce the thickness of the honeydew layer on the leaves but do indeed reduce the leaf area and thus the number of stomata covered with honeydew. The contribution of honeydew deposition to aphid damage is usually established in experiments in which broad-spectrum fungicides are applied to control diseases (Rabbinge *et al.*, 1981; Rossing, 1991). The effect of honeydew on wheat yields will in the presence of the naturally occurring yeasts probably be smaller than calculated from such experiments.

The increase in kernel yields due to maneb in 1987 and to maneb/anilazin in 1988 was most likely due to the reduction of infection by *Septoria tritici*, although maneb might have influenced kernel yields directly, as found for oats by Clark *et al.* (1986). The effect on *S. tritici* was rather unexpected, because evaluation of five years of field experiments in the Netherlands by the Plant Protection Service (Internal Report, 1986) did not reveal any effect of maneb on diseases. In those experiments however, maneb was added to other fungicides, whereas in the experiments described here only maneb or anilazin were applied and the frequency of spraying was greater. The influence of honeydew on necrotrophic pathogens and on the activity of prochloraz in the experiment at 'De Eest' in 1987 could not be analysed properly, because the expected stimulating effect of honeydew accumulation on infection was counteracted by the inhibiting effect of maneb. In the experimental design that was used, interference of honeydew with prochloraz activity could only have been revealed for a pathogen that was not influenced by maneb. No effect of the accumulation of honeydew on the flag leaves on maneb activity was found, presumably because no *S. tritici* infection was observed on the flag leaf in this experiment. More elaborate experiments are needed to confirm the interference of honeydew with the effectiveness of fungicides against necrotrophic leaf pathogens, which was found under controlled conditions (Dik *et al.*, 1991), in the field.

From the experiments described, we conclude that the naturally occurring saprophytic microflora on wheat leaves, especially the yeasts, can prevent accumulation of aphid honeydew in dry periods and thereby reduce aphid damage in wheat. This occurs especially when the aphid population does not grow too rapidly in a period when saprophyte population densities are very low.

The removal of nutrients by the yeasts also applies for other nutrients on the leaves, such as pollen and exudates from leaves and fungal spores. Although these nutrients may not have a direct effect on the functioning of the leaves, their ability to stimulate infection by necrotrophic pathogens is apparent and competition for these nutrients by the yeasts has been shown to reduce infection (Fokkema, 1971, 1973). The beneficial effect of naturally occurring saprophytes demonstrated here can be used in wheat cultivation by eliminating fungicides that harm the saprophytes and this may be equally valid for other field crops.

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