*Plant and Soil* **145**: 17–27, 1992.

PLSO 9171

# Relationships between take-all, soil conduciveness to the disease, populations of fluorescent pseudomonads and nitrogen fertilizers

A. SARNIGUET, P. LUCAS and M. LUCAS
*INRA, Station de Pathologie végétale, Centre de Recherches de Rennes, B.P. 29 F-35650 Le Rheu, France*

Received 9 April 1991. Revised April 1992

*Key words:* ammonium, *Gaeumannomyces graminis* var *tritici*, nitrate, nitrogen fertilizers, fluorescent pseudomonads, root system, soil conduciveness, wheat

**Abstract**

Take-all of wheat, caused by *Gaeumannomyces graminis* var *tritici* (*Ggt*), is reduced by ammoniacal fertilizers as compared to nitrate sources. This influence of nitrogen on the disease is only observed on nodal roots at flowering. But soil conduciveness to take-all, as measured in a soil bioassay, is modified earlier. Forty days after nitrogen application at early tillering, the $NH_4$-treated soil became less conducive than the $NO_3$-treated one. When nitrogen applications are done at sowing and at tillering, differences in disease propagation between the two soils are enhanced. Results from four years of experimentation show that when the level of natural soil inoculum is high, disease severity is reduced by ammonium, showing an effect on the parasitic phase of *Ggt*. At a low level of natural inoculum the effect of the source of nitrogen is mainly observed on the percent of infected plants, indicating that the saprophytic and preparasitic phases are affected. Rhizospheric bacterial populations increase from sowing to tillering, but differences on take-all conduciveness after tillering are not correlated with differences in the amounts of aerobic bacteria or fluorescent pseudomonads isolated from soils treated with different sources of nitrogen. Qualitative changes in fluorescent *Pseudomonas* spp. populations, like in vitro antagonism, are more likely to explain differences in soil conduciveness to take-all than are quantitative changes in this group. Nevertheless, the introduction of *Ggt* in a cropped soil leads to a greater increase in fluorescent pseudomonads populations than in total aerobic bacteria.

The delay between reducing soil conduciveness and reducing disease in the field with ammonium nitrogen fertilization, the qualitative change of fluorescent pseudomonads populations and the role of necroses in rhizobacteria multiplication, provide information leading to our representation of a dynamic model based on the differentiation of the wheat root system into seminal and nodal roots.

## Introduction

Take-all of wheat is a root disease caused by the soilborne fungus *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker (*Ggt*). There are no effective sources of cultivar resistance or chemical control. However, cultural practices such as crop rotations and nitrogen fertilizer management have some impact on the disease. Application of an ammonium ($NH_4^+$) source reduces take-all in most situations, whereas this is not true for ($NO_3^-$) nitrate sources (Huber, 1969, 1981; Hornby and Goring, 1972; McNish and Speijers, 1982). Uptake of $NH_4^+$ by roots decreases the pH of the rhizosphere and, from a study of soil sterilized or not with methyl bromide, Smiley and Cook (1973) suggest that decreasing the pH has an indirect inhibition on

take-all through changes in rhizosphere microflora between pH 5 and 7, and a direct effect on the fungus for pH below 5. Subsequently, Smiley (1978, a, b) reported that the application of a $NH_4^+$ source of nitrogen leads to a higher proportion of the rhizosphere pseudomonads, which are in vitro antagonistic to *Ggt*, than does an application of $NO_3^-$. Although Cook and Rovira (1976) suggested that strains of fluorescent pseudomonads may be involved in suppression of *Ggt*, few studies have been conducted on the relations between nitrogen fertilization and control of take-all by pseudomonads. Attention has rather mainly been focussed on the mechanisms of action of these bacteria (Weller and Cook 1986) or on the improvement of control of take-all by use of different sources of $NH_4^+$ (Christensen and Brett 1985).

In this work, we attempt to establish the importance of the modifications induced in fluorescent *Pseudomonas* spp. populations due to different sources and timing of nitrogen fertilizers on field crops. The results will be compared with disease progress in the field and soil conduciveness to take-all as measured in a pot bioassay (Lucas et al. 1989). From that, a hypothesis of dynamic build up of microbial antagonism on the wheat root system is discussed.

## Materials and methods

### *Fields experiments*

Field experiments were conducted at Le Rheu (western France) on a soil (clay 15%, silt 70%, sand 15%) previously cropped with wheat in order to increase the amount of natural soil inoculum for *Ggt*. Fields were planted with wheat for one year prior to the 1985–86 and 1986–87 experiments, and for two consecutive years prior to 1987–1988 and 1988–89 experiments. The treatments consisted of different forms and timing of nitrogen fertilizers as shown in Table 1. The experimental design was a split plot with forms of nitrogen as main plots and time of application as subplots. Plots measured 4.5 m × 2.6 m and were replicated four times.

