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Phyllosphere yeasts antagonize penetration from appressoria and subsequent infection of maize leaves by Colletotrichum graminicola

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Abstract

In growth cabinet experiments, the common phyllosphere yeasts *Sporobolomyces roseus* and *Cryptococcus laurentii* var. *flavescens* were sprayed as a mixture (1:1) onto the fourth leaves of maize plants (*Zea mays)* two-three days prior to inoculation with *Colletotrichum graminicola.* In four experiments the average yeast population of the treated leaves at the time of pathogen inoculation varied between 5×10^4 and 8×10^5 cells cm^{-2} leaf, whereas on the untreated leaves the yeast population varied from $< 10^3$ to 10^4 cells cm^{-2} leaf. The yeasts reduced lesion density and necrosis from *C. graminicola* infection by approximately 50%. Contrary to findings with other necrotrophic pathogens, conidial germination, superficial mycelial growth and appressorium formation were not affected. Instead, the reduction of infection could only be explained by a reduced number of penetrations from the normally formed appressoria, a site of interaction not previously recorded.

Additional keywords: biological control, *Sporobolomyces roseus, Cryptococcus laurentii* var. *flavescens.*

Introduction

Saprophytic phyllosphere bacteria and yeasts can antagonize various necrotrophic leaf pathogens by nutrient competition. This affects the superficial development of the pathogens and thus the number of penetrations and the extent of subsequent necrosis (Blakeman and Fokkema, 1982). Contradictory observations, however, are reported concerning the response of pathogenic *Colletotrichum* species to phyllosphere saprophytes. Leben (1964) found that a bacterium, isolated from healthy cucumber leaves, reduced infection by *C. orbiculare* (cucumber anthracnose) in the glasshouse. There is circumstantial evidence that naturally occurring fungal saprophytes limit development of coffee berry disease caused by *C. coffeanum* (Firman and Waller, 1977).

On the other hand, in vitro studies showed that bacteria reduced conidial germination slightly, but stimulated appressorium formation of *C. gloeosporioides* (Lenné

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and Parbery, 1976). When conidia were incubated with several *Pseudomonas* species known to be highly competitive for amino acids on detached beetroot leaves, germination remained unaffected but the formation of appressoria was promoted in *C. dematium* f. sp. *spinaceae* (Blakeman and Brodie, 1977) and *C. acutatum* (Blakeman and Parbery, 1977). Blakeman and Brodie (1977) suggested that nutrient-limiting conditions promote appressorium formation and may thus stimulate rather than reduce infection by *Colletotrichum* species, but this has not been demonstrated. The suppression of lesion formation by *C. lindemuthianum* on bean hypocotyls in the presence of orange extract (Mercer et al., 1970) supports such a role for nutrients.

As yeasts are a common and stable component of leaf microfloras (Fokkema, 1981), the effect of yeasts on the infection of maize leaves by *Colletotrichum graminicola* (Ces.) Wilson was studied. In preliminary experiments, Fokkema and De Nooij (1981) found that although *Sporobolomyces roseus* Kluyver and Van Niel reduced infection of *C. graminicola*, conidial germination and appressorium formation were not affected, indicating the need for further experiments to determine the site(s) of interaction.

In this paper, the in vivo interactions between two common saprophytic yeasts, *S. roseus* and *Cryptococcus laurentii* var. *flavescens* Lodder and Van Rij, and the maize pathogen *C. graminicola* are described and a new site of antagonism is elucidated.

Materials and methods

Fungi and plants. C. graminicola, S. roseus and *C. laurentii* var. *flavescens* were grown on potato dextrose agar slants (PDA) at 23 °C in the dark. Inoculum and yeast cell suspensions were obtained by flooding 10 to 14-day-old cultures with 0.01% Tween 80, scraping the colony surface and filtering the suspension through nylon gauze. The concentration of *C. graminicola* conidia was adjusted to 1-2 $\times$ 10^5 conidia per ml. The concentration of cells in each yeast suspension was adjusted to 2 $\times$ 10^7 cells per ml. Equal volumes of the suspensions of *S. roseus* and *C. laurentii* var. *flavescens* were combined to make a mixed suspension for spraying, in which the concentration of each of the two yeasts was 10^7 cells per ml.

