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Antagonism of yeastlike phyllosphere fungi against Septoria nodorum on wheat leaves

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Abstract

Aureobasidium pullulans, *Sporobolomyces roseus*, and *Cryptococcus laurentii* var. *flavescens*, added to the inoculum, reduced the superficial mycelial growth of *Septoria nodorum* and the infection of wheat leaves by 50% or more. The mycelial growth was affected similarly in vitro, on slides covered with water agar. The antagonistic effect on germination was slight. The concentration of the saprophytes on the leaves after inoculation was comparable to population densities occurring on field-grown wheat.

Introduction

The saprophytic leaf mycoflora of anemophilous plants can considerably reduce the stimulatory effect of pollen on perthotrophic pathogens (Warren, 1972; Fokkema, 1973; Fokkema, et al., 1975). Antagonism by phyllosphere fungi is not limited to a reduction of stimulation by pollen, since, in the absence of pollen, infection of onion leaves by *Alternaria porri* and *Botrytis cinerea* was reduced by *Aureobasidium pullulans* and *Sporobolomyces roseus* in a similar way (Fokkema and Lorbeer, 1974). To elucidate the mechanism of antagonism and to examine the possibilities for biological control in the field, similar experiments were performed with the pathogen *Septoria nodorum* Berk. on spring wheat, *Triticum aestivum* L. 'Jufy'. Results of preliminary indoor 'mixed-inoculation' experiments are presented here.

Materials and methods

Sixteen penultimate leaves were inoculated with a mixture of cells of either *Aureobasidium pullulans* (De Bary) Arnaud, *Sporobolomyces roseus* Kluyver at van Niel, or *Cryptococcus laurentii* var. *flavescens* Lodder et van Rij (each in a concentration of $3 \times 10^7 - 10^8$ cells/ml) and of conidia of *S. nodorum* ($10^7 - 10^8$ cells/ml). The saprophytes were grown for 14 days on PDA slants in darkness at 22°C. The pathogen was grown on oatmeal agar slants under normal light conditions at room temperature. As control, 16 leaves were inoculated with only *S. nodorum*. The leaves, which remained attached to the plants, were inoculated and incubated as described earlier (Fokkema, 1973). During incubation the temperature varied from 15–20°C and relative humidity from 90–95%. The inoculated leaves were wetted with a mist of water at the end of each afternoon. After 3 days incubation 8 leaves of each group were collected. A part of each leaf was used for examining the spore germination and the superficial mycelium of the pathogen (Fokkema, 1971). The remaining leaf parts were

individually shaken in water and the suspensions plated on agar to determine the saprophytic colonization (Fokkema, 1971). After 8–9 days incubation, infection of the remaining leaves was determined by measuring the necrotic leaf area with a planimeter.

Results and discussion

The superficial mycelial growth and the degree of necrosis were significantly reduced with all saprophytes tested (Table 1). Germination, however, was less affected. Though a significant effect on germination shortly after inoculation may not be excluded, such an effect is apparently easily overcome. In several other experiments similar results were obtained, however, the antagonistic effect was sometimes less pronounced unless there was a delay of 3–6 days between the application of the antagonist and the inoculation with *S. nodorum*. The reduction of infection can be explained by the decrease in the superficial mycelial growth, which is probably due to nutrient competition, consequently reducing the number of penetration sites. This is in agreement with previous observations (Fokkema, 1973; Fokkema and Lorbeer, 1974). When the mixed-inoculum was applied to slides coated with water-agar (Fokkema, 1971), the mycelial growth of *S. nodorum* was similarly reduced (Table 2).

In presence of rye pollen (30 mg per ml inoculum), the saprophytes did not reduce mycelial growth or necrosis significantly. This is in contrast with experiments with *Cochliobolus sativus* as a pathogen on rye leaves (Fokkema, 1973). Since, in absence of pollen, the *Septoria* infection on wheat was already very high compared to the *Cochliobolus* infection on rye, addition of pollen to the wheat leaves apparently created a nutrient supply sufficient for optimal development of both *S. nodorum* and the saprophytes.

