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Effect of seventeen fungicolous fungi on sporulation of cucumber powdery mildew

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Abstract

Nineteen isolates of 17 different fungal species thriving upon other fungi were tested for their ability to control sporulation of cucumber powdery mildew, *Sphaerotheca fuliginea*. More than half of the fungi reduced the number of healthy conidiophores to less than 10%. *Tilletiopsis albescens* was superior to *Ampelomyces quisqualis*. Three species, viz. *A. quisqualis*, *Aphanocladium album* and *T. albescens*, were selected for further greenhouse experiments.

Additional keywords: biological control, hyperparasites, *Sphaerotheca fuliginea*.

Introduction

Ampelomyces quisqualis has long been known as a hyperparasite of powdery mildews (Blumer, 1967). Various attempts have been made to use this fungus in the biological control of cucumber powdery mildew (Sundheim, 1982; Philipp et al., 1984). However, parasitism of powdery mildews by other fungi seems to be more widespread than previously assumed (Hijwegen and Buchenauer, 1984). Recently, biological control of cucumber powdery mildew by *Tilletiopsis minor* was reported (Hijwegen, 1986). This raised the question whether or not there might be other, more effective parasites. Each year long lists of new mycoparasites are published (e.g. Commonwealth Mycological Institute, 1986). Most mycoparasites also do not seem te be very specialized. However, they sometimes loose parasitic activity rather rapidly in culture (Hijwegen, unpublished results). Experiments were therefore performed using fresh isolates of fungi thriving upon other fungi isolated during the autumn of 1985. This paper presents the results of a comparison of the effectivity of these isolates against cucumber powdery mildew studied under optimal conditions for cucumber growing.

Materials and methods

Inoculation of plants with powdery mildew. Four-week-old cucumber plants, *Cucumis sativus* L. cv. Lange Gele Tros, with three to four expanding leaves were inoculated with cucumber powdery mildew, *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll., by dusting with conidia from infected leaves inoculated 2 weeks earlier. They were subsequently transferred to a climate cabinet under an alternating day/night temperature regime of

23/17 °C with a relative humidity of 75% during daytime and 90% at night. Light (HPL 340, 6 Klux) was given during 12 hours per day.

Fungi. *Acremonium alternatum* Link: Fr. was obtained from Dr N.E. Malathrakis, Crete, Greece and *Ampelomyces quisqualis* Ces. from Dr L. Sundheim, Ås, Norway. *Tilletiopsis minor* Nyland strain B was isolated from *Erysiphe martii* Lév. and used previously (Hijwegen, 1986). All other fungi were freshly isolated in the autumn of 1985, viz. *Acremonium strictum* W. Gams from *Laccaria* sp.; *Aphanocladium album* (Preuss) W. Gams, *Peziza ostracoderma* Korf, *Tilletiopsis albescens* Gotzhale and *Verticillium lecanii* (Zimm.) Viégas strain II from *Sphaerotheca fuliginea*; *Calcarisporium arbuscula* Preuss from *Lactarius necator* (Bull. em Pers.: Fr.) Karsten; *Cladobotryum varium* Nees: Fr. from *Armillaria* sp.; *Paecilomyces farinosus* (Holm: Gray) A.H.S. Brown & G.Sm. from *Boletus* sp.; *Penicillium chrysogenum* Thom from *Gymnopilus penetrans* (Fr.: Fr.) Murr.; *Scopulariopsis brevicaulis* (Sacc.) Bain. from *Paxillus involutus* (Batsch) Fr.; *Sepedonium chrysospermum* (Bull.: Fr.) Link from *Boletus* sp.; *Sesquicillium candelabrum* (Bon.) W. Gams from *Collybia* sp.; *Tilletiopsis minor* Nyland strain W from *Erysiphe heraclei* (DC.) St.-Am.; *Trichoderma viride* Pers: Fr. from *Clavulinopsis helvola* (Fr.) Corner; *Verticillium lecanii* (Zimm.) Viégas strain I and *V. fungicola* (Preuss) Hassebr. var. *fungicola* from *Boletus* sp.

