SOIL-BORNE DISEASES OF WHEAT IN THE NETHERLANDS AND RESULTS OF SEED BACTERIZATION WITH PSEUDOMONADS AGAINST GAEMANNOMYCES GRAMINIS VAR. TRITICI

J.G. Lamers, B. Schippers, F.P. Geels

Research station for arable farming and field production of vegetables, Lelystad, The Netherlands

1) Willie Commelin Scholten, Phytopathological Laboratory, Baarn, The Netherlands
2) Present address: Mushroom Experimental Station, Horst, The Netherlands

Summary

In the Netherlands wheat and barley are affected to a limited extent by soil-borne pests and diseases like Meloidogyne naasi, Pseudocercosporella herpotrichoides, Rhizoctonia cerealis and Gaemannomyces graminis var. tritici. In 1984 take-all was observed in wheat in 10% of the fields sampled (5% infected culms). Experiments have been set up to investigate the possibilities of biological control of take-all in wheat by Pseudomonas fluorescens. In the first year of monoculture of spring wheat, Pseudomonas isolates were used as a seed treatment in take-all conducive soil with or without inoculation of G. graminis. The inoculation with oat kernel inoculum caused 100% diseased plants and resulted in a yield of 10% compared to untreated. Bacterization of the seed with P. fluorescens strain WCS 532 or strain Co 2-79 together with inoculation of the soil with G. graminis increased the yield significantly up to 15%. In the second year, without additional treatments in the experiment, the untreated plots showed 27% of white heads, while after G. graminis inoculation in the preceding year the soil had become take-all suppressive and no white heads were observed. The bacterization with P. fluorescens strain WCS 417 in the preceding year had limited the natural build-up of G. graminis. Only 6% white heads had developed in these fields and yield was increased significantly.

Keywords: bacterization, biological control, Gaemannomyces graminis var. tritici, Pseudomonas fluorescens, soil-borne pathogens, take-all, Triticum aestivum.

Introduction

Biological control has become a major topic in research on take-all disease of wheat and barley, caused by the fungus Gaemannomyces graminis var. tritici (Hornby, 1987). Control by resident soil micro-organisms occurs naturally under certain cropping regimes (Gerlach, 1968) and may be manipulated to some extent. Of the resident organisms, Pseudomonas-bacteria have been a favoured explanation for take-all decline. Take-all decline is accredited with helping many farmers in various parts of the world to grow wheat or barley continuously. A survey is presented of the severity of some soil-borne diseases of wheat in the Netherlands leading to symptoms at the stem base or at the roots. In field experiments the biological control of take-all was studied; special attention was given to long lasting effects of seed-treated Pseudomonas fluorescens.
incidence of stem base and root diseases

In the Netherlands the cereals play a minor part in crop rotation. Most farmers grow wheat or barley once in two or three years. On marine clay soil in the northern region of the Netherlands more cereals are grown (45% of the arable land) soil as well as on loess in the south of the Netherlands (33%). The cereal acreage consists mainly of winter wheat (65%) and spring barley (20%).

The significance of stem base diseases of winter wheat can be derived from the presence of various fungi in fields and from the average incidence of these fungi. Stol (1985) and Hoek (1986) made a survey of all cereal diseases in the Netherlands during the growing season. They sampled 40 culms from 100-150 fields during grainfilling for disease assessments. Pseudocercosporella herpotrichoides was the most important fungus and was observed in 79% of the fields in the period of 1979-1986 (table 1). In these fields the average eyespot incidence was low and amounted to 18% (table 2). An eyespot incidence of more than 70% is considered as being harmful for grain yield. Therefore, it is assumed for many fields that P. herpotrichoides will not have caused yield losses. The low incidence must be ascribed to the low frequency of cereal cropping, as well as to the effectiveness of chemical sprayings. According to the Epire-programme (Reinink, 1986) 12% of the fields were sprayed. Eyespot was commonly controlled by carbendazim-products. Resistance against this chemical was found only in the northern part of the Netherlands (Sanders et al, 1986). Rhizoctonia cerealis was present in 30% of the fields. The fields with sharp eyespot symptoms had an incidence of 8%. Fusarium spp. attacked 8% of the culms in 58% of the fields. The development of G. graminis var. tritici seems to be restricted to special years. Only in 1984 the presence was significant, when take-all symptoms were found on 5% of the culms in 10% of the fields.