The population densities of the saprophytes on the inoculated leaves compared favourably with those on the flag leaves of field-grown wheat (Table 3). In the field, the

Table 1. Effect of yeastlike phyllosphere fungi on the prepenetration development of *Septoria nodorum* on wheat leaves, 3 days after inoculation, and on the subsequent necrosis, 9 days after inoculation. Mean results of two experiments.

Treatment	Saprophytic colonization (propagules $\times 10^3$ per cm^2)		Number of germ tubes per 100 spores		Superficial mycelium ($\mu m/mm^2$)		Necrotic leaf area (%)	
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
water (control)	–	–	167	119	3400	13800	71	62
Aureobasidium pullulans	13	10	159	127	1700*	3200*	12*	26*
Sporobolomyces roseus	30	8	142	81	1200*	2000*	36*	12*
Cryptococcus laurentii	32	13	143	79	800*	2200*	17*	32

* $P \leq 0.05$, level of significance for the differences with the control treatment.

Tabel 1. Effect van fyllosfeergisten op de prepenetratie-ontwikkeling van Septoria nodorum op tarwebladeren, 3 dagen na inoculatie, en op de daaropvolgende necrose, 9 dagen na inoculatie. Gemiddelde resultaten van twee experimenten.

Table 2. Effect of yeastlike phyllosphere fungi on spore germination and mycelial growth of *Septoria nodorum* on agar-coated slides. Mean results (n = 8) of two experiments.

Treatment	Number of germtubes per 100 spores		Mycelium density ($\mu m/mm^2$)	
	exp. 1	exp. 2	exp. 1	exp. 2
water (control)	144	127	36400	55000
A. pullulans	120	97	15000*	40700
S. roseus	116	101	16200*	27100*
C. laurentii	121	106	19500 *	38600*

* $P \leqslant 0.05$, level of significance for the differences with the control treatment.

Tabel 2. Effect van gistachtige fyllosfeerschimmels op sporekieming en myceliumgroei van Septoria nodorum op met agar bedekte objectglaasjes. Gemiddelde resultaten (n = 8) van twee experimenten.

Table 3. Development of the saprophytic microflora on the flag leaves of field-grown wheat during July 1974. Mean results, assessed by cultural methods (Fokkema, 1971), of 8 leaves per sample.

Micro-organism	Number of propagules × 10^3 per cm^2 of leaf surface			
	5 July	12 July	19 July	30 July
Aureobasidium pullulans	3	3	2	1
Sporobolomyces spp.	40	48	48	33
'white yeasts'[1]	37	91	60	58
Cladosporium spp.	10	13	23	11
Bacteria	140	368	561	301

[1] Mainly *Cryptococcus* spp.

Tabel 3. Ontwikkeling van de saprofytische microflora van het vlagblad van tarwe gedurende juli 1974. Gemiddelde resultaten, verkregen met behulp van cultuurmethodes (Fokkema, 1971), van 8 bladeren per monster.

saprophytic mycoflora of wheat leaves may have a buffering effect on *Septoria* infection similar to that demonstrated on rye leaves with pollen against infection by *Cochliobolus sativus* (Fokkema et al., 1975).

Samenvatting

Antagonisme van gistachtige fyllosfeerschimmels tegen Septoria nodorum op tarwebladeren

Aureobasidium pullulans, *Sporobolomyces roseus* en *Cryptococcus laurentii* var. *flavescens* toegevoegd aan een conidiënsuspensie van *Septoria nodorum* verminderden de oppervlakkige myceliumgroei van *Septoria* en de infectie van de bladeren tot de helft of meer (Tabel 1). Het effect op de sporekieming was gering. In vitro, op zgn. agarglaasjes, werd de myceliumgroei op vergelijkbare wijze geremd (Tabel 2). De concentratie van de saprofyten na inoculatie kwam overeen met in het veld voorkomende populatiedichtheden (Tabel 3).

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