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Effect of Oxafun T Seed Dressing on Bacteria in Rhizosphere and non-Rhizosphere Soil

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Abstract

On plots under barley culture from seeds treated and untreated with the fungicidal preparation Oxafun T, the total numbers of oligotrophic and copiotrophic bacteria, as well as the numbers methylotrophic and *Pseudomonas* bacteria, were determined in rhizosphere and in non-rhizosphere soil. It has been found that in non-rhizosphere soil the application of the seed dressing did not significantly change the mean number of the studied bacteria for the period of barley culture. It did however, contribute to the proliferation of these microorganisms in the rhizosphere zone. Rhizosphere effect of barley was increased to the degree dependent on the group of determined bacteria.

Keywords: fungicide, barley, rhizosphere, bacteria

Introduction

Fungicides, irrespective of the method of application, always reach the soil where in microbial communities they may induce unintended effects. They not only control fungal plant pathogens, but also act in a different way on saprophytic microorganisms, among them on bacteria, i.e. on their survival, growth, interrelations and processes that they carry out [3, 17].

From the review by Domsch [3] concerning studies of many authors it follows that in the case of fungicide application at rates not exceeding field doses, the discussed side effects are noted comparatively seldom, if the sensitivity of the system nodule bacteria-leguminous plant is omitted. This statement may, however, result from the fact that the soil of experimental objects in these studies was examined as a whole, without distinguishing the zone in the nearest neighborhood of a cultured plant, i.e. rhizosphere. Rhizosphere seems to be a zone in which fungicide action is particularly concentrated, among others, as a consequence of seed, tuber, bulb and plant root dressing with these preparations. Besides, in the case of application plant spraying with systemic fungicides, some of them are excreted in the unchanged form from plants through the roots into the soil [24]. In rhizosphere, where conditions are especially favourable to the development of microorganisms, the applied fungicides are decomposed more intensively than in non-rhizosphere soil, which leads to accumulation of cells of the microorganisms causing these processes [23]. Moreover, it is nearly a textbook information that spraying of plants with pesticides modifies their metabolism, which, among others, determines root development. It also determines a quantitative and qualitative composition of root excretions, which are easily assimilable sources of carbon and energy for rhizosphere microorganisms. This is detected not only after the application of herbicides [6], as pesticides acting on plants by nature, but also after the application of fungicides [1, 20].

This study has also been devoted to side microbiological effects of fungicide application. It presents results of one-year studies on the action of the seed dressing Oxafun T at the dose recommended by agricultural service on the number of barley rhizosphere bacteria from different groups, which are more or less characteric of that environment.

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Material and Methods

The experiments were carried out in 1999 in barley crop of the cv. Polo, on experimental fields in Ziotniki belonging to the Experimental-Didactic Department of A. Cieszkowski University of Agriculture in Poznan. The experiments were arranged in a randomized block design, with four replications, on light loamy sand at pH H_2O 5.6, containing 0.7% organic C and 12.5% silt and clay jointly.

Barley was grown on plots 25 m x 2.25 m in size. Some plots were sown with seeds (400 seeds/m^2, with rows spaced at 11 cm) dressed with Oxafun T (250 g fungicide/100 kg seed), others were sown with untreated seeds (control plots). Oxafun T contained 37.5% carboxine and 37.5% thiuram.

At the developmental stages of tillering, shooting, end of heading, blooming, milk and ripe maturity, samples were taken from plots between the rows and from soil closely adhering to plant roots. Soil samples from row-spacings were collected by Egner's sampling stick and recognized for non-rhizosphere soil, despite the awareness of this inaccuracy. To obtain samples of soil closely adhering to plant roots, the root systems were taken out from the earth, transferred to a laboratory and after a slight drying and shaking off loosely attached soil particles, they were rinsed with tap water and the obtained rinsed soil was recognized as a rhizosphere soil. Thus prepared samples of rhizosphere and non-rhizosphere soils were used for a serial-dilution set to determine bacterial numbers by the plate method. The following numbers were determined:

