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Reduced chlamydospore formation and lysis of macroconidia of Fusarium solani f. cucurbitae in nitrogen-amended soil

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Abstract

Reduction of chlamydospore formation and of lysis of macroconidial cells of *F. solani* f. *cucurbitae* comparable to that found in chitin-amended soil was obtained with additions of NH_4Cl or KNO_3 to soil. NH_4-nitrogen and NO_3-nitrogen levels in these soils were kept comparable to those detected in chitin-amended soil. Combined addition of NH_4Cl and KNO_3 was most effective. Reduction of chlamydospore formation or macroconidial lysis in nitrogen-amended soils was not correlated with an increase or decrease in germination of macroconidia.

Introduction

In a previous paper (Schippers and de Weyer, 1972) it was reported that the addition of chitin to soil reduced chlamydospore formation from macroconidia of *Fusarium solani* f. *cucurbitae*. Chitin also initially inhibited the complete lysis of macroconidia resulting in high numbers of macroconidial structures staying viable over a period of 4 to 7 weeks. It was suggested that both of these phenomena could be caused by an increase in the availability of simple nitrogen substrates in soil, resulting from chitin decomposition.

This possible role of ammonium-nitrogen and nitrate-nitrogen in inhibiting chlamydospore formation and lysis in chitin-amended soil was further examined. The amounts of NH_4-nitrogen and NO_3-nitrogen released in soil in the course of the decomposition of added chitin were determined and by the addition of NH_4- and NO_3-inorganic salts it was attempted to maintain NH_4-nitrogen and NO_3-nitrogen levels in soil comparable to those detected in chitin-amended soil. The fate of macroconidia in these nitrogen-amended soils and in chitin-amended soil was compared. The supposition that reduced chlamydospore formation as well as reduced macroconidial lysis in chitin-amended and nitrogen-amended soil originates from an inhibition of macroconidial germination (Schippers and de Weyer, 1972) was examined. A brief report of some of these results has been published earlier (Schippers, 1972).

Materials and Methods

The soils. A 'Vleuten sandy loam' and a 'Lienden sandy loam', the characteristics of which have been described earlier (Schippers and de Weyer, 1972) were passed through

a 2 mm and 1 mm mesh stainless steel sieve. After mixing the soil with various compounds or with conidia, as described later, soil moisture content was adjusted to 30–33% on a dry weight basis. For each treatment, amounts of 100 g of soil were incubated in 225 ml plastic beakers at 20 °C. Beakers were covered with a polyethylene sheet to prevent moisture losses.

The pathogen – soil inoculation technique. Single spore cultures of *Fusarium solani* (Mart.) Appel & Wollenw. f. *cucurbitae* Snyder & Hansen were grown on potato dextrose agar (PDA) for one week at 23–26 °C. During this period cultures were subjected to alternating 12 hour periods of dark and light (6000 Lux). Macroconidia of the pathogen were washed twice in distilled water and subsequently evenly sprayed through soil with an atomizer to reach a final concentration of 10^6 conidia/g moist soil.

NH_4- and NO_3-nitrogen in chitin-amended soil. Amounts of NH_4- and NO_3-nitrogen in chitin-amended and non-amended soils were determined at intervals over a 70 day period. Of 400 g of sieved soil, 200 g were mixed thoroughly with 2 g chitin (BDH) and 200 g were left untreated. After one week incubation, 100 g samples of chitin-amended and of non-amended soil were inoculated with macroconidia, the rest was kept uninoculated.

Concentrations of NO_3- and NH_4-nitrogen were determined in duplicate for all treatments. Samples of 15 g of soil were taken at various intervals with the first sample being taken about two hours after chitin amendment. Soil samples were shaken in 60 ml 1 N K_2SO_4 solution for 30 minutes. After centrifuging the soil suspension for 10 min at 5000 rev/min, the supernatant was purified and sterilized by filtration through a Sartorius membrane filter (0.2 μm).

NH_4-nitrogen in soil was then determined colorimetrically in the sterile water-extracts using sodium phenoxide as a reagent (Growther and Large, 1956). NO_3-nitrogen was determined colorimetrically in the sterile water-extracts using o-xylenol as a reagent according to Alten et al. (1936). The amounts of NH_4- and NO_3-nitrogen determined are expressed as μg N/g moist soil.

All experiments were repeated at least three times.