Maize plants(*Zea mays* L. cv. Balda) were grown individually in a sandy compost soil in 8 cm diameter plastic pots in the glasshouse at 17 to 22 °C.

Inoculation procedure. In preliminary experiments different inoculum concentrations were applied to newly unfolded third, fourth or fifth leaves of 3-wk-old maize seedlings. The results (Fig. 1) showed that inoculation of the fourth-formed leaf with an inoculum containing 1-2 $\times$ 10^5conidia per ml was suitable for experimentation because this combination produced a level of necrosis (50 to 60%) which could potentially be stimulated or inhibited by synergistic or antagonistic interactions, respectively.

At the beginning of each experiment, the fourth leaf of each plant was carefully drawn between the moistened thumb and index finger to increase the wettability of the leaf, thereby ensuring an even distribution of spray droplets over the leaf surface. Because leaves continued to grow during these experiments, the treated part of each leaf was marked off with a felt-tipped pen. The yeast mixture was sprayed onto the adaxial leaf surfaces with a pump-operated glass chromatography reagent sprayer, two

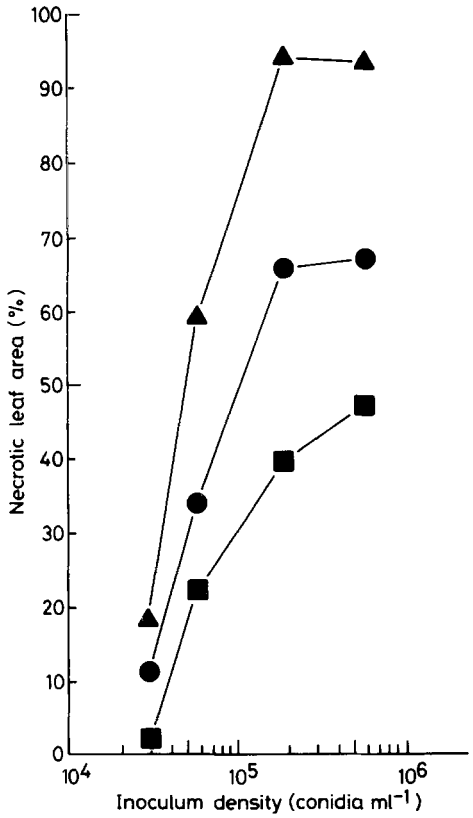

Fig. 1. Effect of *C. graminicola* inoculum density on the necrosis of the third (▲), fourth (•) and fifth (■) leaves of 3-wk-old maize plants (n = 5).

to three days prior to pathogen inoculation. The control leaves were sprayed with a 0.01% Tween 80.

After yeast treatment, all plants were put into polyvinyl chloride infection boxes which were lined with moist filter paper and housed within a climate cabinet with a 10 h day: 14 h night cycle at 90% relative humidity and 22 °C under fluorescent light (45 Watt m^{-2} on top of the canopy). After two to three days incubation, all the plants were inoculated with the pathogen and returned to the infection boxes for a further three days incubation. Thereafter, the plants were exposed to the environment of the climate cabinet for another 7 to 10 days.

Analysis of the phyllosphere microflora. Just before inoculation with the pathogen, five yeast-treated and five control leaves were collected and the phyllosphere saprophyte populations were estimated by culturing yeast cells and bacteria washed (in 0.01 % Tween 80, 0.5 % bactopeptone) from the leaves (Fokkema et al., 1979). Bactopeptone was added to the shaking medium to promote the survival of bacteria (Straka and Stokes, 1957). After three to five days incubation at 23 °C, the colonies were counted and colonization was expressed as the number of colony forming units (CFU) per cm^2 leaf.

Assessment of the pre-penetration development and penetration of C. graminicola. One to four days after inoculation with the pathogen, yeast-treated and control leaves

were collected to determine percentage germination, number and length of germ tubes, appressorium formation and penetration of the host. These parameters were assessed using one or more of the following three techniques: (1) epi-illumination fluorescence microscopy of fresh leaf tissues (FM), (2) light microscopy of leaf tissues cleared and stained in alcoholic lactophenol cotton blue (ALCB) and (3) polystyrene leaf surface replicas (PR).