### *Disease assessment*

Plants were dug from eight randomly selected positions in each plot, each sample consisting of all plants collected on a 10 cm length row. Fifty plants were analyzed for each plot. The plants were rinsed in order to remove adhering soil from the root systems and were evaluated for incidence and severity of take-all. Each plant

*Table 1.* Nitrogen sources, rates, and timing of application in field experiments

| Year | Nitrogen source | N ($kg\ ha^{-1}$) | | |
|---|---|---|---|---|
| | | Sowing | Early tillering | Early stem elongation |
| 85–86 | $NH_4NO_3$ | 0 | 50 | 60 |
| | $(NH_4)_2SO_4$ | 40 | 50 | 40 |
| 86–87 | $NH_4NO_3$ | 0 | 50 | 50 |
| | $(NH_4)_2SO_4$ | 0 | 50 | 50 |
| | $(NH_4)_2SO_4$ | 40 | 50 | 50 |
| 87–88 | $Ca(NO_3)_2$ | 0 | 50 | 70 |
| | $Ca(NO_3)_2$ | 50 | 50 | 50 |
| | $(NH_4)_2SO_4$ | 0 | 50 | 70 |
| | $(NH_4)_2SO_4$ | 50 | 50 | 50 |
| | $NH_4NO_3$ | 50 | 50 | 50 |
| 88–89 | $Ca(NO_3)_2$ | 0 | 50 | 70 |
| | $Ca(NO_3)_2$ | 50 | 50 | 50 |
| | $(NH_4)_2SO_4$ | 0 | 50 | 70 |
| | $(NH_4)_2SO_4$ | 50 | 50 | 50 |

was assigned to one of the five disease severity classes (0, 1, 2, 3, 4) corresponding to nil, 1 to 10, 11 to 30, 31 to 60, 61 to 100% of the root system with take-all lesions. A disease index (DI) was calculated with the formula:

$$DI = \sum_{i=0}^{4} (n_i \times xi) \times \left( \sum_{i=0}^{4} n_i \right)^{-1}$$

where i = severity class and ni = number of plants assigned to the class i. The disease severity index (SI) was calculated in the same way but by taking only the diseased plants in account ($i \geq 1$). It reflected the intensity of disease when the plant is infected:

$$SI = \sum_{i=1}^{4} (n_i \times i) \times \left( \sum_{i=1}^{4} n_i \right)^{-1}$$

The percentage of diseased plants was also considered and reflected the spread of the disease or the rate of successful infections.

All the data were analysed with analysis of variance and the calculated means were compared for significance using the Newman-Keuls test.

*Measure of soil conduciveness*

The method was the same as described by Lucas et al. (1989). It consists of measuring the ability of a soil to allow expression of pathogenicity by increasing rates of inoculum, in a population of susceptible host plants (Alabouvette et al. 1982). Wheat rhizosphere soil was collected from each plot, 40 days after the nitrogen applications, at sowing and also at early tillering. Cores of soils (4 cm diameter, 15 cm depth) were collected in the row, and the roots and stones were removed by hand as all soil samples from the same treatment were composited. They were then air-dried before grinding into 5 mm particles or smaller.

Inoculum of *Ggt* was grown on barley grains soaked in water (w/v = 1) and sterilized twice in 24 hours (1 h, 120°C). After 3 weeks of incubation at 20°C, the colonized grains were air-dried, ground and passed through sieves in order to obtain propagules (infectious particles) of 1 to 1, 6 mm. The propagules were introduced and mixed into soils at the rates of 0, 300, 1000, 3000 units per 2 kg, necessary to fill 4 pots of 500 g, each one considered as a replicate. Each pot was then seeded with 5 caryopses of wheat cv Talent. The caryopses had been previously superficially sterilized 1 minute with bleach (1.25% chlorine), rinsed with sterile water and put on sterile humidified filter paper. Only the germinating caryopses were used. Pots were maintained for 5 weeks at 15°C day, 10°C night, photoperiod 14 h and 80–90% relative humidity. Soil moisture was adjusted to the pot water holding capacity (35% water against 45% for the field whc). After this period plants were harvested, roots were gently washed under running water and root necrosis was noted according to the scale described earlier. A disease index (DI) for each rate of inoculum and each soil was calculated.

*Analysis of the bacterial microflora*

At the end of the biological test for measuring soil conduciveness and just before washing roots systems, adhering soil clumps were collected. For the doses 0 (0 propagule) and 3 (3000 propagules) of *Ggt* the bacterial microflora was evaluated. Soil suspensions (1:10) were prepared by mixing 20 g soil with 180 mL sterile distilled water and they were shaked 15 minutes. From these master suspensions, other dilutions were prepared by transfering 2 mL to 18 mL distilled water and so on, down to dilution $10^{-5}$. Suspensions were agitated for 10 s just before each pipetting. From dilutions $10^{-3}$, $10^{-4}$ and $10^{-5}$, 0.1 mL of suspension was spread over the surface of three replicate plates of: either Tryptic soy agar (Difco, 1/10 strength) (TSA) for assessment of Gram-positive and Gram-negative aerobic bacteria (Martin 1975), or King's medium B supplemented with novobiocin (45 $\mu g\, mL^{-1}$), penicillin G (75 Units $mL^{-1}$) and cycloheximide (75 $\mu g\, mL^{-1}$) (KMB NPC) for assessment of fluorescent pseudomonads (Sands and Rovira 1970). After the agar had absorbed free liquid, the plates were incubated aerobically at 22°C. Populations of aerobic bacteria were enumerated after 5 days incubation and fluorescent *Pseudomonas* spp. after 2 days (under UV lights at 365 nm). Only data from dilutions which gave from 50 to 100 CFU per plate were consid-

ered and analyzed, after a log transformation, by analysis of variance.

*Antagonistic activity of fluorescent pseudomonads to Ggt*

For each soil sample, after counting, fluorescent *Pseudomonas* spp. colonies were randomly sampled from plates and their antagonistic activity on the growth of *Ggt* was assessed on potato dextrose agar (PDA, BioMerieux). PDA plates were inoculated with *Ggt* at the center, and 2 days later, with bacteria to be tested on 2 opposite edges. After incubation for 4 days at 22°C, growth inhibition of *Ggt* was evaluated.