Fig. 1. gives a representative example of the changes in NH_4- and NO_3-nitrogen concentrations in chitin-amended 'Vleuten' soil over a period of 70 days after addition of chitin on day O. In chitin-amended soil, the NH_4-nitrogen concentration rapidly increased from undetectable quantities at day O to about 120 μg N/g soil within 8 days and then rapidly decreased, becoming undetectable after 18 days of incubation. The NO_3-nitrogen concentration slowly increased from 150 μg at day 0 to about 220 μg N/g soil within 8 days and then rapidly increased to 550 during the next 12 days, thereafter slowly increasing to about 700 μg N/g soil at the end of the incubation period.

In non-amended soil, the NO_3-nitrogen concentration varied between 150 and 175 μg N/g soil, while NH_4-nitrogen could not be detected.

The presence of 10^6 macroconidia/g soil did not notably influence the concentration of NH_4- and NO_3-nitrogen in soil.

Additions of NH_4- and NO_3-nitrogen to soil. On the basis of the NH_4- and NO_3-nitro-

Fig. 1. Changes in water-extractable NH_4-nitrogen and NO_3-nitrogen concentrations in chitin-amended and non-amended soils determined colorimetrically. NH_4-nitrogen was not detected in non-amended soil samples.

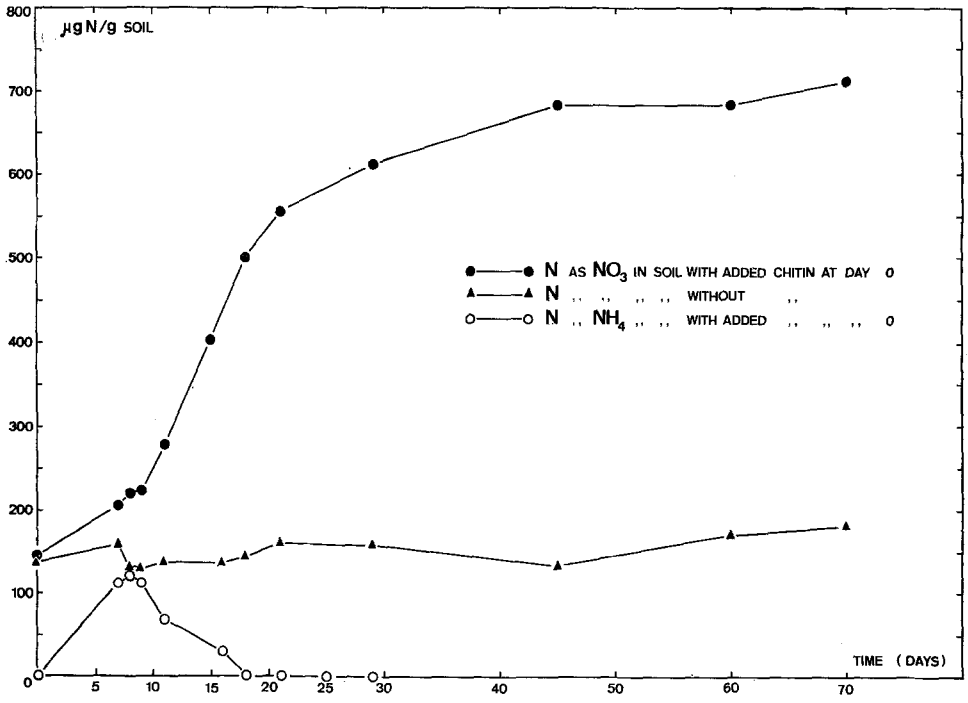

Fig. 1. Veranderingen in de NH_4-stikstof- en NO_3-stikstofconcentraties in met chitine behandelde (1 g/100 g grond) en onbehandelde grond, colorimetrisch bepaald met respectievelijk natrium-fenoxide en o-xylenol reagens.

gen levels found in the chitin-amended soil (Fig. 1) it was attempted to duplicate these levels in chitin free soil samples by additions of inorganic salts. Macroconidia were evenly distributed through all soil samples at day O, within 2 h after the first nitrogen-amendments.

The required levels of NH_4 and NO_3 were maintained in the soils by regular colorimetric determinations of the NH_4- and NO_3-nitrogen levels of the soil samples during the incubation period. The NH_4-nitrogen level in one soil was maintained by additions of NH_4Cl, the NO_3-nitrogen level in a second soil maintained by adding KNO_3, and levels of both NH_4- and NO_3-nitrogen were maintained in a third soil by additions of both of these salts. Amounts up to 1 ml of solutions containing 10 mg N/ml of NH_4Cl or KNO_3 were sprayed onto 100 g soil samples at two day intervals and thoroughly mixed with the soil. Some soil samples were treated with distilled water rather than salt solutions to duplicate mechanical disturbance of soils during nitrogen treatments. Other samples were amended with 1% chitin one week before macroconidia were added, and treated with distilled water at two day intervals.