Each leaf was cut into six pieces of equal length, three of which (1, 3 and 5) were coated with a thin layer of 5% polystyrene (from fragments of plastic Petri dish) in toluene (w:v). About 0.5 h later, the polystyrene peels were stained in 0.01% aniline blue (aqueous), washed and mounted in 50% glycerol for microscopic examination. The method is adapted from Lingappa and Lockwood (1963) using a modified polystyrene mixture (J.L. Lockwood, personal communication). The remaining pieces of leaf tissue were mounted fresh in 50% glycerol, examined microscopically with transmitted and fluorescent (FM) light and/or cleared and stained in ALCB (Shipton and Brown, 1962), washed in water and mounted in 50% glycerol for microscopic examination.

Germination was considered to have taken place when either an appressorium had formed or a germ tube with a length greater than the conidium width could be seen protruding from the conidium.

Penetration was assessed by one or more of the three methods. When using FM, the presence or absence of a fluorescent spot, in the cell or cell wall beneath 100 appressoria was recorded on each of three leaf pieces per leaf. In ALCB-treated tissues, the presence of an infection hypha in the host cell beneath an appressorium indicated successful penetration. The presence of a distinct hole in the appressorium, observed in appressoria on PR preparations, was used on one occasion as a check for the amount of penetration recorded in FM. Although FM is not a direct measure of pathogen penetration but simply an indicator of pathogen activity, it enabled rapid assessment of activity beneath appressoria.

The significance of differences in the development of *C. graminicola* on leaves in the presence and absence of saprophytic yeasts was analysed using the Wilcoxon two sample test (Sokal and Rohlf, 1969).

Assessment of disease. The amount of disease was assessed by counting the number of the lesions per cm^2 in non-necrotic (NN) leaf tissue (Fig. 2) of attached leaves 6 to 10 days after inoculation with the pathogen. In experiment 1, lesion density was determined from 10 randomly selected 1 cm^2 quadrats, whereas in all other experiments assessment involved counting the number of lesions in 1 cm^2 quadrats at three sites (A, B, C) on either side of the leaf midrib in the non-necrotic area of the leaf (Fig. 2). At the end of each experiment leaves were collected and the whole leaf and its necrotic area were traced out on transparent tracing paper.

The amount of necrosis was determined by measuring the necrotic leaf area or the percentage of the leaf length shrivelled (S). S was determined using the following index (Fig. 2):

$$S = \frac{FS + (PS/2)}{N + NN} \times 100$$

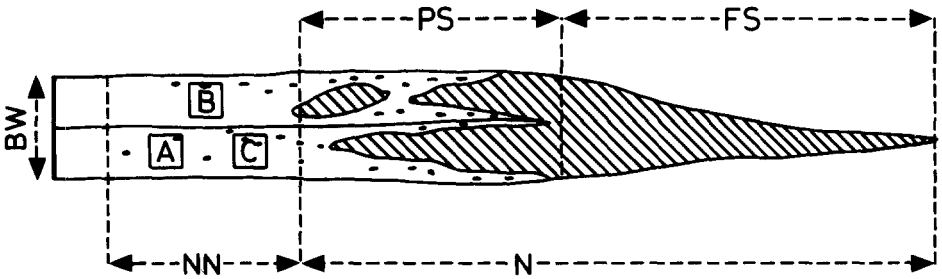

Fig. 2. Diagram illustrating the positions of the lesion density quadrats A, B and C in the non-necrotic area of the leaf (NN) and the components of the infected leaf used for calculating the percentage of the leaf length shrivelled in the necrotic area of the leaf (N), where BW is the basal width of the leaf (for details see text), PS is the length of the leaf partially shrivelled and FS the length of the leaf fully shrivelled.

where FS is the length of the leaf which is fully shrivelled, PS is the leaf length which is partially shrivelled, N is the length of the necrotic part of the leaf (FS + PS = N), NN is the length of the non-necrotic part of the leaf, N + NN is the treated length of the leaf.