## Results

*Development of the disease in the field*

The black necrosis characteristic of take-all were observed only on the seminal roots at mid tillering (Growth Stage (GS) 23, Zadocks et al. 1974), on both seminal and nodal roots at beginning of stem elongation (GS 31), and only on nodal roots at flowering (GS 65).

Plants treated with different sources of nitrogen did not have significant differences in disease expression when sampled at tillering and at stem elongation, on seminal or on nodal roots (data not shown). The differences were only noticed at flowering on nodal roots (Table 2). For the years 1985–86 and 1986–87, the frequency of infected plants by *Ggt* was very low (from 15 to 36%). The disease index and the percentage of infected plants decreased when the soil was fertilized with $(NH_4)_2SO_4$ compared to $NH_4NO_3$ ($p = 0.05$). The additional early fertilization at sowing did not have significant effect, as compared to $(NH_4)_2SO_4$ applied at tillering and stem elongation (data not shown, Sarniguet, 1990).

For the years 1987–1988 (Table 2), the percentages of infected plants at flowering were very high and homogeneous (about 100% for all the situations). The effect of $NH_4$ was beneficial ($p = 0.05$) (disease index and disease severity were lower) compared to $NO_3$.

For the years 1988–89, the percentages of infected plants on the nodal roots were high and similar (71 to 87%). As for the preceeding years, the disease severity was lower after fertilization with $(NH_4)_2SO_4$ compared to $Ca(NO_3)_2$.

*Soil conduciveness to take-all*

In the year 1987–88, the three sources of nitrogen were compared for their effect on soil conduciveness. The soils fertilized with $Ca(NO_3)_2$ and $NH_4NO_3$ showed a significantly higher disease index than the $(NH_4)_2SO_4$-treated soil. The $Ca(NO_3)_2$- and $NH_4NO_3$-treated soils differed only for the higher rate of introduced *Ggt* (Fig. 1). Thus the $(NH_4)_2SO_4$-treated soil was the less susceptible to the disease. The $Ca(NO_3)_2$-treated soil was slightly more susceptible than the $NH_4NO_3$-treated one, but this difference was not significant. Only the extreme soils, $(NH_4)_2SO_4$- (the less susceptible) and $Ca(NO_3)_2$- treated soil (the most susceptible), were further studied.

*Table 2.* Influence of nitrogen sources on take-all of nodal roots at the time of plant flowering in he field at Le Rheu

| Year | Disease index (o to 4) | | | | Severity index (1 to 4) | | | | Frequency of infected plants (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $S_0$[a] | $S_1$ | $S_0$ | $S_1$ | $S_0$ | $S_1$ | $S_0$ | $S_1$ | $S_0$ | $S_1$ | $S_0$ | $S_1$ |
| | **$(NH_4)_2SO_4$** | | **$NH_4NO_3$** | | **$(NH_4)_2SO_4$** | | **$NH_4NO_3$** | | **$(NH_4)_2SO_4$** | | **$NH_4NO_3$** | |
| 85–86 | nd | 0.18b | 0.47a | nd | nd | 1.55a | 1.30a | nd | nd | 18b | 36a | nd |
| 86–87 | 0.16b | 0.28b | 0.23a | 0.31a | 1.09a | 1.09a | 1.16a | 1.12a | 14.6b | 16.5b | 26a | 27a |
| | **$(NH_4)_2SO_4$** | | **$Ca(NO_3)_2$** | | **$(NH_4)_2SO_4$** | | **$Ca(NO_3)_2$** | | **$(NH_4)_2SO_4$** | | **$Ca(NO_3)_2$** | |
| 87–88 | 2.26b | 2.16b | 2.63a | 2.72a | 2.29b | 2.20b | 2.68a | 2.73a | 98.2a | 98.3a | 97.8a | 99.5a |
| 88–89 | 1.26b | 1.26b | 1.62ab | 1.75a | 1.64a | 1.68ab | 1.98b | 2.00b | 71.1a | 78.7a | 80.1a | 87.1a |

[a] $S_0$ = no fertilizer applied at sowing, and $S_1$ = fertilizer applied at sowing. Values with the same letter are not significantly different at $p = 0.05$, based on Newman–Keuls test. nd = not determined.

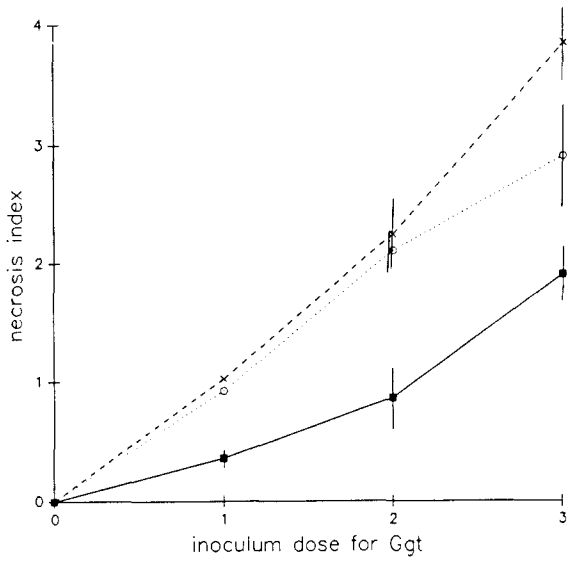


*Fig. 1.* Influence of different nitrogen sources on soil conduciveness to take-all; $(NH_4)_2SO_4$ —■—, $NH_4NO_3$ ···○···, $Ca(NO_3)_2$ --- × ---. Soils were sampled 40 days after a second fertilizer application at tillering.

