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Biological control of cucumber powdery mildew by Tilletiopsis minor

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Tilletiopsis spp., basidiomycetous yeasts, are commonly cited as constituents of the phylloplane microflora, especially when the leaves are infected by powdery mildews (Last, 1970).

Tilletiopsis minor Nyland was isolated as a hyperparasite from *Erysiphe martii* growing on *Lupinus polyphyllus* (Hijwegen and Buchenauer, 1984).

In preliminary experiments *T. minor* showed some promise in the biological control of cucumber powdery mildew *(Sphaerotheca fuliginea* (Schlecht.: Fr.) Poll.). This combination has been investigated on a single occasion previously, under rather artifial circumstances (Hoch and Provvidenti, 1979). These authors inoculated detached cucumber leaves and maintained them in covered plastic dishes exposed to continuous fluorescent light in an atmosphere where the relative humidity approached 100%. In addition, the emphasis in these experiments was placed on the histology of the powdery mildew-hyperparasite interaction utilizing electron microscopy techniques, rather than investigating the optimal conditions for the establishment of hyperparasitism. As the conditions they employed were rather unnatural, it was decided to explore the control of cucumber powdery mildew utilizing *T. minor* under conditions more consistent with those practised in modern cucumber growing in the Netherlands.

Four-week-old cucumber plants with 3-4 expanding leaves were transferred to a Weiss climate cabinet under an alternating day/night temperature regime of 23/17 °C within which the relative humidity of 75% during daytime was elevated to 90% at night. These conditions are recommended for cucumber growing in the Netherlands (J. van Uffelen, Naaldwijk, personal communication). Artificial light was given during the 16 hours daylight period. Additional experiments were performed in the greenhouse under less controlled conditions.

Cucumber plants were inoculated with cucumber powdery mildew by dusting with conidia obtained from infected leaves.

These cucumber plants were sprayed with various concentrations of *T. minor* 1 day prior to and 4 and 7 to 9 days after inoculation with powdery mildew. Disease incidence was assessed by estimating the percentage of apparently healthy conidiophores with conidia compared to the non-treated control with the aid of a binocular microscope. In some experiments a second spray containing either water or various concentrations of *T. minor* was applied.

T. minor was grown in shake culture in 300 ml Erlenmeyer flasks in a medium containing 100 ml 2% malt extract (Oxoid L39) and 0.2% mycological peptone (Oxoid L40) for 8 days at 23 °C and then stored at 5 °C. A concentration of 1-2 $\times$ 10^9 fungal propagules ml^{-1} could easily be obtained by this technique.

Under the aforementioned optimal conditions for cucumber growing *T. minor* proved to be effective in limiting the development of *S. fuliginea* at concentrations between 10^6 and 2 $\times$ 10^8 propagules ml^{-1} (the highest concentration tested) applied 7 to 9 days after the initial inoculations with powdery mildew. Concentrations below 10^{-6} propagules ml^{-1} were less effective.

Inoculum of *T. minor* originally harvested from 8-day-old actively growing cultures was consistently the most effective in controlling powdery mildew: it reduced (at 10^7 propagules ml^{-1}) the number of apparently healthy conidiophores with conidia of cucumber powdery mildew sometimes to 1% of the level found on untreated plants.

Inoculum obtained from cultures stored for 1 to 4 weeks at 5 °C was less effective, although a reduction in the incidence of apparently healthy conidiophores to 10 and 20% the levels found on untreated plants was obtained at 2 $\times$ 10^7 and 2 $\times$ 10^6 propagules ml^{-1}, respectively. (12 replicates with 4 plants each having 3-4 expanding leaves. Results varying from 5-30%, usually 10% at 2 $\times$ 10^7 propagules ml^{-1}, and from 10-40%, usually 20% at 2 $\times$ 10^6 propagules ml^{-1}).