As it is impossible to accurately measure the necrotic area of the leaf when it is dead and shrivelled, the necrotic leaf area was determined by measuring the non-necrotic area of each treated leaf with a planimeter and subtracting this value from the area of a template leaf with the same basal width (BW) and leaf length as the treated leaf. Template leaves were leaves obtained from plants raised and treated in the same manner as the controls in the experiments except that they were not inoculated with the pathogen. When these leaves were harvested (after 8 to 13 days according to the duration of the experiment) the outlines of the leaves were traced on tracing paper giving a template of each leaf. The basal width of the treated area of each leaf was marked onto the template, this point being the line marked onto the leaf with a felt-tipped pen at the beginning of the experiment. Necrosis was expressed as a percentage of the area of the template leaf. The Wilcoxon two sample test (Sokal and Rohlf, 1969) was used to analyse the significance of the differences in lesion number and necrosis of the treated and control leaves.

Results

The effect of yeasts on infection. In all experiments, the amount of necrosis expressed as percentage necrotic area or percentage leaf length shrivelled, was about 50% less (Table 1) and lesion density about 65% less on yeast-treated leaves than on control leaves (Table 2). Analyses of fungal population densities (Table 3) showed that yeast-treated leaves had an established population of *C. laurentii* var. *flavescens* and *S. roseus* of at least 50 000 CFU per cm^2 leaf. Examination of leaf surface replicas and cleared leaf tissues showed that most yeasts grew in the grooves formed at the junctions of the anticlinal walls of the epidermal cells.

Because fungi, including *Alternaria, Cladosporium* and *Penicillium* species, were present in variable but approximately equal densities on both the control and yeast-

Table 1. Effect of *S. roseus* and *C. laurentii* var. *flavescens* on necrosis[1] of maize leaves[2] following infection by *C. graminicola.*

Exp.	Days post inoculation	n	Average necrosis ± sd				% reduction	
			control[3]		+ yeasts[4]			
			NA	S	NA	S	NA	S
1	10	5	36 ± 17	46 ± 24	19 ± 16*[5]	26 ± 29*	47	43
2	8	12	73 ± 26	–[6]	33 ± 27*	–	55	–
3	7	24	–	46 ± 34	–	22 ± 27**	–	52
4	7	10	–	57 ± 27	–	22 ± 12**	–	61

[1] Necrosis is expressed as a percentage of necrotic leaf area (NA) or as a percentage of the leaf length shrivelled (S).
[2] The fourth-formed leaf of 3-week-old plants grown in the glasshouse.
[3] Control leaves were sprayed with 0.01% Tween 80 solution, 2 to 3 days prior to pathogen inoculation.
[4] + Yeasts leaves were sprayed with a mixture: (1 : 1) of *S. roseus* and *C. laurentii* var. *flavescens* in 0.01% Tween 80 with a total yeast concentration of 2×10^7 cells per ml, 2 to 3 days prior to pathogen inoculation.
[5] $P < 0.05$, ** $P < 0.01$, levels of significance for the differences in necrosis between yeasts-treated and control leaves.
[6] – = not determined.

Table 2. Effect of *S. roseus* and *C. laurentii* var. *flavescens* on the density of *C. graminicola* lesions on maize leaves[1]

Exp.	Days post inoculation	n	Lesion number cm^{-2} ± sd		% reduction
			control[2]	yeasts[3]	
1	10	5	18 ± 1	2 ± 1*[4]	89
3	7	24	15 ± 4	7 ± 3**	53
4	5	10	27 ± 10	10 ± 4**	63

[1] See [2] Table 1.
[2, 3] See [3] and [4] Table 1, respectively.
[4] * $P < 0.05$, ** $P < 0.01$, levels of significance for the differences in lesion number per cm^2 between the yeast-treated and control leaves.

treated leaves in most experiments, it is not likely that these organisms interfered with the experiments. Although larger populations of bacteria were found on the yeast-treated leaves, these differences in population density were not significant in three of the four experiments. The highest density recorded was low when compared to the densities required for antagonism by bacteria (Sleesman and Leben, 1976).

Effect of yeasts on pre-penetration development of C. graminicola. After *C. graminicola* conidia had been sprayed onto leaves, germination began within a few

Table 3. Average population densities of saprophytes on five yeast-treated leaves[1] and five control leaves[1] at the time of pathogen inoculation[2].