In barley about the same severity of stem base diseases is found as in wheat. Other soil-borne diseases attacking foliage or ears are not mentioned in this paper.

A survey of harmful eelworms in the northern part of the Netherlands in fields with a high percentage of cereals in the crop rotation, revealed that only 3 of the 20 fields had been infested by Meloidogyne naasi and 2 of the 20 fields by Heterodera avenae (Brinkman en De Moel, 1985). So it seemed that eelworms are not a major problem in cereal growing.

Table 1. Percentage of fields with symptoms of different wheat stem diseases.

<table>
<thead>
<tr>
<th></th>
<th>79</th>
<th>80</th>
<th>81</th>
<th>82</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. herpotrichoides</td>
<td>87</td>
<td>85</td>
<td>89</td>
<td>74</td>
<td>92</td>
<td>74</td>
<td>80</td>
<td>52</td>
<td>79</td>
</tr>
<tr>
<td>R. cerealis</td>
<td>13</td>
<td>28</td>
<td>22</td>
<td>33</td>
<td>28</td>
<td>47</td>
<td>34</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>60</td>
<td>43</td>
<td>63</td>
<td>92</td>
<td>60</td>
<td>45</td>
<td>55</td>
<td>45</td>
<td>58</td>
</tr>
<tr>
<td>G. graminis var. tritici</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Percentage of diseased stems in fields with symptoms of wheat stem base diseases.

<table>
<thead>
<tr>
<th></th>
<th>79</th>
<th>80</th>
<th>81</th>
<th>82</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
<th>average</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. herpotrichoides</td>
<td>23</td>
<td>9</td>
<td>23</td>
<td>13</td>
<td>38</td>
<td>13</td>
<td>20</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>R. cerealis</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>13</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>G. graminis var. tritici</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

135
Biological control of take-all

Materials and methods

At the Willy Commelin Phytopathological Laboratory of the Universities of Utrecht and Amsterdam fluorescent pseudomonads have been isolated from lesions of wheat roots growing in take-all decline soil. The isolation and selection of the pseudomonads for control of take-all was similar to that of plant growth promoting rhizobacteria for control of deleterious micro-organisms in a soil with a high frequency of potato cropping (Geels and Schippers, 1983a and 1983b). Many isolates were screened in the field for control of take-all. In this experiment Pseudomonas fluorescens strain WCS 417 and P. fluorescens strain WCS 532 were used. R.J. Cook kindly provided subcultures of one of their most promising P. fluorescens strain Co 2-79.

In vitro tests showed that these strains produced siderophores and antibiotics or other growth inhibiting substances.

Wheat seeds were surface-sterilized by 3% sodium hypochlorite. The bacteria were grown on King's Medium B, suspended in a solution of 1.0% methyl cellulose and mixed with wheat seeds (Weller and Cook, 1983).

For the preparation of inoculum of G. graminis var. tritici (ggt), a virulent isolate growing on PDA-plates was used for the infection of autoclaved whole oat seeds. Before drilling in the field 13 g/m² of these infested oat kernels were mixed with 15 g/m² of wheat seeds.