The effect of the nitrogen treatments of soil described above was as follows. NH_4-nitrogen concentrations increased from 50 µg/ N/g soil at day 0 to 130 µg N/g soil at day 7 falling to undetectable levels at day 9. The NO_3-nitrogen concentrations in

Table 1. Mean number of different types of propagules $\times 10^4$/g moist soil seen 24 h and 14 days after introduction of macroconidia of *F. solani* f. *cucurbitae* in chitin-amended, nitrogen-amended and non-amended soils by microscopic examination of soil smear preparations. Figures in parenthesis present percentages of the total number of propagules seen at 24 h after introduction of macroconidia in soil.

Types of propagules	Amendment									
	H_2O		chitin		KNO_3		NH_4Cl		$KNO_3 + NH_4Cl$	
	24 hr	14 d	24 h	14 d	24 h	14 d	24 h	14 d	24 h	14 d
non-lysed macroconidia	43(41)	0(0)	72(72)	2(2)	77(71)	4(4)	83(77)	4(4)	96(96)	59(59)
partly lysed macroconidia	35(34)	13(13)	20(20)	63(63)	14(13)	37(34)	15(14)	75(73)	3(3)	28(28)
chlamydospores	26(25)	59(57)	8(8)	23(23)	18(16)	20(18)	10(9)	24(22)	1(1)	3(3)
total numbers	104(100)	72(70)	100(100)	88(88)	109(100)	61(56)	108(100)	103(99)	100(100)	90(90)

Tabel 1. Gemiddeld aantal van verschillende typen propagula $\times 10^4$/g grond waargenomen door microscopische analyse van gronduitstrijkprepraraten, 24 uur en 14 dagen na toedienen van macroconidiën van F. solani f. cucurbitae aan onbehandelde, met chitine, of met stikstofzouten behandelde grond. De getallen tussen haakjes geven de aantallen weer in percentages van het totaal aantal propagula dat 24 uur na toedienen van de macroconidiën aan grond werd waargenomen.

KNO_3-amended soils increased from about 150 μg at day 0 to 300 μg at the 2nd and to about 800 μg N at the 14th day after introduction of the conidia into the soils.

Microscopic examination of the pathogen in soil smear preparations. Counts of morphologically distinguishable propagules derived from macroconidia in soils were made at various intervals by microscopic examination of soil smears. Soil smears were prepared as described by Schippers and de Weyer (1972). All soil treatments were done in duplicate. Each time, 4 soil smears of each treatment were examined at × 1000 magnification.

Three types of propagules were distinguished: 1) non lysed macroconidia; 2) partly lysed macroconidia, i.e. macroconidia of which some cells had lost their contents and may have completely disappeared and 3) chlamydospores, i.e. one- or more-celled loose chlamydospores and chlamydospores still inside macroconidial structures or outside these structures, but connected with them, with or without a short germtube (Schippers and de Weyer, 1972, Fig. 2).

Results

Lysis and chlamydospore formation. Mean numbers of the three different types of propagules of the pathogen were microscopically determined in soil smears prepared at 24 h and 14 days after introduction of the macroconidia into soils with added water, chitin, KNO_3, NH_4Cl or both NH_4Cl and KNO_3. Results are calculated per gram of soil and as percentage of the number of propagules seen after 24 h of incubation of the macroconidia in soil (Table 1, Fig. 2).

Fig. 2. Percentage of non-lysed macroconidia, partly lysed macroconidia and chlamydospores recovered from the macroconidia of *F. solani* f. *cucurbitae* 14 days after their introduction in soil treated with water, chitin, KNO_3, NH_4Cl or $NH_4Cl + KNO_3$. The rest of the macroconidia had disappeared by complete lysis.

