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# Ammonia, a fungistatic volatile in chitin-amended soil

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Chlamydospore formation and mycolysis of macroconidia of *Fusarium solani* f. *cucurbitae* is inhibited by the addition of chitin to soil (Schippers and De Weyer, 1972). This effect has been ascribed to a sudden increase of nitrogen, and especially of ammonium originating from the microbial degradation of chitin (Schippers, 1972). This easily available nitrogen was supposed to reduce chlamydospore formation and lysis of macroconidial cells by compensating a nutrient deficiency – the nutritional hypothesis (Ko and Lockwood, 1970) – or by suppressing enzyme activity involved in nitrogen metabolism essential for mycolysis and the formation of chlamydospores (Schippers and Old, 1973). Volatiles in chitin-amended soil have been demonstrated to suppress fungal activity (Hora and Baker, 1972; Sneh and Henis, 1972). Schippers and Bouman (1973) reported a volatile factor formed in chitin-amended and non-amended 'Lienden'-soil that inhibits the germination of macroconidia of *F. solani* f. *cucurbitae* and of conidia of *Aspergillus flavus*. Because ammonia is formed in chitin-amended soil, the effect of this gaseous compound on germination of spores was determined.

Modifying the soil emanation method used by Hora and Baker (1972), about 200 conidia of both fungi were incubated on each of a number of wateragar disks above a sandy loam 'Lienden', pH 7.4 (Schippers and De Weyer, 1972) in Conway vessels. The incubation lasted 44 h at 15 °C for *Fusarium* and 22 h at 20 °C for *Aspergillus*. In contradiction to the procedure followed by Hora and Baker, the wateragar disks were not preactivated above the soil before the conidia were placed on to them. The Conway vessels were used to obtain a closed micro-atmosphere. The outer ring of the vessels contained sterile water (controls) or moistened soil (33 % on dry weight basis) with or without chitin-amendment (10,000 ppm). The inner ring contained sterile water (controls) or a 0.65 M boric acid solution or neither of them. Quantitative absorption of $NH_3$ by boric acid gives a standard micro-analytical method for measuring ammonia production (Bremner, 1965). The soil moisture content and the soil pH, which was measured with 0.01 M $CaCl_2$ (1:5) as suggested by Smiley and Cook (1972), changed hardly during the experiments.

A volatile fungistatic factor was observed only in the chitin-amended soil (Table 1) in contradiction to previous experiments (Schippers and Bouman, 1973). The volatile factor was completely absorbed by the boric acid solution. This suggested that ammonia might be the volatile fungistatic factor. Because of their more sensitive reaction only conidia of *Aspergillus* were used in the following experiment. In order to examine the influence of ammonia on germination of these conidia similar experi-

Table 1. Percentage of non-germinated conidia of *F. solani* f. *cucurbitae* and *A. flavus* above 'Lienden'-soil (— C), chitin-amended 'Lienden'-soil (+ C), silversand (— N) and $NH_4Cl$-amended silversand (+ N) in the presence of sterile water (A) or boric acid (B), or without (A) or (B).

| | 'Lienden'-soil | | Silversand | |
|---|---|---|---|---|
| | *Fusarium* | *Aspergillus* | | *Aspergillus* |
| + C, A | 12* | 25* | + N, A | 100* |
| + C, B | 3 | 8 | + N, B | 9 |
| — C, A | 2 | 1 | — N, A | 3 |
| — C, B | 2 | 3 | — N, B | 1 |
| + C | 9* | 23* | | |
| — C | 2 | 0 | | |
| A | – | 1 | | |
| B | – | 1 | | |

*Differ significantly from results of the same series at a P = 0,05 level according to the Wilcoxon two sample test. Average of 6 replicates.

*Tabel 1. Percentage niet gekiemde conidia van F. solani* f. *cucurbitae en A. flavus boven 'Lienden'-grond (— C), 'Lienden'-grond met chitine toegevoegd (+ C), zilverzand (— N) en zilverzand met $NH_4Cl$ (+ N) in aanwezigheid van steriel water (A) of boorzuur (B), of zonder (A) of (B).*

ments as described above were performed with a pure sand without organic material or micro-organisms (silversand, sandy component nearly 100%). $NH_4Cl$ was added at 200 μg $NH_4$-N/g silversand. Thus only ammonia was present in the micro-atmosphere. A fungistatic effect on spore germination was clearly recognizable; it was almost completely neutralized by the boric acid solution (Table 1).

Using the sodium phenoxy – sodium hypochlorite method for the determination of ammonia as modified by Growther and Large (1956), the ammonia uptake by both sterile water and boric acid with 'Lienden' soil (+ and – chitin-amendment) was measured (Table 2). From these data it is concluded that in our experiments ammonia is at least a component of the volatile fungistatic factor in chitin-amended soil. Other volatiles like methylamine and ethylamine, which may be formed during degradation of chitin, are also completely absorbed by boric acid. They do not react with the reagents using the method of Growther and Large (1956). Their contribution to the volatile fungistatic factor cannot completely be excluded.

Hora and Baker (1972) observed volatile fungistatic activity in soils amended with

Table 2. Total amount of ammonia in μg $NH_4$-N from 'Lienden'-soil (— C) and chitin-amended 'Lienden'-soil (+ C), absorbed in sterile water (A) or boric acid (B) during 24 h or 7 days. Figures are averages of 3 replicates.

| | 24 h | 7 days |
|---|---|---|
| + C, A | 3,4 | 46,2 |
| + C, B | 8,4 | 81,9 |
| — C, A | 0 | 0 |
| — C, B | 0 | 0 |

*Tabel 2. Totale hoeveelheid ammoniak in μg $NH_4$-N afkomstig uit 'Lienden'-grond (— C) en 'Lienden'-grond met chitine (+ C), geabsorbeerd in steriel water (A) of boorzuur (B) gedurende 24 uur of 7 dagen. Waarden zijn de gemiddelden van 3 waarnemingen.*

chitin, but little activity in those amended with glucose or cellulose. They noticed that volatile fungistatic activity was maximum in neutral or alkaline soils. These observations are also in favour of a role of ammonia as a fungistatic volatile factor in soil.


## Samenvatting

*Ammoniak, een fungistatische vluchtige stof uit grond waaraan chitine is toegevoegd*

Uit Lienden-grond, waaraan chitine is toegevoegd, komt een vluchtige substantie vrij die kiemremmend werkt op conidia van *Fusarium solani* f. *cucurbitae* en *Aspergillus flavus* (Tabel 1). Deze vluchtige stof wordt ten dele door steriel water en volledig door een boorzuuroplossing geabsorbeerd (Tabel 2).

Op grond van het kiemremmend effect van zilverzand, waaraan $NH_4Cl$ was toegevoegd en van kwantitatieve bepalingen van het ammoniumgehalte in de absorptiemiddelen (steriel water en boorzuur) wordt geconcludeerd dat ammoniak een component is van de vluchtige kiemremmende factor in grond, waaraan chitine is toegevoegd.


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