The field trial was situated in a rotation experiment (Lamers, 1981) at a marine loam soil near Lelystad. The soil pH was 7.5. The spring wheat variety Stratos was used and fertilised with 60 kg N/ha (nitrochalk). During 11 years preceding the experiment only potatoes and sugar beets were grown. In the first year of wheat monoculture (1984), treatments included bacterization of seeds with P. fluorescens strains and inoculation of field soil with ggt oat seed inoculum. In the second year (1985), spring wheat was sown on the same fields, but seeds were not treated with P. fluorescens strains and the soil was not inoculated with ggt. Between the first and second year of monoculture, the soil was ploughed late on 7 February 1985 and spring wheat was sown early on 28 February 1985. The fields measured 4 x 13 m² and 30 m² was combine harvested. The experimental design was a randomized block with 4 replicates. Analysis of variance was carried out on the results of the measurements and a separate analysis was made for the results of 1984 to test the significance of individual P. fluorescens treatments compared to the treatment with inoculation of ggt.

Results

In the 1984 experiment, marked differences in crop development were shown to be due to ggt inoculation. In the inoculated fields significant more wheat plants carried runner hyphae on seminal roots (table 3). These roots were almost dead, while those of untreated fields were slightly affected. The plants in the inoculated fields were stunted (0.33 m plant length) and many did not develop ears. The yield was significantly reduced to 10% of the control, caused by a significant lower number of ears per m² (30% of the control) and a significant lower weight of the grains (60% of the control).

Seed treatment with P. fluorescens strain WCS 532 and Co 2-79 reduced the effects of take-all inoculation. The number of plants with runner hyphae on seminal roots was reduced from 98 to 80% (strain WCS 532 significant at p = 0.10), the length of plants increased to 0.38 m (significant at p = 0.10) and yield increased significantly from 10% to resp. 15% and 14% for P.
fluorescents strain WCS 532 and Co 2-79. This yield increase was the result of an increase in the number of ears per m².

The P. fluorescents strain WCS 417 with or without inoculation of ggt did not have an effect on take-all incidence or plant growth when compared to the inoculated resp. untreated fields.

In the second year 1985, in the same plots the residual effects of the seed bacterization and soil inoculation with ggt were studied.

Table 3. Runner hyphae on seminal roots of plants, crop development and grain yield after G. graminis var. tritici inoculation and seed bacterization with different strains of P. fluorescents in 1984.

<table>
<thead>
<tr>
<th>Take-</th>
<th>P.</th>
<th>Runner</th>
<th>Plant</th>
<th>Ears</th>
<th>Grain</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>all flou.</td>
<td>hyphae</td>
<td>height</td>
<td>(no./m²)</td>
<td>weight</td>
<td>(g/m²)</td>
<td>(mg)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>45</td>
<td>58.1</td>
<td>480</td>
<td>48.0</td>
<td>615 (100)</td>
</tr>
<tr>
<td>-</td>
<td>WCS 417</td>
<td>98*</td>
<td>33.0*</td>
<td>145*</td>
<td>28.1*</td>
<td>59* (10)</td>
</tr>
<tr>
<td>+</td>
<td>WCS 417</td>
<td>33.0*</td>
<td>114*</td>
<td>26.9*</td>
<td>63* (10)</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>WCS 532</td>
<td>80</td>
<td>38.1*</td>
<td>200*</td>
<td>26.3*</td>
<td>90* (15)</td>
</tr>
<tr>
<td>+</td>
<td>Co 2-79</td>
<td>80</td>
<td>38.0*</td>
<td>193*</td>
<td>24.8*</td>
<td>83* (14)</td>
</tr>
</tbody>
</table>

* significant (p = 0.05) compared to untreated
+ significant (p = 0.05) compared to G. graminis var. tritici inoculation without bacterization.

Table 4. Soil cover (17 May), white heads (31 July), yield components and yield in the second year of spring wheat monoculture (1985). Treatments were carried out in 1984.