Exp.	Treatment	Number of colony forming units ($\times 10^3$) cm^{-2} ± sd			
		S. roseus	*C. laurentii* var. *flavescens*	other fungi	bacteria
1	control[3]	<1	<1	<1	82 ± 14
	+ yeasts[4]	294 ± 100	489 ± 186	16 ± 11	110 ± 30
2	control	<1	<1	24 ± 20	10 ± 15
	+ yeasts	81 ± 30	120 ± 52	16 ± 22	67 ± 8
3	control	6 ± 7	4 ± 5	15 ± 5	6 ± 8
	+ yeasts	21 ± 6	30 ± 8	24 ± 16	19 ± 6
4	control	<1	2 ± 5	<1	2 ± 2
	+ yeasts	118 ± 35	6 ± 6	<1	2 ± 1

[1] The fourth-formed leaf of 3-wk-old plants grown in the glasshouse and incubated in a climate cabinet for two to three days after yeast treatment or control treatment with water.
[2] The leaves were inoculated with the pathogen on the same day that five other leaves from each treatment were sampled for the estimation of saprophyte population densities.
[3, 4] See Table 1.

Table 4. Effect of *S. roseus* and *C. laurentii* var. *flavescens* on the pre-penetration development of *C. graminicola* on maize leaves.

Exp.[1]	Hours[2]	Observ[3].	Percentage ± sd			
			conidial germination		appressorium formation[6]	
			control[4]	+ yeasts[5]	control[4]	+ yeasts[5]
1	96	1 × 50/5	98 ± 1	98 ± 2	100 ± 0	100 ± 0
2	9	1 × 100/5	77 ± 12	80 ± 6	89 ± 8	87 ± 9
	36	6 × 50/5	97 ± 2	97 ± 1	100 ± 0	100 ± 1
3	48	3 × 50/6	85 ± 9	94 ± 3	–[7]	–
4	48	3 × 100/5	99 ± 0	98 ± 2	100 ± 0	100 ± 0

[1] Measurements made from tissues of the fourth-formed leaf cleared and stained in alcoholic lactophenol cotton blue (Exps 1 and 4) and from polystyrene replicas of the surface (Exps 2 and 3).
[2] Hours post inoculation.
[3] Number of observations, where 1 × 50/5 = 1 sample of 50 conidia from each of 5 leaves.
[4, 5] See [3] and [4] Table 1 respectively.
[6] Percentage of germinated conidia with appressoria.
[7] – = not determined.

hours and usually resulted in the formation of sessile appressoria. Rarely was more than one appressorium formed per conidium. Most appressoria were produced in the grooves at the junctions of the epidermal cells where the yeasts also grew, a response apparently determined by leaf topography (Lapp and Skoropad, 1978).

Irrespective of when measurements were made between 9 h and 96 h post inoculation, neither conidial germination nor appressorium formation was affected by the presence of the yeasts (Table 4). Therefore, the reduction in necrosis cannot be explained by a negative effect of yeasts on the pre-penetration development of the pathogen.

Effect of yeasts on penetration from appressoria, lesion formation and necrosis. The data presented in Table 5 clearly show that the penetration of leaf tissue from appressoria of *C. graminicola* was affected by the presence of yeasts. Penetration from *C. graminicola* appressoria was reduced by half in the presence of the mixed population of *C. laurentii* var. *flavescens* and *S. roseus*. Furthermore, these saprophytes did not simply delay penetration, because measurements made four days after inoculation (Exp. 1, Table 5) correspond to the measurements made at earlier times (Exps 2 and 4, Table 5). After having successfully penetrated the epidermal cell of the host, the infection hyphae ramified through neighbouring epidermal cells and underlying mesophyll cells, causing death of cells which could be observed in a few days as small, translucent flecks in the maize leaf.

Ultimately the maize leaves became necrotic. The percentage reduction in penetration from appressoria (Table 5) was similar to the percentage reduction in lesion density in the presence of yeasts (Table 2). Subsequently the lesions coalesced to form larger necrotic areas whose final size remained reduced in the presence of yeasts and similar in degree (Table 1) to the reduction in lesion density.

Table 5. Antagonistic effect of *S. roseus* and *C. laurentii* var. *flavescens* on penetration from appressoria of *C. graminicola* on maize leaves[1].