### *Influence of the number of nitrogen fertilizer application and the date of soil sampling*

When nitrogen was applied at sowing there was no significant difference in the disease index between the nitrogen sources, for all levels of inoculum on samples collected 40 days later (Fig. 2a). Thus 40 days after sowing the $NH_4$- and $NO_3$-treated soils had the same high level of conduciveness to take-all. On the other hand, 40 days after a second fertilization applied at tillering, the disease indexes were lower in the $NH_4$-treated soil for the doses 1, 2 and 3 of *Ggt* (Fig. 2b). Without inoculum, a very weak disease index was observed, but only for the $NH_4$-treated soil. The $NH_4$-treated soil appeared less susceptible than the $NO_3$-treated one 40 days after tillering.

When no fertilization was applied at sowing but only at tillering, there was no significant difference in necrosis index between the two nitrogen sources, for doses 2 and 3 of *Ggt* (Fig. 3a). The disease index was weaker with $NH_4$- than in the $NO_3$-treatment only at the dose 1 of *Ggt*. Without artificial inoculum introduced (dose 0 *Ggt*), some necrosis caused by naturally present *Ggt*, was observed on the plant roots from the $NH_4$-treated soil. Thus a single fertilizer application at tillering decreased only slightly the soil conduciveness of the $NH_4$-treated soil compared to the $NO_3$-treatment. However, when the fertilizer was applied both at sowing and at tillering, and when soil samples were collected 40 days after the second nitrogen application, the necrosis index was lower in the $NH_4$-

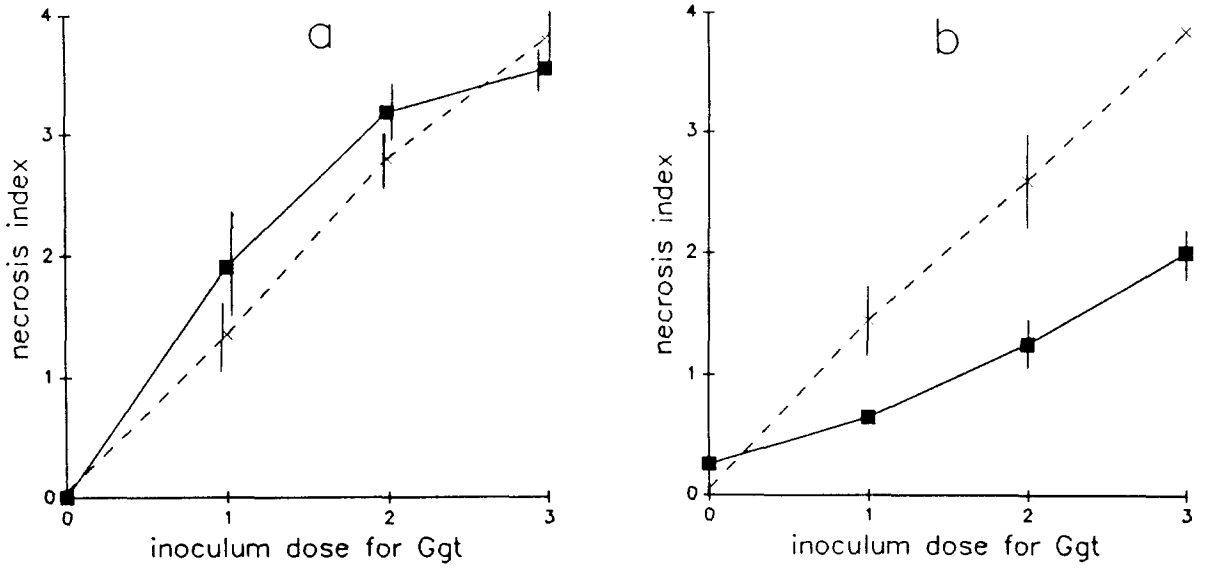


*Fig. 2.* Conduciveness to take-all in soils fertilized with $(NH_4)_2SO_4$ —■— or $Ca(NO_3)_2$ --- × ---, when soils were sampled **a)** 40 days after sowing (one fertilizer application) or **b)** 40 days after tillering (two fertilizer applications). (Le Rheu 1987–88).