<table>
<thead>
<tr>
<th>Take-</th>
<th>P.</th>
<th>Soil</th>
<th>White</th>
<th>Ears</th>
<th>Grains</th>
<th>Grain</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>all flou.</td>
<td>cover</td>
<td>heads</td>
<td>(no./m²)</td>
<td>(no./ear)</td>
<td>weight</td>
<td>(g/m²)</td>
<td>(%)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>67</td>
<td>26.6</td>
<td>371</td>
<td>39.6</td>
<td>41.9</td>
<td>613 (100)</td>
</tr>
<tr>
<td>-</td>
<td>WCS 417</td>
<td>65</td>
<td>6.2*</td>
<td>432*</td>
<td>37.1</td>
<td>41.4</td>
<td>661* (108)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>44*</td>
<td>0.1*</td>
<td>448*</td>
<td>36.6</td>
<td>42.7</td>
<td>698* (114)</td>
</tr>
<tr>
<td>+</td>
<td>WCS 417</td>
<td>39*</td>
<td>0.1*</td>
<td>456*</td>
<td>34.9*</td>
<td>42.8</td>
<td>681* (111)</td>
</tr>
<tr>
<td>+</td>
<td>WCS 532</td>
<td>49*</td>
<td>0.0*</td>
<td>433*</td>
<td>37.4</td>
<td>43.3</td>
<td>698* (114)</td>
</tr>
<tr>
<td>+</td>
<td>Co 2-79</td>
<td>48*</td>
<td>0.1*</td>
<td>442*</td>
<td>36.0*</td>
<td>43.3</td>
<td>688* (112)</td>
</tr>
</tbody>
</table>

L.S.D. 1) 17.3 (5.9) 51 3.2 2.4 43

* significant (p = 0.05) compared to untreated. 1) according to the Studentized range test of Tukey (p = 0.05)

On 17 May 1985 the estimated soil cover by wheat was significantly reduced from 67% to 44% in fields inoculated with ggt in 1984. However, on 31 July these fields had become take-all decline as no white heads had developed (table 4). In the untreated fields 27% of the ears showed white heads. After initial inoculation the yield was significantly increased to 114% because of a significant higher number of ears per m².

None of the P. fluorescents strains showed significant effects on yield (components) in the second year if combined with ggt inoculation. P. fluorescents strain WCS 417 without ggt inoculation did not affect the soil cover in May, but did reduce the percentage of white heads in July highly significantly from 27% to 6%. The grain yield increased significantly to 108% by a higher number of ears per m². P. fluorescents strain WCS 532 and Co 2-79 did not show an effect in 1985.
Discussion

In 1984 and 1985 wheather was favourable for take-all (table 1 and 2). In 1984, a dramatic effect on plant development and yield occurred as a consequence of the inoculation with ggt. In the beginning of 1985, the negative effects of the inoculation with ggt in the preceding year still existed as a lower soil cover was measured in May. The thin stand in 1985 indicated disappearance of plants. Tillering and ear formation must have overcompensated for this negative effect rapidly, as the number of ears at harvest was high. In the second year take-all developed intensively at low temperatures early in the season on seminal roots leading to some plant death but was controlled by antagonists on nodal roots. In 1984 after one month of growth, residual ggt occupied 45% of the seminal roots of the wheat plants in untreated fields. This early presence was not reflected in a growth reduction of the crop. Also in May 1985 less inoculum was present in the untreated soil as soil cover was good, compared to the inoculated fields. However, during the period of tillering and stem elongation the natural developed inoculum must have destroyed the nodal roots as many white heads appeared. It may be expected that in the third year take-all severity in winter wheat will decline.

Rovira and Wildermuth (1981) proposed that take-all decline develops because of a gradual build-up of inhibitory bacteria (among which pseudomonads) in infested root debris with several years of monoculture. These bacteria are supposed to retard the growth of ggt from the debris towards the root and/or to cause lysis of the hyphae. These suppressive bacteria are thought to build-up initially in lesions on roots of living plants as secondary colonists of the infected tissues and then carry over with the pathogen in crop residue where they limit the ability of the pathogen to attack the next crop. Kloepper et al. (1980) demonstrated that fluorescent pseudomonads had at least a partial role in take-all decline. Also Weller (in Cook and Baker, 1963) showed that the number of fluorescent Pseudomonas spp. inhibitory to ggt were greater on roots in suppressive than in conducive soils.