Exp.	Prep[2].	Hours[3]	Observ[4].	% Penetration ± sd		% Reduction
				control[5]	+ yeasts[6]	
1	ALCB	96	1 × 50/5	95 ± 3	45 ± 4**[7]	53
2	PR	36	6 × 50/4	91 ± 3	43 ± 2*	53
	FM	48	3 × 100/6	86 ± 6	45 ± 4**	48
4	FM	28	3 × 100/5	51 ± 4	25 ± 4**	51
	ALCB	48	3 × 100/5	63 ± 7	29 ± 4**	54

[1] See [2] Tabel 1.

[2] Preparation type: ALBC = tissues cleared in alcoholic lactophenol cotton blue, PR = polystyrene replicas of the leaf surface and FM = fresh tissues examined with epi-illumination fluorescence microscopy (see text for details).

[3] Hours post pathogen inoculation.

[4] Number of observations, where 1 × 50/5 = 1 sample of 50 conidia from each of 5 leaves.

[5, 6] See [3] and [4] Table 1, respectively.

[7] * $P<0.05$, ** $P<0.01$, levels of significance for the differences in penetration from appressoria between yeast-treated and control leaves.

The formation of large necrotic areas was more rapid at the leaf tip than at the leaf base (youngest tissue). As found in the preliminary experiments with leaves of different ages (Fig. 1), in older tissues necrosis was always more extensive than in younger tissues.

Discussion

In the experiments described above, infection was reduced by about 50% in the presence of the common phyllosphere yeasts *S. roseus* and *C. laurentii* var. *flavescens*. This reduction in necrosis is comparable to the levels obtained in the antagonism of phyllosphere saprophytes against other cereal leaf necrotrophs (Blakeman and Fokkema, 1982). In many instances, the antagonistic effect of yeasts on infection could be explained by a reduction in both conidial germination and superficial prepenetration growth of the pathogen, which resulted in fewer infection courts (Fokkema, 1981). However, in the yeast-*C. graminicola* interaction studied, as penetration from appressoria was the only stage in the development of the pathogen that was affected, inhibition of this stage must have been responsible for the reduction in infection. In contrast to interactions between bacteria and *Colletotrichum* spp. (Lenné and Parbery, 1976; Blakeman and Brodie, 1977; Blakeman and Parbery, 1977), the number of appressoria formed was not increased. Other experiments (M.A. Williamson, unpublished results) have demonstrated that added nutrients stimulated the superficial mycelial growth of *C. graminicola*. Occasionally, however, this caused a delay in the formation of appressoria whose number eventually doubled in the presence of nutrients, resulting in more infection. This demonstrates that the number of appressoria should not be assessed too early, but only when hyphal growth has stopped. Failure to do so may explain contradictory results in literature.

The halving of the number of successful penetrations from appressoria on the yeast-treated leaves suggests that the yeasts affected appressorium germination and/or the emergence and extension of the penetration peg. The yeasts used in this study have antagonized other necrotrophic pathogens by competition for exogenous and endogenous nutrients, with no indication of an in vivo production of toxic metabolites or an induced host resistance (Blakeman and Fokkema, 1982). Using radio-isotopes Blakeman and Brodie (1976, 1977) demonstrated that leaf bacteria competed for both exogenous nutrients and leaked endogenous nutrients from *Botrytis cinerea* conidia and that a negative relationship existed between amino acid uptake by bacteria and *Sporobolomyces roseus* and the germination of *B. cinerea* conidia. With in vitro experiments Fokkema (1984) showed that the germination of *Cochliobolus sativus* conidia, which was strongly inhibited in the presence of yeasts, could be restored by transferring the *C. sativus* conidia to a yeast-free environment. This indicates that yeasts may act as a nutrient sink, creating nutrient stress in the conidia. The same stresses are likely to be experienced by germinating appressoria.

In both control and yeast treatments, conidia germinated and formed sessile appressoria which suggests that the requisites for mycelial growth were already limited on glasshouse-grown maize leaves. This explanation is supported by the prolific growth response of mycelium on the leaf surface when nutrients were added (M.A. Williamson, unpublished results). *C. graminicola* may need some exogenous nutrients or endogenous reserves from the conidium or appressorium for penetrating the host.