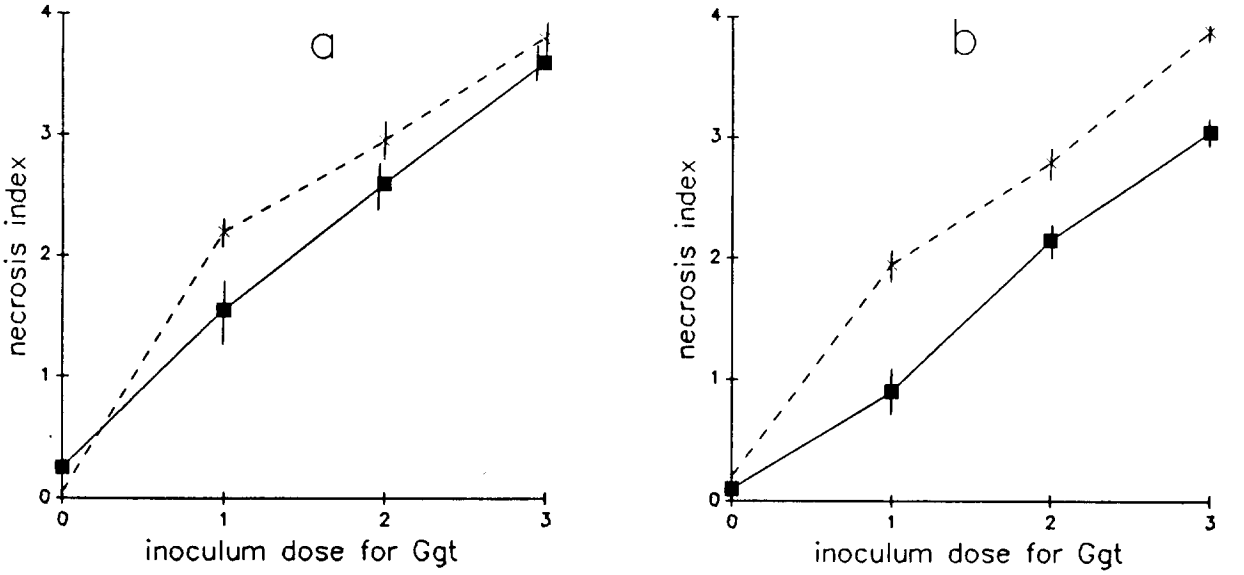


*Fig. 3.* Conduciveness to take-all in soils fertilized with $(NH_4)_2SO_4$ —■— or $Ca(NO_3)_2$ --- × ---, when soils were sampled 40 days after fertilization at tillering; fertilizer applied **a)** only at sowing or **b)** at sowing and tillering. (Le Rheu 1988–89)

treated soil for doses 1, 2 and 3 of *Ggt* (Fig. 3b). Without artificial inoculum (*Ggt* dose 0) there was also minor necrosis of roots in the $NH_4$-treated soil. This soil conduciveness was highly reduced in the $NH_4$-treated soil when nitrogen was also applied early at sowing and then at tillering.

### *Rhizospheric bacterial populations*

Bacterial counts were conducted on soils fertilized with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ and collected from the rhizosphere of wheat plants used to measure soil conduciveness.

In the 1987 study, when no inoculum (*Ggt* dose 0) was introduced, the numbers of fluorescent pseudomonads were significantly lower ($p = 0.05$) in $NH_4$- than in $NO_3$-treated soil, if these soils had been fertilized at sowing (Table 3). However, after a second fertilization at tillering, their amount increased but did not differ significantly among sources of nitrogen (Table 3). The results were the same in 1989 (Table 4).

*Table 3.* Bacterial counts after assessment of conduciveness to take-all of soils fertilized with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ (Le Rheu, 1987): total number and frequency of antagonistic fluorescent pseudomonads to *Ggt* (56 tested strains on PDA medium for each treatment). The soils were collected from the field 40 days after each fertilizer application. Values with the same letter are not significantly different at $p = 0.05$, based on Newman–Keuls test

| | 40 days after | Nitrogen source | Fluorescent pseudomonads $10^5$ CFU $g^{-1}$ dry soil | Frequency (%) of antagonistic fl. ps. |
|---|---|---|---|---|
| *Ggt* dose 0 | Sowing | $(NH_4)_2SO_4$ | 2.5a | 18 |
| | | $Ca(NO_3)_2$ | 4.4b | 23 |
| | Tillering | $(NH_4)_2SO_4$ | 4.2b | 77 |
| | | $Ca(NO_3)_2$ | 4.9b | 49 |
| *Ggt* dose 3 | Sowing | $(NH_4)_2SO_4$ | 11.4c | 17 |
| | | $Ca(NO_3)_2$ | 14.4d | 32 |
| | Tillering | $(NH_4)_2SO_4$ | 29.2e | 46 |
| | | $Ca(NO_3)_2$ | 30.4e | 32 |

*Table 4.* Bacterial counts after assessment of conduciveness to take-all of soils fertilized with $(NH_4)_2SO_4$ or $Ca(NO_3)_2$ (Le Rheu 1989): number of fluorescent pseudomonads (isolated on King B + CNP medium), total number of aerobic bacteria (1/10 strength TSA medium), ratio fluorescent pseudomonads/total bacteria (%), and frequency of antagonistic fluorescent pseudomonads (56 tested strains). The soils were collected from the field 40 days after the fertilizer application at tillering. Values with the same letter are not significantly different at $p = 0.05$, based on Newman–Keuls test

| | Nitrogen source | Fluorescent pseudomonads $10^5$ CFU $g^{-1}$ dry soil | Total bacteria $10^6$ CFU $g^{-1}$ dry soil | Fl. pseudomonads × 100 total bacteria | Frequency (%) of antagonistic fl. pseudomonads |
|---|---|---|---|---|---|
| *Ggt* dose 0 | $(NH_4)_2SO_4$ | 5a | 58.8c | 0.85 | 34 |
| | $Ca(NO_3)_2$ | 4a | 68.8c | 0.58 | 16 |
| *Ggt* dose 3 | $(NH_4)_2SO_4$ | 18b | 87.6d | 2.05 | 36 |
| | $Ca(NO_3)_2$ | 17b | 103.6d | 1.7 | 24 |

The percentage of antagonistic fluorescent pseudomonads on PDA medium was quite similar for the two nitrogen sources after sowing (18 to 23%; Table 3), but it became higher in the $NH_4$-treated than in the $NO_3$-treated soil after tillering (77% compared to 49% in 1987, 34% compared to 16% in 1989).