All fungi, except *T. minor* and *T. albescens*, were grown on malt agar slants (Oxoid CH 59) for two weeks at 22 °C in diffuse daylight. *T. albescens* and *T. minor* were grown in shake culture in 300-ml Erlenmeyer flasks containing 100 ml 2% malt extract (Oxoid L 39) and 0.2% mycological peptone (Oxoid L 40) in the dark for 8 days at 23 °C and used immediately.

High concentrations of conidia were obtained quite easily from most fungi. It was hardly possible, however, to obtain concentrations above 2×10^6 ml $^{-1}$ from *Cladobotryum varium* with very large two-celled conidia and *Sepedonium chrysospermum* with thick-walled aleuriospores and few thin-walled phialospores.

Inoculations with fungi. At 4 and 10 days after inoculation with powdery mildew, i.e. before sporulation of the mildew and when sporulation was abundant, respectively, the cucumber plants were sprayed with spores of different fungi in various concentrations.

Disease assessment. Disease incidence was assessed by estimating the percentage of apparently healthy conidiophores with conidia compared to the non-treated control, where leaves were covered for approximately 40% with powdery mildew, using a dissecting microscope six days after the sprayings before sporulation of the mildew or four days after the sprayings during sporulation, respectively. The same day all treated plants were sprayed with water and again examined four days later.

For every fungus applied two plants, each having three to four expanding leaves with several thousands of conidiophores, were examined. Percentages are based upon 2000 examined conidiophores per leaf.

In the first experiments nine hyperparasites chosen at random were compared within one experiment. In later experiments fungi giving the best results were matched against each other.

Results

In Table 1 the results are listed. Average as well as minimum and maximum effect is given as observed in the experiments. From these data it can be seen that more than half of the species listed reduced the proportion of healthy conidiophores of cucumber powdery mildew to less than 10% when sprayed during sporulation after rewetting.

Tilletiopsis albescens (Fig. 1) was the best in all treatments, giving almost complete control under the prevailing conditions, followed by *Ampelomyces quisqualis*. *Sesquicillium candelabrum* gave a very poor performance, followed by *Penicillium chrysogenum*. *Trichoderma viride* was ineffective on mycelium without conidiophores, but gave good control on sporulating mycelium, though applied at a concentration of 10^7 spores ml^{-1}. All other treatments showed intermediate effectivity. In most cases rewetting six or four days later greatly enhanced control.

Discussion

Ampelomyces quisqualis was applied in relatively low concentrations by Philipp et al. (1984). It was therefore tested in the present experiments at 2×10^6 spores ml^{-1} on non-sporulating mycelium. *Cladobotryum varium* and *Sepedonium chrysospermum* were tested at the same concentration. All other fungi were applied at a concentration of 10^7 spores ml^{-1} on non-sporulating mycelium.