There was no visual indication that yeasts inhibited melanin biosynthesis which could have explained the reduced penetration (Wolkow et al., 1983).

Politis and Wheeler (1973), in their ultrastructural study of infection of maize leaves by *C. graminicola,* found that when appressoria germinated the outer electron-dense wall of the appressorium in the area attached to the host epidermis disappeared and a new electron-lucid wall was formed in the basal area of the appressorium. They suggested that enzymes bound to the lomasome-like structures, found only in this area, may facilitate the dissolution of the electron-dense wall of the appressorium and assist in the penetration of the epidermal wall of the host. Suzuki et al. (1981, 1982) reported that appressoria of *C. lagenarium* formed in the presence of cycloheximide were unable to penetrate nitrocellulose membranes, probably because of a disturbance in cellulase production. Recently, Kolattukudy (1984) demonstrated that leaves of corn seedlings were well protected against infection by *C. graminicola* when the inoculum was applied with the cutinase inhibitors diethyl trichloropyridyl phosphate or hinosan. Their results indicate the importance of cutinase in the infection process of *C. graminicola.*

Our study demonstrates that in the yeast-*C. graminicola* interaction, the main stage of the infection process affected was the penetration from appressoria. In view of the possible involvement of enzymes such as cellulase and cutinase in the penetration process, it is possible that a nutrient sink created by the yeasts may affect energy-requiring processes such as the pathogen's enzyme production. Further experiments in this direction are currently being carried out.

In the field, it is likely that naturally occurring yeast populations may have a moderating effect on maize anthracnose, especially in conjuction with selected fungicides (Fokkema and De Nooij, 1981) which permit the normal growth of saprophytes. As yeasts exhibit dessication tolerance, they offer greater potential as naturally occurring control agents than do bacteria (Bashi and Fokkema, 1977; Leben et al., 1965).

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Samenvatting

Fyllosfeergisten remmen de penetratie vanuit appressoria en de daaropvolgende infectie van maisbladeren door Colletotrichum graminicola

In klimaatkastexperimenten werden maisbladeren (4e blad) twee-drie dagen voor inoculatie met *Colletotrichum graminicola* bespoten met een mengsel (1:1) van de algemeen voorkomende fyllosfeergisten *Sporobolomyces roseus* en *Cryptococcus laurentii* var. *flavescens.* In vier experimenten varieerde de gemiddelde gistpopulatie op de behandelde bladeren, op het moment van inoculatie met het pathogen, van 5×10^4 tot 8×10^5 cellen cm^{-2} blad, op de onbehandelde bladeren van $< 10^3$ tot 10^4 cellen cm^{-2} blad. De gisten reduceerden de lesiedichtheid en het necrotisch bladoppervlak tengevolge van de *C. graminicola* infectie voor ongeveer 50%. De stadia in de ontwik-

keling van andere necrotrofe pathogenen, die gewoonlijk gevoelig zijn voor antagonisme door gisten, zoals sporekieming, oppervlakkige myceliumgroei en vorming van appressoria, werden bij *C. graminicola* niet beïnvloed. De waargenomen reductie van infectie kon alleen verklaard worden door een remming van de penetratie vanuit normaal gevormde appressoria. Interactie in dit stadium van het infectieproces is nog niet eerder waargenomen.

References

Bashi, E. & Fokkema, N.J., 1977. Environmental factors limiting growth of *Sporobolomyces roseus,* an antagonist of *Cochliobolus sativus,* on wheat leaves. Trans. Br. Mycol. Soc. 68: 17-25.

Blakeman, J.P. & Brodie, I.D.S., 1976. Inhibition of pathogens by epiphytic bacteria on aerial plant surfaces. In: Dickinson, C.H. & Preece, T.F. (Eds), Microbiology of aerial plant surfaces. Academic Press, London. p. 529-557.

Blakeman, J.P. & Brodie, I.D.S., 1977. Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetrot leaves. Physiol. Pl. Path. 10: 29-42.

Blakeman, J.P. & Fokkema, N.J., 1982. Potential for biological control of plant diseases on the phylloplane. A. Rev. Phytopath. 20: 167-192.