1. The total number of copiotrophic bacteria, using strength nutrient broth, the so-called NB medium [18], and a 5-day incubation of plates.
2. The total number of oligotrophic bacteria, using a 100-fold diluted broth medium, the so-called DNB-100 medium, and a 21-day incubation of plates.
3. The number of bacteria assimilating methanol as a sole carbon and energy source, using a medium ace. to Urakami and Kamagata [21], modified by the addition of 5 ml methanol per 1 1 [15], and a 5-day incubation of plates. The number of these bacteria was considered as an indicator of the number of methylotrophic bacteria.
4. The number of pseudomonads, using a selective medium ace. to Grant and Holt [5] and a 2-day incuba tion of plates. To distinguish fluorescent and nonfluorescent forms, bacteria from 100 randomly selected colonies were transferred onto King's B medium [17] and after 3 days of incubation examined for fluorescence under ultraviolet rays.

In all the bacteria counts the plates were incubated at 28°C.

Results and Discussion

Results of determinations performed in the soil of control plots (Tables 1-3) do not indicate that barley is a plant creating considerable rhizosphere effects. Since in these determinations the soil between rows (which is not so distant from plant roots and, therefore, is probably incompletely devoid of them) was recognized for non-rhizosphere soil, we suggest that the determined rhizosphere effect has been somewhat underestimated. At particular times of analyses, mostly only several times more bacteria from the studied groups were found in the rhizosphere than in the non-rhizosphere soil. Exceptions were values usually found at full maturity stage, when the most increases in the bacteria number were noted more than several times. However, since plant roots looked at this time like dying or dead, we have doubts whether these large effects can be called rhizosphere i.e. generated by the roots of growing plants. Their appearance was rather indicative of their microbiological decomposition. For that reason, the discussed values have not been taken into account in calculations of average rhizosphere effects for the whole period of barley culture. Especially large rhizosphere effects were also found in the case of nonfluorescent pseudomonads at the time of barley tillering and shooting stage.

The particular groups of bacteria responded to a grown plant to a different degree. Numerously, as in the previous studies [4, 15], occurring methylotrophs (on the level of 10^6/g of soil) were microorganisms with the weakest response (Table 1). Their number increased on average no more than by about 30%. Though methylotrophs, due to utilize carbon substances of type Ci, chiefly methane, are independent of root exudates, their increased activity is found in the rhizosphere of some plants, especially rice [2]. A reduced oxygen content in soil resulting from root respiration is favourable to the production of that gas, which on aerobic and anaerobic zone borders becomes a good carbon and energy source for many methylotrophs. It should, however, be emphasized that our studies were conducted with light, mineral soil, of a low content of substrates required for methane production and of good aeration.

Table 1. Influence of Oxafun T seed dressing on the number of methylotrophic bacteria (10^6/g dry wt) in the rhizosphere (R)* and non-rhizosphere (S)** soil under barley crop.

Plant growth-phase	Control plots			Oxafun plots		
	S	R	R:S	S	R	R:S
Tillering	1.4	1.6	1.1	3.0	7.1	2.9
Shooting	9.2	11.7	1.3	10.3[x]	16.4	1.6
End of heading	1.1	2.2	2.0	3.6	11.6	3.2
Blooming	4.9	7.6	1.5	1.8	8.0*	4.4
Milk maturity	1.2	0.9	0.8	1.6	4.2	2.6
Full maturity	1.4	12.8	9.1	1.4[x]	10.1	7.4
on average***	3.6	4.8	1.3	4.1[x]	9.4	2.9

* Soil closely adjacent to the roots
** Soil between the rows
*** Data for full maturity omitted in calculation
[x] Numbers of bacteria in the oxafun plots, followed by the symbol are not statistically different (at P > 0.05) from those in the control plots.

In contrast to methylotrophs, the total number of copiotroph bacteria was in the rhizosphere, as might be expected, more markedly increased - over threefold (Table 2).

Table 2. Influence of Oxafun T seed dressing on the number of copiotrophic and oligotrophic bacteria (10^6/g dry wt) in the rhizosphere (R)* and non-rhizosphere (S)** soil under barley crop.