In 1989, the total amount of aerobic bacteria did not differ for the two nitrogen sources. The ratio of fluorescent pseudomonads versus total bacteria (0.85% to 0.58%) in the two treatments when sampled after tillering was not significantly different.

When inoculum of *Ggt* (dose 3) was added, the numbers of fluorescent pseudomonads were always higher than in non-inoculated soils (Tables 3 and 4). This was less pronounced in the soil sampled after sowing (3.2- to 3.5-fold more) than in the soil sampled after tillering (7-fold more) (Table 3).

Fluorescent pseudomonads remained significantly less numerous in the $NH_4$-treated soil taken after sowing (Table 3), but their number did not differ for the two nitrogen sources after tillering (1987 and 1989) (Tables 3 and 4).

The percentage of antagonistic fluorescent pseudomonads was lower in $NH_4$- than $NO_3$-treated soils sampled after sowing (17% compared to 32%) but became higher in $NH_4$-soil sampled after tillering (46% compared to 32% in 1987, 36% compared to 24% in 1989).

The total numbers of bacteria were also increased by the introduction of inoculum of *Ggt*, but less than were the fluorescent pseudomonads (1.5-fold more instead of 4-fold more for the fluorescent pseudomonads in 1989) (Table 4).

## Discussion

The use of nitrogen fertilizers in the field showed that an ammonium ($NH_4^+$) source ($(NH_4)_2SO_4$) reduced the disease index compared to a nitrate ($NO_3^-$) source ($Ca(NO_3)_2$) or a mixed one ($NH_4NO_3$). These differences were not recorded at tillering or at the stem elongation, either on seminal or on nodal roots. The results observed at flowering confirmed those of others (Huber, 1969, 1981; McNish and Speijers 1982).

The splitting of the disease index into disease severity and disease frequency of infected plant gave complementary information on disease development in the field. During the two first years (1985–86 and 1986–87), the frequency of infected plants was low (14 to 27%). The frequency of disease was higher in the $NO_3$-treated soil than $NH_4$-treated soil, but there was no effect on disease severity.

In 1987–88 and 1988–89, the frequency of infected plants was very high (98 and 80%) and did not differ for the nitrogen sources. The severity index was lower in the $NH_4$-treated soil than in the $NO_3$-treated soil. A high frequency of infected plant is undoubtedly related to a high infectious potential of the soil. Actually, the two first experiments were conducted after only one earlier culture of wheat had been produced. So the natural level of infestation by *Ggt* was weak. In order to increase the level of natural inoculum in the soil, the experiments during the two following years were performed after two wheat croppings. It was not possible to determine the level of native inoculum because of the lack of reliable methods to directly assess the amount of soil resident inoculum (Hornby 1975).

From inoculum in soil, the infectious process of *Ggt* occurs in three major phases. Mycelium of *Ggt* grows from its nutritive base towards roots and then colonizes the surface of roots. These two steps correspond respectively to the saprophytic and to the preparasitic phases of *Ggt*. Then differentiated microhyphae enter the root cortex and cause necrosis: this is the parasitic phase (Skou, 1981). The first two phases condition the fungus for successful root infection. The third phase is more related to the extension of the root lesions.

For a similar level of inoculum, a low frequency of infected roots may result from a low amount of growth of *Ggt* from its nutritive base, or from a low amount of ectotrophic colonization of the roots by the pathogen. As the amount of *Ggt* inoculum becomes higher, the probability of a meeting between the infectious propagules and the root also increases, so the importance of the saprophytic and (or) preparasitic phases becomes reduced as the amount of inoculum is increased. In years when the inoculum level was high, a large number of roots were reached and this was independent of the nitrogen sources. In years when inoculum level was low, $NH_4$-N as the sole nitrogen source led to a reduced frequency of infected roots. This effect of nitrogen source must be considered to influence both the saprophytic and (or) preparasitic phases.

The disease severity provides information about the infected plants, while the disease index is related to all the plants of the sampling. The disease severity directly reflects the importance of the root necrosis. The number and the length of the lesions are integrated in the severity rating. The extension of the lesions is directly related to the parasitic activity of *Ggt*. When the inoculum pressure is high, the disease index is also high. But in this last case the disease severity is lower in the $NH_4$-treated soil compared to $NO_3$-treatment, showing an impact of the source of nitrogen on the parasitic phases.

The differences between levels of natural infestation in the field help to understand that when the inoculum pressure is low, the nitrogen source works more on saprophytic and preparasitic phases of *Ggt*. In contrast when the inoculum pressure is high, the nitrogen source acts more on preparasitic and parasitic phases.

Take-all development in the field is the result of early events which can be assessed by the measure in pots of soil conduciveness to disease. The measure of soil conduciveness 40 days after fertilization demonstrated at first the indirect influence of nitrogen sources. Thus, the early induced differences remained 'inscribed' in the soils and remained, independent of further evolution of nitrogen forms and pH values (from 5.9 to 6.3). After the second fertilizer application at tillering, the $(NH_4)_2SO_4$-treated soil is less susceptible than the $Ca(NO_3)_2$- and $NH_4NO_3$-treated soils. This rank of the conduciveness is the same as that observed for the disease in the field. The $(NH_4)_2SO_4$-treated soil is the less susceptible soil and the $Ca(NO_3)_2$-treated soil is the more susceptible one.