When the inhibitory P. fluorescens strain WCS 532 was introduced into conducive soil on the seed and sown together with oat kernels colonized by ggt a significant (p=0.10) decrease in the number of plants with runner hyphae on seminal roots and a significant increase in yield was measured. Strain Co 2-79 behaved similarly as WCS 532. The inoculation with take-all however overruled the effects of bacterization.

During the first year and at the beginning of the second year no effect of seed bacterization with P. fluorescens strain WCS 417 on plant development was observed. However, the natural build-up of take-all was significantly controlled by strain WCS 417 and no peak in take-all severity was seen suggesting the soil was made suppressive. The yield was significantly increased. These effects two years after application are surprising (Lamers et al, 1986) because the strain WCS 417 did not show any control of inoculated ggt in the first year. As strain WCS 532 and Co 2-79 were more suppressive the first year, an even better control by these isolates was expected in the second year.

Cook and Weller (1987) do not expect that introduction of a single strain of Pseudomonas may suppress take-all as consistently or to the same degree as occurs in soils where take-all has declined. This study indicates that suppressive strains must be introduced from the onset of ggt establishment in the wheat crop, that is from the very beginning when wheat or barley is grown continuously. A selection of inhibitory pseudomonads within the lesion will take place from the start. An introduction of suppressive
strains in the second or later years might give less results as more
natural selected inhibitory pseudomonads may overrule the effects of the
introduced strain. This may explain why Hornby (1987) found no control of
take-all with strain Co 2-79 and why Cook (1985) got a positive response in
only one-half to two-thirds of his trials. Five other experiments with P.
fluorescens in conducive and suppressive soils at Lelystad did show some
significant influences on take-all control, but in most experiments take-
all was not severe enough to show many white heads or important yield
depressions.

References

Brinkman, H. & C.P. de Moel, 1985. Het voorkomen van het graswortelknob-
belaatje bij intensieve teelt van granen in het noordelijk kleigebied en

Cook, R.J., 1985. Biological control of plant pathogens: theory to appli-

control of plant pathogens. American Phytopathological Society, St. Paul,
Mn, 539 pp.

crops of wheat or barley. In: I. Chet.: Innovative approaches to plant
disease control. John Wiley & Sons, USA. 41-76.

Geels, F.P. & B. Schippers, 1983a. Selection of antagonistic fluorescent
Pseudomonas spp. and their root colonization and persistence following

Geels, F.P. & B. Schippers, 1983b. Reduction of yield depressions in high
frequency potato cropping soil after seed tuber treatments with antago-

Garlagh, M. 1968. Introduction of Ophiobolus graminis into new polders

Proefstation voor de Akkerbouw en Groenteteelt in de Vollegrond, Lelystad,
13 pp.

Hornby, D., 1987. Field testing putative biological controls of take-all:

Microbiol. 4: 317-320.

Lamers, J.G., 1981. Potatoes and sugar-beets in monoculture and intensive
rotations. Publikatie nr. 12, Proefstation voor de Akkerbouw en de Groente-
teelt in de Vollegrond, Lelystad, 64 pp.


Reinink, K., 1986. Experimental verification and development of Epiprep, a
92: 3-14.

Rovira, A.D. & G.B. Wildermuth, 1981. The nature and mechanisms of
suppression. In Asher, M.J.C. & P.J. Shipton: Biology and control of take-

Sanders, P.L., M.A. de Waard & W.M. Loerakker, 1986. Resistance to car-
bendazim in Pseudocercosporella herpotrichoides from dutch wheat fields.

Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond,
Lelystad, 15 pp.

treatments with fluorescent pseudomonads. Phytopathology 73: 463-469.
Cereal breeding related to integrated cereal production

Proceedings of the conference of the Cereal Section of EUCARPIA (European Association for Research on Plant Breeding), Wageningen, Netherlands 24-26 February 1988

M.L. Jorna and L.A.J. Slootmaker (compilers)

Pudoc Wageningen 1988