Blakeman, J.P. & Parbery, D.G., 1977. Stimulation of appressorium formation in *Colletotrichum acutatum* by phylloplane bacteria. Physiol. Pl. Pathol. 11: 313-325.

Firman, I.D. & Waller, J.M., 1977. Coffee berry disease and other *Colletrotrichum* diseases of coffee. Phytopath. Pap. 20: 1-53.

Fokkema, N.J., 1981. Fungal leaf saprophytes, beneficial of detrimental? In: Blakeman, J.P. (Ed.), Microbial ecology of the phylloplane. Academic Press, London. p. 433-454.

Fokkema, N.J., 1984. Competition for endogenous and exogenous nutrients between *Sporobolomyces roseus* and *Cochliobolus sativus.* Can. J. Bot. 62: 2463-2468.

Fokkema, N.J., Houter, J.G. den, Kosterman, Y.J.C. & Nelis, A.L., 1979. Manipulation of yeasts of field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum.* Trans Br. Mycol. Soc. 72: 19-29.

Fokkema, N.J. & Nooij, M.P. de, 1981. The effects of fungicides on the microbial balance in the phyllosphere. EPPO Bull. 11: 303-310.

Kolattukudy, P.E., 1984. Fungal penetration of defensive barriers of plants. In: Duggar, W.M. & Bartnicki-Garcia, S. (Eds), Structure, function and biosynthesis of plant cell walls. Proc. Seventh. Symp. Bot., Univ. Calif. Riverside p. 302-343.

Lapp, M.S. & Skoropad, W.P., 1978. Location of appressoria of *Colletotrichum graminicola* on natural and artificial barley leaf surfaces. Trans. Br. Mycol. Soc. 70: 225-228.

Leben, C., 1964. Influence of bacteria from healthy cucumber leaves on two leaf diseases of cucumber. Phytopathology 54: 405-408.

Leben, C., Daft, G.C., Wilson, J.D. & Winter, H.F., 1965. Field tests for disease control with an epiphytic bacterium. Phytopathology 55: 1375-1376.

Lenné, J.M. & Parbery, D.G., 1976. Phyllosphere antagonists and appressorium formation in *Colletotrichum gloeosporioides.* Trans. Br. Mycol. Soc. 66: 334-336.

Lingappa, B.T. & Lockwood, J.L., 1963. Direct assay of soils for fungistasis. Phytopathology 53: 529-531.

Mercer, P.R., Wood, K.S. & Greenwood, A.D., 1970. The effect of orange extract and other extracts on the anthracnose of French beans by *Colletotrichum lindemuthianum.* Ann. Bot. 34: 593-604.

Politis, D.J., & Wheeler, H., 1973. Ultrastructural study of penetration of maize leaves by *Colletotrichum graminicola.* Physiol. Pl. Path. 3: 465-471.

Shipton, W.A. & Brown, J.F., 1962. A whole leaf clearing technique to demonstrate host-pathogen relationships of wheat stem rust. Phytopathology 52: 1313.
Sleesman, J.P. & Leben, C., 1976. Microbial antagonists of *Bipolaris maydis.* Phytopathology 66: 1214-1218.
Sokal, R.R. & Rohlf, F.J., 1969. Biometry. W.H. Freeman and Co., San Francisco.
Straka, R.P. & Stokes, J.L., 1957. Rapid destruction of bacteria in commonly used dilutants and its elimination. Appl. Microbiol. 5: 21-25.
Suzuki, K., Furusawa, I., Ishida, N. & Yamamoto, M., 1981. Protein synthesis during germination and appressorium formation of *Colletotrichum lagenarium* spores. J. Gen. Microbiol. 124: 61-69.
Suzuki, K., Furusawa, I., Ishida, N. & Yamamoto, M., 1982. Chemical dissolution of cellulose membranes as a prerequisite for penetration from appressoria of *Colletotrichum lagenarium.* J. Gen. Microbiol. 128: 1035-1039.
Wolkow, P.M., Sisler, H.D. & Vigil, E.L., 1983. Effects of inhibitors of melanin biosynthesis on structure and function of appressoria of *Colletotrichum lindemuthianum.* Physiol. Pl. Path. 23: 55-71.