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Biological control of cucumber powdery mildew with Tilletiopsis minor under greenhouse conditions

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Abstract

In growth cabinet and greenhouse experiments the efficacy of *Tilletiopsis minor* in controlling cucumber powdery mildew decreased as humidity was lowered. This effect could be counteracted by formulation of the mycoparasite with lipophilic substances, like Hora Oleo 11E and lipids from milk. These formulations, without the mycoparasite, were also found to be deleterious to powdery mildew development. In the long run, however, formulations with *T. minor* gave better biological control of cucumber powdery mildew than formulations alone.

Additional keywords: mycoparasites, *Sphaerotheca fuliginea*, formulations, Hora Oleo 11E.

Introduction

Performance of *Tilletiopsis minor* Nyland in biological control of cucumber powdery mildew, *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll., under growth cabinet conditions has been published (Hijwegen, 1986, 1988). Other papers on the antagonism of *Tilletiopsis* species towards powdery mildews have appeared (Klecan et al., 1990; Knudsen and Skou, 1990; Hajlaoui and Bélanger, 1991). These reports all deal with biological control under rather artifical circumstances.

However, before biological control can be applied in practice under greenhouse conditions, the performance under these conditions has to be studied. This is the aim of the present investigation. A preliminary report has been published (Stout and Hijwegen, 1990).

Materials and methods

Inoculation of plants with powdery mildew. Four-week-old cucumber plants, *Cucumis sativus* L. cv Sporu, with three to four expanded leaves were inoculated with cucumber powdery mildew, *Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll., by dusting with conidia taken from infected leaves inoculated two weeks earlier.

Growth cabinet experiments. Inoculated plants were subsequently transferred to a growth cabinet under an alternating day/night temperature regime of 23/17 °C with a relative humidity of 75% during daytime and 90, 80 or 70% at night. Light (HPL 340, 6 klux at plant level) was given for 12 hours a day. Ten days after inoculation, when

sporulation was abundant, the cucumber plants were sprayed with spores of *Tilletiopsis minor* at concentrations of 10^6 or 5×10^6 spores/ml. Effects of treatments were assessed four days after spraying by estimating the percentage of apparently healthy conidiophores with conidia under a dissecting microscope. The same day all treated plants were sprayed with water and examined again four days later. For every treatment, two plants with three to four leaves were examined. The experiment was repeated three times.

Greenhouse experiments. Inoculated plants were transferred to a greenhouse where temperatures ranged from 16 to 35 °C (from 20 to 28 °C during 80% of the duration of the experiment) and relative humidity varied between 40 and 95% (between 60 and 80% during 80% of the time). At the onset of powdery mildew sporulation, after 6–7 days, plants were sprayed with *T. minor* at a concentration of 10^7 spores/ml in a) water, b) water to which Hora Oleo 11E, a formulated paraffin-oil, had been added at a final concentration of 0.5 or 0.1%, or c) coffee cream (Nutricia, containing 9% fatty substances, 9% protein and 13% carbohydrates) adjusted to a final concentration of 0.1% fatty substances.

Effects of treatments were assessed as mentioned above seven days later. Spraying and assessment was repeated twice at weekly intervals. For every treatment five plants were examined.

Cultivation of the fungus. *Tilletiopsis minor* was 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 eight days at 23 °C and used immediately (Hijwegen, 1988).

Statistics. Analysis of variance and tests for least significant difference (LSD) were used for comparison of data.

Results

Growth cabinet experiments. Results at 90% relative humidity and 10^6 spores/ml were in good agreement with those published previously (Hijwegen, 1988). After spraying with 5×10^6 spores/ml and rewetting, control was almost 100%. However, lowering the relative humidity to 80 or 70% at night immediately resulted in much poorer control even at 5×10^6 spores/ml (Table 1). Results of treatments below 80% relative humidity did not reach an acceptable level of powdery mildew control.

These results are similar to those obtained with *T. washingtonensis* on rose powdery mildew (Hajlaoui and Bélanger,1991) and *T. albescens* on cucumber powdery mildew (Knudsen and Skou,1990).

Greenhouse experiments. Performance of *T. minor* alone, even at a concentration of 10^7 spores/ml, was poor. The percentage of healthy conidiophores could only be reduced to about 60–70% of the control even after spraying three times at weekly intervals (Table 2). A better result was obtained when *T. minor* was formulated with 0.5 or 0.1% Hora Oleo 11E, primarily meant to enhance the microclimatic conditions for the mycoparasite, in accordance with results published by Philipp et al. (1990).

Table 1. Effects of treatments in a growth cabinet at various relative humidities (RH).

RH at night	% Healthy conidiophores*			
	10^6 spores/ml		5×10^6 spores/ml	
	I	II	I	II
90	25.0 a	2.5 a	2.5 a	0.1 a
80	40.0 ab	22.5 b	27.5 b	8.75 ab
70	50.0 b	30.0 b	35.5 b	15.0 b
LSD (0.05)	16.3	10.0	8.3	12.1

* Average of four experiments. I = first assessment, II = second assessment.
Values with different letters are significantly different at the $P \leqslant 0.05$ level.

Table 2. Effects of treatments in the greenhouse.