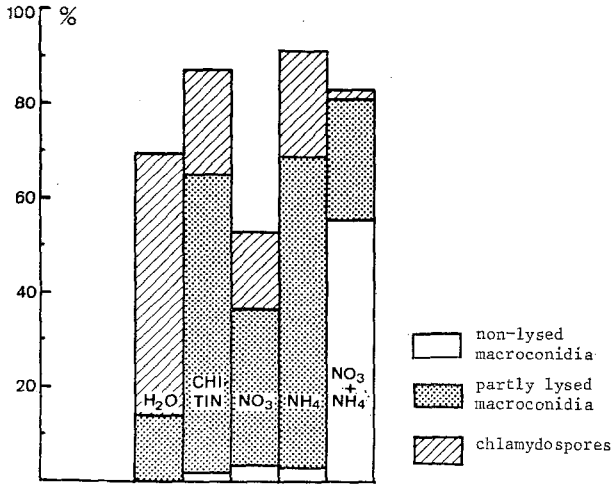

Fig. 2. Percentage niet gelyseerde macroconidiën, gedeeltelijk gelyseerde macroconidiën en chlamydosporen afkomstig van aan grond toegevoegde macroconidiën van F. solani f. cucurbitae (10^6/g grond) na 14 dagen incuberen in met water-, chitine-, KNO_3-, NH_4Cl-, of $NH_4Cl + KNO_3$- behandelde grond. De rest der macroconidiën was geheel gelyseerd.

Within the first 24 h of incubation no decrease in numbers of propagules of the pathogen occurred in any soil treatments and total numbers still amounted to approximately 10^6/g soil (Table 1). The number of non-lysed macroconidia, however, was already much lower in water-treated soils than in chitin- and N-amended soils. Numbers of non-lysed macroconidia were highest in soils amended with both NH_4Cl and KNO_3. Numbers of chlamydospore-like structures were higher in water-treated soils than in chitin- and all other nitrogen-amended soils and lowest in soils amended with both NH_4Cl and KNO_3.

After 14 days of incubation no non-lysed macroconidia were observed in water – treated soils. A few were observed in NH_4Cl- and KNO_3-amended soils, while more than half of the numbers added to soils treated with both NH_4Cl and KNO_3 still looked unchanged. Numbers of partly lysed macroconidia by this time were significantly higher in chitin-amended and all nitrogen-amended soils than in water-treated soils. More than half the number of macroconidia added to water-treated soils had formed chlamydospores. Chlamydospore formation in chitin-amended, NH_4Cl-amended, KNO_3-amended soils and in soils amended with both NH_4Cl and KNO_3 appeared to be less than half of that in water-treated soils and lowest in soils added with both NH_4Cl and KNO_3.

Germination. The percentages of germinating macroconidia recovered from the macroconidia introduced into soils supplemented by water, KNO_3, NH_4Cl or with both NH_4Cl and KNO_3, were determined by microscopic examination of soil smears made at 17, 24 and 44 h after introduction of the macroconidia. Four soil smears of each soil treatment containing at least 150 propagules, were examined for germination at each sampling time. Three separate experiments revealed similar results, two of them are presented in Table 2. No significant difference was observed between the percentage of germinating macroconidial structures in water-treated soils and that in nitrogen-amended soils of experiment I. The same holds for the germination of macroconidial structures after 17 h of incubation in soils of experiment II. Germination was, however, significantly higher (10% level) in nitrogen-amended soils, than in water-treated soils of experiment II after 24 h and 44 h of incubation.

Both chlamydospore formation and lysis of macroconidia were reduced in these

Table 2. Percentages of germinating macroconidial structures recovered from macroconidia of *F. solani* f. *cucurbitae* at 17, 24 and 44 h after their introduction in nitrogen-amended and non-amended soil by microscopic examination of soil smear preparations.

Hours after introduction of macroconidia in soil	Amendment							
	H_2O		KNO_3		NH_4Cl		$KNO_3 + NH_4Cl$	
	I	II	I	II	I	II	I	II
17	14	23	7	30	11	26	11	26
24	14	18	17	32	27	35	10	32
44	19	14	12	38	23	27	9	32

Tabel 2. Percentages gekiemde macroconidiumstructuren van het aan de grond toegediend aantal macroconidiën van F. solani f. cucurbitae, waargenomen door microscopische analyse van gronduitstrijkpreparaten, 17, 24 en 44 uur na toedienen van de macroconidiën aan de grond.

nitrogen-amended soils. Mean numbers of chlamydospores determined 16 days after introduction of the macroconidia in soil added with water, KNO_3, NH_4Cl or with both, amounted to 60, 20, 32 and 10×10^4/g soil respectively, in experiment II. Mean numbers of partly lysed macroconidia at that time amounted to 1, 24, 17 and 38×10^4/soil, respectively.

Discussion

The maintenance of water-extractable NH_4- and NO_3-nitrogen in soil by additions of inorganic nitrogen salts at similar concentrations as obtained by decomposition of added chitin (1 g/100 g soil) resulted in a reduction of chlamydospore formation from macroconidia corresponding to that observed in chitin-amended soils. It also inhibited the lysis of macroconidial cells as in chitin-amended soil. These observations indicate that the inhibiting effects of decomposing chitin are due, at least in part, to the gradual increase of simple nitrogen substrates in soil.