Forty days after a fertilizer application at sowing, the level of conduciveness does not differ between the $NH_4$- and $NO_3$-treated soils. A real difference is only observed after two fertilizer applications at sowing and tillering. The change is soil conduciveness is a dynamic process which settles down slowly. Two $NH_4$-fertilizations are necessary to increase significantly the resistance of these soils to take-all. On the other hand, in spite of its lower conduciveness, the $NH_4$-treated soil always induces some necrosis without artificial inoculum (*Ggt* dose 0). In the field, the $NH_4$-source would also benefit to the development of the natural inoculum.

The differences in soil conduciveness observed after tillering do not lead to the same magnitude and relationships for differences in the disease index in the field at the same stage. The beneficial effect of $NH_4$-source is observed only at flowering. This result fits with the idea of a dynamic evolution of soil resistance. The resistance increased slowly until a sufficient level is established to control the disease in the field. *Ggt* in the rhizosphere takes advantage of $NH_4$ fertilization and the decrease of conduciveness is reflected later as a reduced disease index. The period corresponds certainly to changes in the composition and numbers of soil microflora, like bacteria.

Without introduced inoculum, the number of fluorescent pseudomonads was lower in $NH_4$-treated soil than in $NO_3$-treated soil sampled 40 days after a fertilizer application at sowing. The

percentage of fluorescent pseudomonads which were antagonistic to *Ggt* in vitro was low for both nitrogen sources, and the soils exhibited equally high levels of conduciveness. Forty days after a second fertilization at tillering, the number of fluorescent pseudomonads was equal in the both fertilizer treatments, but the frequency of antagonistic bacteria became 1.6 to 2 times more important in the $NH_4$-treated soil than in the $NO_3$-treated soil. The evolution of soil conduciveness between sowing and tillering is parallel to the evolution of fluorescent pseudomonads. The populations of fluorescent pseudomonads are qualitatively and quantitatively changed during this period. A low level of conduciveness corresponded to a high frequency of in vitro antagonistic bacteria.

When inoculum of *Ggt* was introduced the number of bacteria increased. With a single fertilizer application at sowing which did not induce a difference in soil conduciveness, the numbers of fluorescent pseudomonads increased in parallel to the development of necrosis, but remained higher in $NO_3$-treated soil. Yet, after a second fertilizer application at tillering the numbers of fluorescent pseudomonads did not significantly differ in these soils.

In return, the frequency of antagonistic pseudomonads increased markedly in the $NH_4$-treated soil after tillering (respectively, 46 and 36% in 1987 and 1989) compared to the $NH_4$-treated soil after sowing (17%), and became higher than in $NO_3$-treated soil at tillering (32 and 24%).

A qualitative evolution of fluorescent pseudomonads could also be related to the differences in disease index. More than nitrogen sources, the development of root necrosis could play a role of induction. It is worthy to note that we had a 4.6 to 6 fold increase in numbers of fluorescent pseudomonads after introduction of *Ggt* (after fertilization at sowing and tillering). In 1989, this increase varied from 3.6 to 4.2 when total aerobic bacteria were increased only 1.5 fold. These results confirm the preferential stimulation of a part of fluorescent pseudomonads correlated to the lesion development. Most certainly, the increasing antagonistic activity of bacteria as measured in vitro is only one among other qualitative changes.

The role of necrosis has already been outlined by Rovira and Wildermuth (1981) to explain the phenomenon of take-all decline. The new sites of exudation resulting from necrotic lesions are

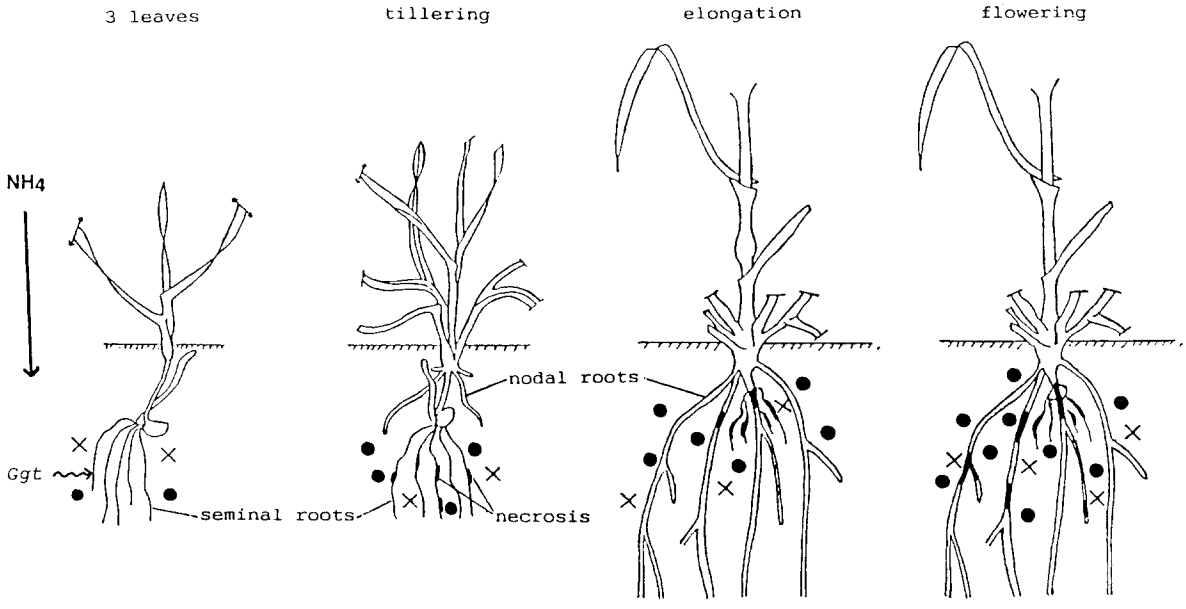


*Fig. 4.* Evolution of take-all on wheat and the dynamic evolution of populations of fluorescent pseudomonads (● antagonistic, ○ others) in a soil fertilized with ammonium nitrogen

privileged sites for the development of rhizobacteria (Vancura, 1980). Our results about field disease development and soil conduciveness allow to focus attention on preparasitic and parasitic phases of *Ggt*, where rhizobacteria seem to have a lead role in disease control.