Treatment	% Healthy conidiophores*		
	I	II	III
T. minor	71.0 a	63.3 a	66.7 a
Hora Oleo 11E 0,1%	56.7 ab	18.3 b	21.7 b
Hora Oleo 11E 0,5%	40.0 ab	5.3 b	3.7 c
T. minor + Hora Oleo 11E 0,5%	43.3 ab	7.7 b	7.0 c
T. minor + Hora Oleo 11E 0,1%	40.0 ab	10.0 b	5.7 c
T. minor + coffee cream 0,1%	30.0 b	8.2 b	4.7 c
LSD (0.05)	33.0	24.5	12.9

* Average of three experiments. I = first assessment, II = second assessment, III = third assessment.
Values with different letters are significantly different at the $P \leqslant 0.05$ level.

These investigators demonstrated that 1% Hora Oleo 11E was effective in reducing the humidity demands of *Ampelomyces quisqualis* during infection, thus enhancing its parasitic performance on cucumber powdery mildew at low relative humidity.

Emulsions of Hora Oleo 11E alone gave good results on established cucumber powdery mildew infections that had not been in contact with the oil before, especially at the higher concentration. At the lower concentration *T. minor* plus Hora Oleo 11E gave significantly better results than Hora Oleo 11E alone. Moreover, it seemed that cucumber powdery mildew adapted rather rapidly to low concentrations of Hora Oleo 11E. Therefore, in one of the experiments, development of powdery mildew was followed on the eighth leaf, which had not been inoculated and could only become infested by secondary infections. Powdery mildew developed conspicuously on these leaves when sprayed with 0.5% Hora Oleo 11E alone in contrast to leaves sprayed with

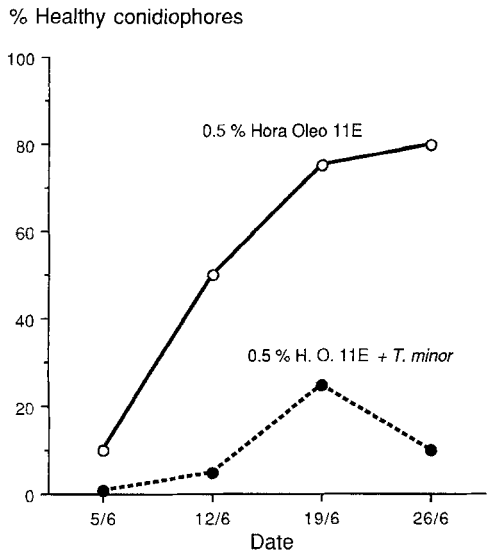

Fig. 1. Powdery mildew development on leaves with secondary infections, compared to the control (= 100%).

both the formulation and the mycoparasite (Fig. 1). A regression analysis of the differences in percentage healthy conidiophores with time between the two treatments was performed. The 95% confidence interval of the slope of the regression line was computed as [1.42, 3.84]. This shows that there is a significant difference between the two treatments. This result suggests that spraying with Hora Oleo 11E alone would not be sufficient for control in the long run.

Results with spore suspensions formulated with coffee cream did not differ significantly from those formulated with Hora Oleo 11E (Table 2).

Discussion

As had been anticipated, reduction of relative humidity immediately resulted in poorer control of powdery mildew, 15% of the conidiophores remaining healthy at 70/75% relative humidity and 5×10^6 spores/ml in the growth cabinet. Greenhouse performance was even poorer. Not less than 60–70% of the conidiophores remained healthy when only *T. minor* was applied. A slightly better result can be obtained by applying spore suspensions twice a week (Knudsen and Skou, 1990; Hijwegen, unpublished), an unacceptable procedure in practice. So, it is necessary to formulate the spore suspensions to obtain better control.

The most conspicuous result is the strong mildew-reducing capacity of the formulated paraffin oil alone. This has also been reported by Schmitz-Elsherif (1990) who used a formulated spore suspension of *Ampelomyces quisqualis*.

According to Calpouzos (1966), the disease-reducing capacity of oils is due not only to a direct deleterious influence on the pathogen but also to changes in the physiology of the host plant. Another reason might be the enhancement of the naturally occurring antagonistic microflora of the leaf. In some experiments in addition to spore suspensions of *T. minor* formulated with 0.1% coffee cream fatty substances, controls

without *T. minor* were applied. Since these emulsions contain lipids, proteins and carbohydrates, an abundant development of fungi, among which *Cladosporium sphaerospermum* and *Penicillium* spp. predominated, was observed with a concomitant reduction in powdery mildew development. Bacteria may also have been present.

Enhancement of natural antagonism after application of Hora Oleo 11E could also explain the reduction in parasitism by *A. quisqualis,* as found by Philipp et al.(1990) and Schmitz-Elsherif (1990).

Diluted coffee cream was chosen as a fatty emulsion that is completely biodegradable. Results with diluted coffee cream did not differ significantly from those obtained with Hora Oleo 11E. Plants treated in this way, however, tended to be slightly more chlorotic.

Results obtained in these short-term experiments seem to be promising. In future experiments, long-term effects on powdery mildew and on cucumber production have to be studied.

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