The addition of NH_4-nitrogen resembled the effect of chitin-amendment on chlamydospore formation and on lysis the best.

The combined addition of both NH_4- and NO_3-nitrogen was most effective in reducing both chlamydospore formation and macroconidial lysis (Fig. 2). Previous studies of macroconidia in chitin-amended soil (Schippers and de Weyer, 1972) have shown that non-lysed and partly lysed macroconidia still present at 14 days after introduction of the macroconidia in soil, succumb after another two to five weeks. A similarly sudden decrease of macroconidial structures must also be expected in nitrogen-amended soil. This then will result, particularly with combined amendments of NH_4- and NO_3-nitrogen, in a notable decrease of the population density of the pathogen in soil.

In a previous paper it was suggested that reduction of chlamydospore formation and macroconidial lysis in chitin-amended soil possibly originated from a reduction of macroconidial germination. This in turn was supposed to be due to the immobilization of simple carbon substrates by the increased activity of microbes utilizing simple nitrogen-subtrates in soil (Schippers and de Weyer, 1972). The necessity of glucose for germination of propagules of *F. solani* f. *phaseoli* in soil has been demonstrated by Griffin (1964) and Cook and Schroth (1965).

The observations on germination of washed macroconidia 17, 24 and 44 h after their introduction in nitrogen-amended soil, however, do not demonstrate that germination is less in nitrogen-amended soils than in the control soil (Table 2). Those of experiment II indicate that germination in nitrogen-amended soil may even be higher, at least after 24 and 44 h of incubation. Observations on the effect of soil treatments on germination of fungal propagules in soil, however, are extremely difficult to interpret. Amendments to soil may not only influence the proportion of germinating propagules, but may also advance or delay germination or accelerate or retard lysis of germtubes. More information is required in this respect before conclusions can be drawn.

Samenvatting

Vermindering van de vorming van chlamydosporen en van lysis van macroconidiën van Fusarium solani f. cucurbitae in grond verrijkt met anorganisch stikstof

Na toedienen van chitine aan grond (1 g/100 g grond) werden de concentraties van met water uit grond extraheerbaar NH_4- en NO_3-stikstof bepaald over een periode van twee maanden (Fig. 1). Indien door het toedienen van anorganische zouten concentraties van NH_4- en NO_3-stikstof werden verkregen, vergelijkbaar met die in chitinerijke grond, dan werd de vorming van chlamydosporen uit macroconidiën van *Fusarium solani* f. *cucurbitae* in een zelfde mate geremd als in chitinerijke grond (Fig. 2, Tabel 1). Evenals in de chitinerijke grond, werd ook de volledige afbraak van macroconidiën geremd, waardoor ongelyseerde, doch vooral gedeeltelijk gelyseerde, veelal tweecellige structuren van kiemkrachtige macroconidiën in de grond aanwezig bleven. De concentratie van anorganisch stikstof blijkt dus van invloed te zijn op vorming van chlamydosporen uit macroconidiën en op de afbraak van macroconidiën.

Het effect van toedienen van NH_4-N komt het meest overeen met het effect van toediening van chitine aan grond op lysis en chlamydosporenvorming.

Gecombineerde toediening van NH_4Cl en KNO_3 remde de chlamydosporenvorming en lysis van macroconidiën het sterkst. Indien de macroconidiën na 4 tot 7 weken tenslotte geheel verdwijnen, zoals in chitinerijke grond werd geconstateerd (Schippers en de Weyer, 1972), zal de gecombineerde toediening van NH_4Cl en KNO_3 tot een aanzienlijke vermindering van de concentratie van het pathogeen in grond leiden.

In een vorige publikatie werd verondersteld, dat de vermindering van chlamydosporenvorming en van lysis van macroconidiën in chitinerijke grond het gevolg zou kunnen zijn van kiemremming (Schippers en de Weyer, 1972). Deze kiemremming zou voortvloeien uit het vastleggen van eenvoudige C-verbindingen, die voor de kieming in grond vereist zijn. Het vastleggen van eenvoudige C-verbindingen is het gevolg van de door de verhoogde N-concentratie toegenomen microbiologische activiteit.

Met enkele oriënterende experimenten kon echter geen duidelijk verband tussen chlamydosporenvorming of lysis van macroconidiën en kieming van macroconidiën worden aangetoond. (Tabel 2).

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