Spraying with water 3 or 4 days after the leaves had been sprayed with a stored spore suspension of *T. minor* gave a further reduction in healthy conidiophores incidence to 5% (varying from 0-10%, usually 5%) after 2 $\times$ 10^7 propagules $ml^{<1}$ had been applied and 10% (varying from 0-20%, usually 10%) after 2$\times$$10^6$ propagules ml^{-1} had been applied (6 replicates).

After spraying twice with 2 $\times$ 10^7 *T. minor* propagules ml^{-1} at an interval of 3 days no secondary infections occurred and the plants remained free from powdery mildew during the following three weeks observation period (4 replicates). However, contrary to the results described by Hoch and Provvidenti (1979) preventive spraying of *T. minor* at 2 $\times$ 10^7 propagules ml^{-1}, 1 day prior to inoculation with cucumber powdery mildew had little effect; a disease incidence of 80% of the level occurring on untreated plants was obtained (2 replicates).

Powdery mildew conidia are more susceptible to *T. minor* than hyphae; spraying 4 days after inoculation, when the mycelium is developing, but prior to conidial formation, did not result in a reduction in the final incidence of mature conidiophores. This result differs with those described for *Ampelomyces quisqualis* by Philipp et al. (1984).

Temperature and relative humidity both appear to be important in the establishment of a hyperparasitic relationship involving *T. minor*. In greenhouse experiments no control was observed at temperatures above 30 °C. Also at a relative humidity below 70% negligible disease control was obtained.

Inoculation of cucumber cotyledons with powdery mildew in a discrete area and then covering this site with a 5-μl droplet of a suspension of 10^6 *T. minor* propagules ml^{-1} before incubation in Petri dishes at 20 °C for 14 days according to the method described by Philipp et al. (1984) for *A. quisqualis*, also resulted in minimal control. Although the centre of the initial inoculum drop remained free from powdery mildew, around its margins hyphal development occurred and subsequently healthy conidiophores with conidia were observed.

To ascertain whether *T. minor* could be used in an integrated programme of disease

control, the sensitivity of *T. minor* to dimethirimol (formulated as Milcurb 125 g active ingredient l^{-1}) at a concentration of 125 μg ml^{-1} and fenarimol (chemically pure) at a concentration of 100 μg ml^{-1} in malt agar (Oxoid CH59) was examined. Hyphal growth rate when dimethirimol was incorporated into the medium was similar to or slightly enhanced compared with the normal rate of growth on the basic malt agar medium. By contrast the fungus was very sensitive to fenarimol. However, after 10 to 12 days growth on this medium was resumed, presumably due to adaptation or mutation of *T. minor*. This 'mutant' was found to have a normal hyphal growth rate on basic malt agar medium and its level of pathogenicity towards cucumber powdery mildew was identical to that of the original culture. This strain of *T. minor* might be used in an integrated control scheme.

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Samenvatting

Biologische bestrijding van komkommermeeldauw door Tilletiopsis minor

Onder optimale omstandigheden kon *T. minor* de ontwikkeling van komkommermeeldauw *(Sphaerotheca fuliginea)* tegengaan.

Spuiten met 2×10^7 sporen ml^{-1} 7 dagen na inoculatie met komkommermeeldauw gaf een reductie van meeldauwontwikkeling van ongeveer 90%. Wanneer een tweede bespuiting met dezelfde concentratie sporen 3 dagen na de eerste werd toegepast bleven de planten vrij van meeldauw tot ze werden opgeruimd 3 weken later.

Bij een R.L. lager dan 70% en een temperatuur boven 30 °C had geen van de behandelingen succes.

T. minor bleek ongevoelig voor dimethirimol (Milcurb) bij een concentratie van 125 μg ml^{-1},terwijl er gemakkelijk een 'mutant' kon worden verkregen, die resistent was tegen 100 μg fenarimol ml^{-1}, bij gelijk blijvende groeikracht en pathogeniteit ten opzichte van komkommermeeldauw, waardoor *T. minor* ingepast kan worden in een schema voor geïntegreerde bestrijding.

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