Bacteria	Plant growthphase	Control plots			Oxafun plots		
		S	R	R:S	S	R	R:S
Copiotrophs	Tillering	3.6	11.7	3.2	4.4[x]	16.8	3.8
	Shooting	4.0	22.3	5.6	7.2	23.8[x]	3.3
	End of heading	2.1	3.6	1.7	1.7[x]	12.3	7.2
	Blooming	4.4	10.6	2.4	2.8	26.2	9.3
	Milk maturity	1.7	6.9	4.1	1.2	4.3	3.6
	Full maturity	0.7	19.5	27.8	0.9[x]	17.3	19.2
	on average***	3.8	11.0	3.4	3.5[x]	17.0	5.4
Oligotrophs	Tillering	7.7	23.7	3.1	15.1	60.7	4.4
	Shooting	19.9	92.8	4.7	14.9	66.2	4.2
	End of heading	8.7	59.5	6.8	7.0[x]	50.4	7.2
	Blooming	17.7	38.5	2.2	12.3	91.3	7.4
	Milk maturity	13.7	12.8	0.9	21.5	58.4	2.7
	Full maturity	10.1	116.4	11.5	6.9	156.6	22.7
	on average***	13.5	45.5	3.5	14.9[x]	65.4	4.6

Explanations as in Table 1.

Table 3. Influence of Oxafun T seed dressing on the total number of pseudomonads (10^3 /g dry wt) in the rhizosphere (R)* and non-rhizosphere (S)** soil under barley crop.

Plant growth-phase	Control plots			Oxafun plots		
	S	R	R:S	S	R	R:S
Tillering	20.2	117.0	5.8	25.3	212.0	8.4
Shooting	36.5	190.0	5.2	42.0[x]	250.0	6.0
End of heading	55.0	78.0	1.4	58.0[x]	480.0	8.3
Blooming	25.3	232.0	9.2	21.9[x]	469.0	21.4
Milk maturity	38.3	91.6	2.4	15.0	69.7	4.6
Full maturity	9.9	89.8	9.1	18.0	56.0	3.1
on average***	35.1	141.7	4.8	32.4[x]	296.1	9.7

Explanations as in Table 1.

Table 4. Influence of Oxafun T seed dressing on the percentage contribution of non-fluorescent forms in the community of pseudomonads as a whole, in the rhizosphere (R)* and non - rhizosphere (S)** soil under barley crop.

Plant growth-phase	Control plots		Oxafun plots	
	S	R	S	R
Tillering	2.7	14.1	11.1	19.7
Shooting	0.0	14.3	19.2	6.6
End of heading	1.2	2.5	24.0	9.0
Blooming	12.1	0.0	9.5	9.0
Milk maturity	5.6	12.5	2.5	2.6
Full maturity	20.9	6.8	14.3	14.2
on average***	4.3	7.2	13.3	10.6

Explanations as in Table 1.

Oligotrophic bacteria, as in other studies [7, 10], constituted the majority of bacterial soil community both in rhizosphere and out of rhizosphere. Their number in rhizosphere multiplied to the same degree as the number of copiotrophs, although the copiotrophs are just the organisms which sensitively respond to increased food amounts by growth. Elucidation of that phenomenon requires separate studies.

The number of *Pseudomonas* (Table 3) was in rhizosphere increased the most, on average, almost five times. However, in our opinion these increases were relatively lower than the expected ones. These bacteria are particularly associated with rhizosphere, metabolizing about 90% of food supplied by plant roots [22], and their number in this environment amounts to 10^6 - 10^7 cells per 1 g of that soil, while in barley rhizosphere studied in this paper it was of the order of 10^5. In *Pseudomonas* community, fluorescent forms were definitely the dominant. Nonfluorescent pseudomonads were relatively few, as in the previous, unpublished studies conducted with different soils. Depending on the time of analyses they also constituted from below 1% to a dozen or so %, whereas in rhizosphere soil they were almost to 21% (Table 4). That corresponded to below 10^2 - 10^4 cells per 1 g of soil (Table 5). The average value of rhizosphere effect for the experimental period was higher in non-fluorescent than in fluorescent forms, but that resulted from a comparelatively stronger increase of their number in rhizosphere only at the initial stages of barley development.