Calcarisporium arbuscula, *Cladobotryum varium* and *Sepedonium chrysospermum*

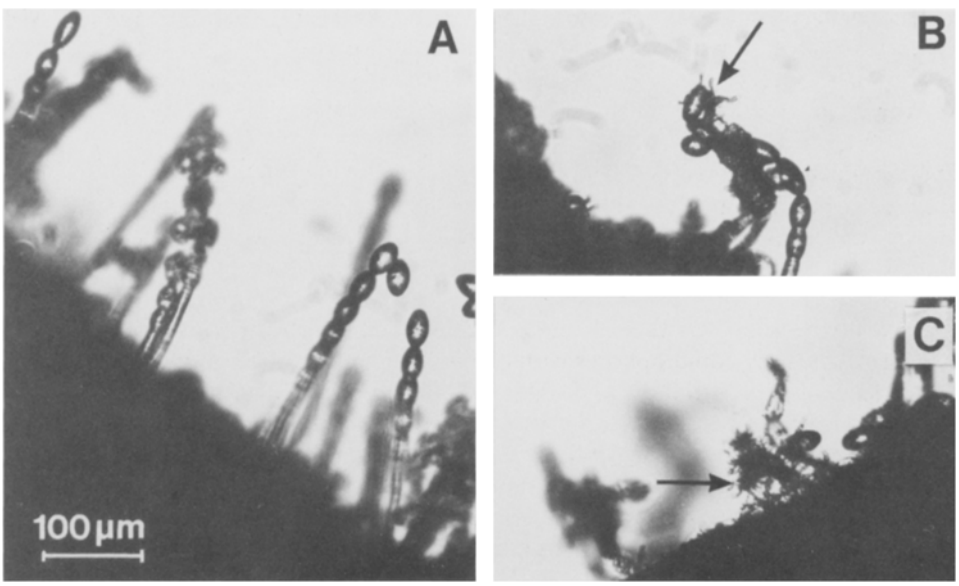

Fig. 1. Sporulating powdery mildew four days after treatment with *Tilletiopsis albescens*. A) control: healthy conidiophores; B) conidiophores slightly affected (will be counted as healthy); C) two conidiophores: one partly collapsed, the other barely recognizable, being completely overgrown with *T. albescens*. Note ballistospores of *T. albescens* (arrow in B and C).

Table 1. Effect of hyperparasites on the percentage of healthy conidiophores of cucumber powdery mildew when applied 4 days (A) or 10 days (B) after inoculation with *Sphaerotheca fuliginea*. First assessment was done 6 days (A) or 4 days (B) after application of the hyperparasite. Second assessment, after rewetting, followed 4 days later.

Hyperparasite applied	Application before sporulation (A)				Application during sporulation (B)			
	number of spores ml^{-1}	% healthy conidiophores[1] 1st assessment	% healthy conidiophores[1] 2nd assessment	number of experiments	number of spores ml^{-1}	% healthy conidiophores[1] 1st assessment	% healthy conidiophores[1] 2nd assessment	number of experiments
Tilletiopsis albescens	10^7	1 (0-2)	0	4	10^6	2 (1-3)	1 (0-2)	4
Ampelomyces quisqualis	2×10^6	10.5 (1-30)	0.5 (0-1)	4	10^6	20 (0-40)	2 (0-5)	6
Sepedonium chrysospermum	2×10^6	2 (1-3)	20 (15-25)	2	10^6	2 (2-2)	3 (1-5)	2
Cladobotryum varium	2×10^6	2.5 (0-5)	15 (10-20)	2	10^6	10 (10-10)	5 (0-10)	2
Paecilomyces farinosus	10^7	90 (90-90)	5 (0-10)	2	10^6	26 (1-50)	5 (0-10)	6
Tilletiopsis minor B	10^7	5 (5-5)	4 (3-5)	2	10^6	5.5 (1-10)	5 (0-10)	2
Tilletiopsis minor W	10^7	10 (10-10)	6 (2-10)	2	10^6	15 (10-20)	6 (2-10)	2
Aphanocladium album	10^7	10 (5-20)	5 (0-10)	4	5×10^6	0.5 (0-1)	1 (0-2)	4
Calcarisporium arbuscula	10^7	2 (1-3)	20 (15-25)	4	5×10^6	2 (0-5)	0.5 (0-1)	4
Verticillium lecanii I	10^7	1.5 (1-2)	10.5 (1-20)	2	5×10^6	2.5 (0-5)	0.5 (0-1)	2
Verticillium lecanii II	10^7	6 (2-10)	4 (2-5)	2	5×10^6	5 (5-5)	0.5 (0-1)	2
Acremonium alternatum	10^7	20 (10-40)	15 (10-20)	2	5×10^6	10 (0-30)	9 (0-15)	5
Acremonium strictum	10^7	6 (2-10)	8 (0-20)	4	5×10^6	10 (1-20)	13 (3-20)	4
Verticillium fungicola	10^7	12 (2-30)	24 (5-40)	4	5×10^6	15 (10-20)	12.5 (0-20)	4
Scopulariopsis brevicaulis	10^7	25 (5-40)	26 (5-60)	4	5×10^6	20 (2-40)	25 (2-60)	4
Peziza ostracoderma	10^7	6 (2-10)	6 (2-10)	2	5×10^6	15 (10-20)	50 (40-60)	2
Trichoderma viride	10^7	90 (80-100)	50 (40-60)	2	10^7	5.5 (1-10)	6 (2-10)	2
Penicillium chrysogenum	10^7	30 (20-40)	40 (40-40)	2	10^7	40 (40-40)	60 (60-60)	2
Sesquicillium candelabrum	10^7	85 (80-90)	80 (70-90)	6	10^7	100 (100-100)	90 (80-100)	4

