# Microorganisms Colonising the Rhizosphere of Winter Wheat Protected with Impact Super 347 SC Fungicide and Bion 50 WG Plant Resistance Stimulator

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**Abstract**

In a field experiment, the effect of plant protection agents on fungal colonies colonising the rhizoplane and the rhizosphere of Elena winter wheat was determined. Impact super 347 SC (flutriafol and chlorotalonil) limited the number of fungi representing *Trichoderma*, promoted the growth of both fungi from the *Fusarium* gene and bacteria of *Pseudomonas* in addition to tricalcium phosphate hydrolysing bacteria. The plant resistance stimulator Bion 50 WG (acybenzolar-s-methyl) strongly reduced the population of *Actinomycetales*. Bacteria representing *Azotobacter* did not respond to the applied agents.

**Keywords**: fungicides; rhizosphere; wheat

## INTRODUCTION

For many years, the negative effect of plant protection agents on microorganisms affecting soil productivity has been observed (GOVINDARAJAN & PURUSHOTHAMAN 1988; MARTYNIUK *et al*. 1991; PIOTROWSKI *et al*. 1995). Attempts at combining chemical control with biological control (HUANG *et al*. 2001) have produced the need for testing the effect of new fungicides on natural plant pathogens antagonists. The aim of the experiment was to evaluate the effect of Bion 50 WG plant resistance stimulator and Impact Super 347 SC fungicide on the natural balance between the plant pathogens and the microorganisms affecting soil productivity and protecting plants against pathogens from the soil.

## MATERIALS AND METHODS

Field experiments were carried out on 6 $m^2$ plots in 1998–1999 in the north-eastern region of Poland. The experiment was established based on the randomly selected blocks in four series. Elena winter wheat was protected with both Bion 50 WG plant resistance stimulator (60 g/ha) and Impact Super 347 SC fungicide (21 g/ha) at the beginning of culm formation.

The microbiological material were plant samples (including roots) taken 48 hours following the application of the agents. Loose soil was removed from the roots by intense shaking. Conical flasks were filled with 90 ml of sterile water and 10 g of roots with the attached soil. The flasks were shaken for 30 min in a table-top shaker type 358S (at a frequency of 170/min and amplitude of 8).

Fungi colonising the rhizoplane were obtained by placing 1 cm fragments of roots on a PDA medium. Each morphologically different colonies were transferred on separate agar-agar slants. After a 30-day incubation, the fungi were identified under a microscope.

The microbiological analysis of the rhizosphere was carried out with the use of an in-depth culture (MARTYNIUK *et al*. 1991) with the use of the following media: Martin's, King B, Burk's, Pikowska's, Graf's (KOBUS *et al*. 1993; PIOTROWSKI *et al*. 1995). The experiment was carried out in 5 series. Based on the number of colonies in the dish, the number of microorganisms in 1 kg of fresh soil was determined. The developing fungal colonies were identified under a microscope.

## RESULTS

In total, 185 fungal colonies (Table 1) were isolated from the surface of the roots of the Elena winter wheat during the phase of culm formation. Potentially pathogenic fungi from the genus of *Fusarium* are worth mentioning. In total, 38 isolated colonies represented six fungal genera: *F. avenaceum, F. nivale, F. culmorum, F. oxysporum, F. sambucinum, F. solani* (Table 1). Fusarium fungi were more frequently isolated from the root surface of the winter wheat protected with the agents than in the control plants. This tendency

Table 1. Fungi colonising the rhizoplane of Elena winter wheat cultivar treated pesticides (Tomaszkowo 1998–1999)