The dynamic aspect of disease development has been studied in this work. The observations of different situations at different times gave greater insight into the process of disease evolution. The delayed relationship between field disease and soil conduciveness and microbial development allow to present a dynamic hypothesis, wherein necrosis plays a central role (Fig. 4).

Thus, in a $NH_4$-treated soil, the seminal roots are infected by *Ggt*. At this stage, no reliable control by the microorganisms can limit the lesion extension. However, on and close to the site of necrosis, the populations of rhizobacteria, including antagonistic fluorescent pseudomonads, increase. This first step involves qualitative and quantitative change in the rhizosphere microflora. Later, the nodal roots grow over the seminal roots, from the crown. These new roots profit from the changed microfloral environment. As *Ggt* infects these roots, the more numerous and still present antagonistic bacteria can develop and limit colonization of roots by hyphae and thereby limit the rates of extension of the necrosis.

Further studies performed on these fluorescent pseudomonads populations show that these qualitative changes are not only related to the antagonistic activity against *Ggt* in vitro but also to a better control of take-all in situ (Sarniguet et al. 1992).

## Acknowledgement

Comments and help from R W Smiley during redaction are gratefully acknowledged.

## References

Alabouvette C, Couteaudier Y and Louvet J 1982 Comparaison de la réceptivité de différents sols et substrats de culture aux fusarioses vasculaires. Agronomie 6, 273–284.

Christensen N W and Brett M 1985 Chloride and liming effects on soil nitrogen form and take-all of wheat. Agron. J. 77, 157–163.

Cook R J and Rovira A D 1976 The role of bacteria in biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol. Biochem. 8, 269–173.

Huber D M 1969 Effect of nitrogen fertilization on take-all of winter wheat. Phytopathology 59, 12.

Huber D M 1981 The role of nutrients and chemicals. *In* Biology and Control of Take-all. Eds M J C Asher and P J Shipton. p 317–341, Academic Press, London.

Hornby D and Goring C A I 1972 Effects of ammonium and nitrate nutrition on take-all disease on wheat in pots. Ann. Appl. Biol. 70, 225–231.

Hornby D 1975 Inoculum of take-all fungus: Nature, measurement, distribution and survival. EOPP Bull. 5, 319–333.

Lucas P, Sarniguet A, Collet J M and Lucas M 1989 Réceptivité des sols au piétin-échaudage (*Gaeumannomyces graminis* var. *tritici*): Influence de certaines techniques culturales. Soil Biol. Biochem. 21, 1073–1078.

Martin J K 1975 Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7, 401–402.

McNish G C and Speijers J 1982 The use of ammonium fertilizers to reduce the severity of take-all (*Gaeumannomyces graminis var. tritici*) on wheat in western Australia. Ann. Appl. Biol. 100, 83–90.

Rovira A D and Wildermuth G B 1981 The nature and mechanism of suppression. *In* Biology and Control of Take-all. Eds M J C Asher and P J Shipton. pp 385–415. Academic Press, London.

Sands D C and Rovira A D 1970 Isolation of fluorescent pseudomonads with a selective medium. Appl. Microbiol. 20, 513–514.

Sarniguet A 1990 Réceptivité des sols au piétin-échaudage du blé: Influence des rotations et de la fertilisation azotée en relation avec certains facteurs physicochimiques et les peuplements de *Pseudomonas* fluorescents. Thèse de doctorat No 1212, Univ. Paris XI-Orsay. 98 p.

Sarniguet A, Lucas P, Lucas M and Samson R 1992 Soil conduciveness to take-all of wheat: Influence of the nitrogen fertilizers on the structure of population of fluorescent pseudomonads. Plant and Soil 145, 29–36.

Skou J P 1981 Morphology and cytology of the infection process. *In* Biology and Control of Take-all. Eds M J C Asher and P J Shipton. pp 317–341, Academic Press, London.

Smiley R W 1978a Antagonists of *Gaeumannomyces graminis* from the rhizoplane of wheat in soils fertilized with ammonium- or nitrate-nitrogen. Soil Biol. Biochem. 10, 169–179.

Smiley R W 1978b Colonization of wheat roots by *Gaeumannomyces graminis* inhibited by specific soils, microorganism and ammonium-nitrogen. Soil Biol. Biochem. 10, 175–179.

Smiley R W and Cook R J 1973 Relationship between take-all of wheat and rhizosphere pH in soils fertilized with ammonium vs. nitrate-nitrogen. Phytopathology 63, 882–890.

Vancura V 1980 Fluorescent pseudomonads in the rhizosphere and their relation to roots exsudates. Folia Microbiol. 25, 168–173.

Weller D M and Cook R J 1986 Suppression of root diseases of wheat by fluorescent pseudomonads and mechanisms of action. *In* Iron, Siderophores, and Plant Diseases. Ed. T R Swinburne, pp 99–107. Plenum, New York.

Zadocks J C, Chang T T and Konzak C F 1974 A decimal code for the growth stage of cereals. Weed Research 14, 415–421.