[1] Figures represent mean values and are followed (in parentheses) by the variation between experiments.

gave good control on four-day-old mycelium, although the effect was short-lasting. Microscopic examination after spraying with water showed a considerable increase in powdery mildew development, probably due to new colonization. Possibly, labile toxins or enzymes are involved.

As noted before (Hijwegen, 1986) parasitic fungi usually were more effective on mycelium *with* than on mycelium *without* conidiophores. Thus at first spore concentrations of 10^6 ml^{-1} were applied to sporulating mycelium (first seven isolates in Table 1). In preliminary experiments, also *Aphanocladium album*, *Calcarisporium arbuscula* and *Verticillium lecanii* were first tested at a concentration of 10^6 spores ml^{-1}. However, efficacy was rather poor. Hence, concentrations of 5×10^6 spores ml^{-1} were chosen for these fungi.

Acremonium alternatum, which has been used for the control of cucumber powdery mildew (Malathrakis, 1985), was less effective than most other fungi, presumably because temperatures were below the optimum for effectivity, i.e. 27 °C (Malathrakis, 1985). In two experiments at 17 °C during night and day the performance was even poorer. However, in these experiments at 17 °C, *Sesquicillium candelabrum*, causing malformations to fleshy fungi in autumn, when temperatures are low, had greater effectivity than at 23/17 °C although not exceeding 70% control (30% healthy conidiophores).

In some cases treatments did not show sufficient effectivity within six or four days. However, after subsequent spraying with water, a much better control was achieved. Rewetting most probably was advantageous to the hyperparasites and disadvantageous to the powdery mildew, thus resulting in better control. Without rewetting powdery mildew tended to increase due to the formation of new healthy conidiophores. The fact that rewetting greatly enhanced the effectivity of treatments suggests that free moisture is very critical for germination and development of the hyperparasites.

This might also explain the great variation between experiments in some cases. This was especially conspicuous for *Paecilomyces farinosus*, *Ampelomyces quisqualis*, *Acremonium alternatum* and *Scopulariopsis brevicaulis*. Even after rewetting variation between experiments with *S. brevicaulis*, which gave 98-40% control of powdery mildew (2-60% healthy conidiophores), was great. An explanation for this phenomenon is not available.

As noticed before (Hijwegen and Buchenauer, 1984) many more fungi are able to thrive upon and control powdery mildews than have been reported. However, several fungi had certain disadvantageous characteristics and were therefore excluded from further experiments. The related fungi *Cladobotryum varium* and *Sepedonium chrysospermum* produce few conidia. Both isolates of *Tilletiopsis minor* were inferior to *Tilletiopsis albescens*. Plants treated with *Paecilomyces farinosus*, *Calcarisporium arbuscula* and *Verticillium lecanii* showed sometimes phytotoxic symptoms. This led to the selection of *Tilletiopsis albescens*, *Aphanocladium album* and *Ampelomyces quisqualis* for further experimentation in the greenhouse.

Samenvatting

Effect van zeventien mycoparasitaire schimmels op de sporulatie van komkommermeeldauw

Negentien isolaten behorend tot 17 verschillende schimmelsoorten werden getoetst op

hun bruikbaarheid voor de biologische bestrijding van komkommermeeldauw aan de hand van effecten op sporulatie. Meer dan de helft van de getoetste isolaten reduceerde het aantal gezonde conidioforen tot minder dan 10%. De werking van *Tilletiopsis albescens* was enigszins beter dan die van de meestal gebruikte hyperparasiet *Ampelomyces quisqualis*. Van de getoetste schimmels werden er drie, n.l. *A. quisqualis*, *Aphanocladium album* en *T. albescens*, geselecteerd voor kasproeven.

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