| Species of fungi | Number of colonies | | | | | | Sum 1998–1990 |
|---|---|---|---|---|---|---|---|
| | Control | | Bion 50WG | | Impact Super 347 SC | | |
| | 1998 | 1999 | 1998 | 1999 | 1998 | 1999 | |
| *Acremonium* spp.* | | | 1 | 1 | | 2 | 4 |
| *Aphanocladium album* (Preuss) W. Gams | 2 | | 1 | | | | 3 |
| *Chrysosporium pannorum* (Link) Hughes | 2 | | 1 | | | | 3 |
| *Cladosporium macrocarpum* Preuss | | | | 1 | | | 1 |
| *Cladosporium sphaerospermum* Penz. | | | | | 1 | | 1 |
| *Cylindrocarpon destructans* (Zins.) Scholten | | | | 1 | 1 | 1 | 3 |
| *Drechslera* sp. | | | | | 1 | | 1 |
| *Fusarium avenaceum* (Fr.) Sacc. | | | | | 1 | 2 | 3 |
| *Fusarium culmorum* (W. G. Sm.) Sacc. | 2 | 1 | 3 | | | 2 | 8 |
| *Fusarium nivale* (Fr.) Ces. **** | | | | | | 1 | 1 |
| *Fusarium oxysporum* Schlecht. | 4 | | 4 | | 2 | | 10 |
| *Fusarium sambucinum* Fuckel | | | 1 | | | | 1 |
| *Fusarium solani* (Mart.) Appel et Wollenw. | | 2 | | 1 | | 3 | 6 |
| *Fusarium* sp. | | 1 | 2 | 1 | 3 | 2 | 9 |
| *Gliomastix murorum* (Corda) Hughes | | | | | | 1 | 1 |
| *Humicola grisea* Traaen | | | | 1 | | 1 | 2 |
| *Mortierella polycephala* Coemans | | | | | 1 | | 1 |
| *Mucorales*** | 3 | | 1 | | 8 | 7 | 19 |
| *Paeciliomyces carneus* (Duche et Heim) Brown et G. Smith | | | | 1 | | | 1 |
| *Penicillium thomii* Maire | 1 | | | | | | 1 |
| *Penicillium waksmanii* Zaleski | 1 | | | | | | 1 |
| *Penicillium* sp. | 2 | 2 | 4 | 2 | | 1 | 11 |
| *Phoma* sp. | | | | 1 | | 1 | 2 |
| *Rhizoctonia solani* Kühn | | | | 1 | | | 1 |
| *Scopulariopsis chartarum* (G. Smith) Morton et G. Smith | | | | | | 1 | 1 |
| *Trichoderma aureoviride* Rifai | | 6 | | 5 | | 2 | 13 |
| *Trichoderma hamatum* (Bon.) Bain. | 2 | 5 | 2 | 7 | 1 | 6 | 23 |
| *Trichoderma harzianum* Rifai | | 6 | 1 | 6 | 1 | 6 | 20 |
| *Trichoderma konigii* Oud. | 2 | 3 | 3 | 3 | | 3 | 14 |
| *Trichoderma longibranchitum* Rifai | | | 1 | | | | 1 |
| *Trichoderma viride* Pers. ex S. F. Gray | 3 | | 1 | | | | 4 |
| *Verticillium tenerum* Ness | | | | | | 2 | 2 |
| Other*** | | 1 | 1 | 1 | | 6 | 9 |
| Dark nonsporulating | | | | 1 | | 1 | 2 |
| Light nonsporulating | 1 | | | | 1 | | 2 |
| Total | 25 | 27 | 27 | 34 | 21 | 51 | 185 |

* *A. charticola* (Lindau) W. Gams, *A. murorum* (Corda) W. Gams, *A. roseo-griseum* (S. B. Saksena) W. Gams, *A. strictum* W. Gams

** *Mucor hiemalis* Wehmer, *Mucor racemosus* Fres., *Rhizopus nigricans* Ehrenberg

*** *Chrysosporium* sp., *Mortierella* sp., *Pseudeurotium* sp., *Pyrenochaeta* sp., *Scopulariopsis* sp., *Spicaria* sp.

**** *Microdochium nivale* (Fr.) Sam. et Hall.

Table 2. Fungi colonising the rhizosphere of winter wheat treated with pesticides (Tomaszkowo 1998–1999)

| Species of fungi | Number of colonies | | | | | | Sum 1998–1999 |
|---|---|---|---|---|---|---|---|
| | Control | | Bion 50WG | | Impact Super 347 SC | | |
| | 1998 | 1999 | 1998 | 1999 | 1998 | 1999 | |
| *Acremonium* spp.* | 3 | | 10 | 2 | | | 15 |
| *Alternaria alternata* (Fr.) Keissler | | | | 1 | | | 1 |
| *Cladosporium herbarum* (Pers.) Link ex S. F. Gray | | | | | | 1 | 1 |
| *Cladosporium macrocarpum* Preuss | | 5 | | 24 | | 6 | 35 |
| *Cladosporium sphaerospermum* Penz. | 11 | | 7 | | | | 18 |
| *Cladosporium* sp. | 1 | | 1 | | | | 2 |
| *Drechslera* sp. | | | | | 1 | | 1 |
| *Fusarium avenaceum* (Fr.) Sacc. | | 2 | | | 1 | 3 | 6 |
| *Gliocladium catenulatum* Gilman et Abbott | | | | 1 | | | 1 |
| *Gliomastix murorum* (Corda) Hughes | 1 | | | | | | 1 |
| *Mucor hiemalis* Wehmer | | | | | 2 | | 2 |
| *Paeciliomyces variotii* Bainier | | | 1 | | | | 1 |
| *Penicillium* sp. | | 13 | | 29 | 2 | 28 | 72 |
| *Pseudeurotium* sp. | 13 | | 1 | | | | 14 |
| *Pyrenochaeta* sp. | | | | 16 | 1 | | 17 |
| *Trichoderma harzianum* Rifai | 2 | | 1 | | 1 | | 4 |
| Yeast-like fungi | 66 | 402 | 32 | 308 | 50 | 386 | 1244 |
| Other** | | | 1 | | 2 | 2 | 5 |
| Dark non-sporulating | 1 | 8 | | 1 | 3 | 7 | 20 |
| Light non-sporulating | | | | | | 1 | 1 |
| Total | 110 | 431 | 89 | 381 | 68 | 441 | 1520 |

* *A. breve* (Sukap. & Thirum) W. Gams, *A. roseo-griseum* (S. B. Saksena) W. Gams, *A. strictum* W. Gams
** *Chaetomella* sp., *Epicoccum* sp., *Mortierella* sp., *Trichocladium* sp.

was particularly clear in plants protected with Impact Super 347 SC fungicide.