A comparison of bacterial numbers from the plots sown with untreated and treated seeds does not indicate that Oxafun T under the conditions of the performed experiments was toxic to bacteria from the studied groups. The toxicity should be evident already at the beginning of the experiment in the form of declines in the numbers of these microorganisms. Meanwhile, instead of

Table 5. Influence of Oxafun T seed dressing on the number of fluorescent and non-fluorescent pseudomonads (10^3/g dry wt) in the rhizosphere (R)* and non-rhizosphere (S)** soil under barley crop.

Pseudomonads	Plant growth-phase	Control plots			Oxafun plots		
		S	R	R:S	S	R	R:S
Fluorescent	Tillering	19.6	100.0	5.1	24.6[x]	170.0	6.9
	Shooting	36.5	185.0	5.1	33.9[x]	233.4	6.9
	End of heading	54.3	> 78.0	0.7	45.0[x]	436.8	9.7
	Blooming	22.2	203.0	9.1	19.8[x]	379.9	19.9
	Milk maturity	36.2	85.4	2.4	14.6	67.9	4.6
	Full maturity	7.8	77.0	9.9	15.8	48.0	3.0
	on average***	32.4	128.9	4.5	27.8	257.6	9.2
Nonfluorescent	Tillering	0.5	16.5.	30.0	11.1	2.8	14.9
	Shooting	< 0.1	4.8	> 48.0	8.1	16.5	2.0
	End of heading	0.7	< 0.1	< 1.0	13.9	115.2	8.3
	Blooming	17.7	29.0	1.6	2.2	42.2	19.2
	Milk maturity	1.4	6.1	4.4	0.4	1.8	4.5
	Full maturity	8.0	12.8	1.6	2.6	8.0	3.1
	on average***	4.1	17.0	7.1	7.1	35.4	9.8

Explanations as in Table 1.

these declines rather the increases were observed at that time. Declines, if they occurred, were found only later and a part of them was statistically insignificant at $P < 0.05$. They may be explained by a repeated development of fungi and/or by qualitative changes in bacterial communities and, therefore, by changes in their interrelationships, which have not been studied in the present work.

Omitting deviations noted in the study period, it might be recognized on the basis of its mean bacterial numbers, that the application of the seed dressing Oxafun T had no significant influence on the occurrence of these microorganisms in the non-rhizosphere soil. An exception could be nonfluorescent *Pseudomonas,* but particularly large dispersions of their numbers during the study period with a simultaneous lack of the exact trend of that variability should be taken into consideration.

In rhizosphere, numeric relations developed in a different manner than out of rhizosphere, since Oxafun T application noticeably increased the bacterial number in it, which was found already from the beginning of the first date of analyses. Even methylotrophic bacteria responded to this preparation by proliferation. Their number has increased almost twofold in comparison to that in the rhizosphere of plants grown up from untreated seeds, so that the rhizosphere effect for them, which was hardly noticed, has become definitely pronounced. As a result of copiotrophic and oligotrophic bacteria increase in the rhizosphere caused by the seed treatment and unchanged bacterial numbers out of rhizosphere, the rhizosphere effect values for these bacteria have also increased - more strongly for copiotrophs (by 54%) than for oligotrophs (by 35%). These differences agree with results of our previous studies [11, 13] conducted with different fungicides under different soil conditions, which showed that, if in general oligotrophs prolifirate under the influence of these preparations, they were always weaker than copiotrophs.

The rhizosphere community of pseudomonads under the effect of Oxafun T has increased to the strongest degree - stronger than that of copiotrophs as a whole, which these bacteria belong to. That resulted first of all from propagation of fluorescent forms.

There might be different reasons for such a response of fluorescent *Pseudomans* to a concentrated fungicide action in the rhizosphere. The most important of them are mycolytic properties of numerous strains of these bacteria [14, 19], due to which shreds of fungi killed by the fungicide became a readily accessible food source. Moreover, fluorescent pseudomonads can also use numerous fungicides as food [3,11], among others, thiuram, which enters into the composition of the seed dressing Oxafun T. A restricted competiveness of fungi as well as changes in barley root excretion favourable to these bacteria cannot be excluded.

An accurate identification of these eventual reasons would require further investigations, exceeding the scope of this paper.

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