At the phase of culm formation, the roots of winter wheat were heavily colonised by fungi from *Trichoderma* (40.5% of the total population). As many as six species were isolated at that time: *T. hamatum, T. harzianum, T. viride* as well as rare species such as *T. koningii, T. longibranchitum* and *T. aureoviride*. The application of Impact Super 347 SC fungicide reduced the fungal population of *Trichoderma*.

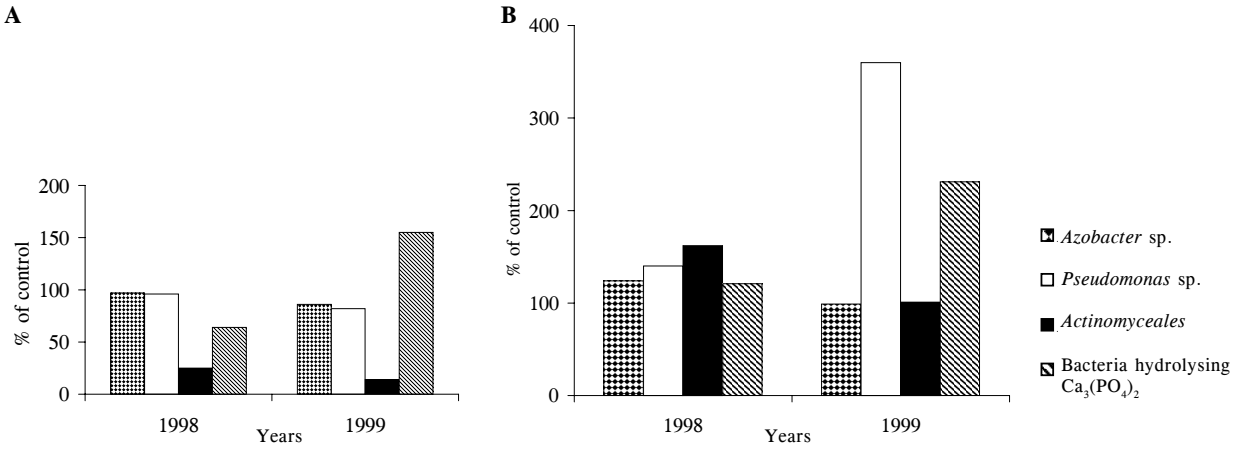


Figure 1. Rhizobacteria colonising Elena winter wheat cultivars treated Bion 50WG (A) and Impact Super 347 SC (B)

The fungal population colonising the rhizosphere was very numerous and varied (Table 2). Yeast-like fungi were dominant and constituted as much as 81.8% of the total isolated population. They were very sensitive to the applied plant protection agents and were eliminated. Their place was taken by other saprophytes such as *Penicillium* and *Cladosporium*.

Tricalcium phosphate hydrolysing bacteria developed more dynamically on the protected plants than on the control plants (Figure 1). The application of plant protection agents did not affect the number of free-nitrogen-fixing bacteria (Figure 1). The strength and the direction of the activity of these agents on *Pseudomonas* and *Actinomycetales* varied. In 1999, the *Pseudomonas* bacteria fed with the changed root secretions developed significantly better in the rhizosphere of the plants protected with Impact Super 347 SC. The number of *Actinomycetales* bacteria was reduced, especially after application of Bion 50 WG plant resistance stimulator (Figure 1).

## DISCUSSION

Similar to experiments by other authors (PIOTROWSKI *et al*. 1995), plant protection agents modified the composition of the microorganism population colonising the root system of winter wheat. The increase in the fungal population, as previously observed (PIOTROWSKI *et al*. 1995; RODGERS-GRAY & SHAW 2001) and the bacteria constant number or their reduction may lead to a decrease in soil productivity. The negative effect of Impact Super 347 SC fungicide on the *Trichoderma* fungi is also disturbing. These fungi are strong antagonists towards plant pathogens (KLEIFELD & CHET 1992).

The experiment did not confirm previous reports of the high sensitivity of *Azotobacter* bacteria to fungicides (MARTYNIUK *et al*. 1991).

The results support the suggestion of HUANG *et al*. (2001) that *Pseudomonas* bacteria can develop better with fungicides. This is a positive phenomena because these bacteria protect wheat against infection with *Gaeumannomyces graminis* (MARTYNIUK *et al*. 1991; HUANG *et al*. 2001).

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