

Effect of glyphosate on the growth and sporulation of several fungi species grown *in vitro*

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Roundup herbicide tested *in vitro* showed fungistatic activity against *Trichoderma hamatum*, *Rhizoctonia solani* and *Gliocladium roseum*. The highest concentration of this herbicide in the medium reduced the growth of *Microdochium nivale* colony, stimulating its plentiful sporulation at the same time. Under the same conditions, sporulation of *Gliocladium roseum* was inhibited and its conidiophors were deformed.

Key words: glyphosate, *Trichoderma hamatum*, *Rhizoctonia solani*, *Gliocladium roseum*, *Microdochium nivale*.

INTRODUCTION

Roundup (glyphosate) is a herbicide that readily translocates from leaves to roots (Levesque and Rahe 1992). It shows a modifying effect on inhabiting fungi population leading to a break of the natural balance among them (Black et al. 1996; Descalzo et al. 1996; Kawate et al. 1997; Różański 1992).

Studies *in vitro* have shown that Roundup suppresses the growth of fungi, especially at higher concentrations, causes changes in the morphology of fungi colonies (Burgiel and Grabowski 1996) and favours the development of certain toxin-forming species leading at the same time to increased production of mycotoxins dangerous to people (Hasan 1994). Roundup can completely inhibit sporulation of certain fungi species (Petrie 1995). It also modifies the virulence of some pathogens (Burgiel and Grabowski 1996).

The weed-killing effect of Roundup depends on blocking in a plant the synthesis of certain amino acids (Levesque and Rahe 1992). The consequence of that is reduced resistance of plants (Burgiel and Grabowski 1996). This in turn leads to increased infestation of plants by fungi resulting in intensification of chemical crop protection (Levesque and Rahe 1992; Decalzo et al. 1996; Pittway 1995).

The objective of this study was to determine *in vitro* conditions the effect of various concentrations of Roundup on the growth and sporogenesis of several fungi species taken from the rhizosphere of grasses.

MATERIALS AND METHODS

Two potentially pathogenic species of *Rhizoctonia solani* Kuühn and *Microdochium nivale* (Fr.) Samules et Sacc. (*Fusarium nivale* (Fr.) Ces.) and two saprophytic species: *Gliocladium roseum* (Link) Bainier and *Trichoderma hamatum* (Bon) Bain were used in the study. Isolates came from the rhizosphere of grasses. They were obtained by the method recommended by Martyniuk et al. (1991). Cultures grown in the Martin's medium were incubated at 23°C for 7 days and then transferred on PDA.

Roundup herbicide (41% isopropylamine N-(phosphonomethyl)-glycine = glyphosate), Monsanto Co. (USA) was added to the PDA medium agar after its sterilization and cooling to 50°C. The following the herbicide concentrations were applied: 5, 50 and 500 mg glyphosate per litre medium (Fabisiwicz and Mikolajska 1992). 15 ml medium containing different concentrations of herbicide were transferred into Petri dishes 9 cm in diameter. Dishes which the medium free of glyphosate were controls.

PDA disks five-mm-diameter, overgrown by old seven-day mycelia of the species tested were placed in the centre of the dishes. Fungi were incubated in the dark at 23°C. Their linear growth was determined by measuring the diameter of the colonies in two perpendicular directions after 24, 48, 72 and 96 hours. The treatments were replicated five times. The data were analysed statistically using the test of Newman-Keuls. Morphological observations of the colonies were performed for 2 weeks.

After 4 days, the sporulation of *M. nivale* and *G. roseum* was estimated. Using platinum loop, fungal hyphae together with spores from the 5-mm zone of colony growth were taken. The spores were counted in each replication within the five ranges of vision. Their number was estimated as high (++++), medium (+++), small (++) and negligible (+) using the following scale:

- ++++ - maximum number of species spores
 +++ - number of spores between 40 and 70% of maximum number of species spores
 ++ - number of spores between 15 and 40% of maximum number of species spores
 + - number of spores below 15% of maximum number of species spores

Since *Trichoderma hamatum* formed numerous conidial spores, which were uncountable under the microscope, compact lumps of its mycelium with high concentration of conidial spores were counted 12 days.

RESULTS AND DISCUSSION

The species studied, characterized by different growth rate, were found to grow worse in the presence of Roundup as compared to the control (Tab. 1). The differences were particularly conspicuous during further observations.

Table 1

The effect of herbicide Roundup on the growth colonies of some fungi species

Species	Days	Concentration of herbicide in medium mg/l				Mean
		Control 0	Glyphosate 5	Glyfosat 50	Glyfosat 500	
<i>Rhizoctonia solani</i>	1	12.8 ^{defg*}	12.8 ^{defgh}	12.1 ^{cdofg}	5.8 ^e	10.9 ^e
	2	37.7 ^h	38.9 ^g	37.0 ^e	11.3 ^{cdof}	31.2 ⁱ
	3	69.1 ^l	69.7 ^l	67.1 ^k	16.9 ^{klm}	55.7 ^l
	4	90 ^o	90 ^o	90 ^o	21.2 ^{no}	72.8 ^k
Mean		52.4 ^h	52.9 ^h	51.6 ^f	13.8 ^d	
<i>Trichoderma hamatum</i>	1	25.7 ^g	28.6 ^h	26.2 ^p	15.4 ^h	23.9 ^h
	2	61.4 ^l	65.9 ^h	63.3 ^w	33.2 ^r	55.9 ^l
	3	90 ^o	90 ^o	90 ^o	50.7 ^h	80.2 ^l
	4	90 ^o	90 ^o	90 ^o	66.6 ^h	84.2 ^m
Mean		66.8 ^h	68.6 ^l	67.4 ^h	41.5 ^e	
<i>Microdochium nivale</i>	1	6.0 ^a	5.9 ^a	5.9 ^a	5.0 ^a	5.7 ^a
	2	10.2 ^{cd}	10.6 ^{cd}	10.7 ^{cd}	8.0 ^b	9.9 ^b
	3	13.1 ^{efghi}	13.6 ^{ghi}	13.6 ^{ghi}	10.8 ^{cd}	12.8 ^d
	4	18.4 ^{lm}	19.0 ^m	18.1 ^{lm}	14.5 ^{hij}	17.5 ^f
Mean		11.9 ^{bc}	12.3 ^{bc}	12.1 ^{bc}	9.6 ^a	
<i>Gliocladium roseum</i>	1	5.5 ^a	5.7 ^a	5.4 ^a	5.8 ^a	5.6 ^a
	2	10.7 ^{cd}	11.7 ^{cdofg}	10.4 ^{cd}	9.9 ^c	10.6 ^c
	3	16.5 ^{kl}	17.4 ^{klm}	15.1 ^{hijk}	13.8 ^{ghi}	15.7 ^a
	4	22.5 ^{no}	23.0 ^o	20.8 ^h	17.1 ^{klm}	20.9 ^e
Mean		13.8 ^d	14.5 ^d	12.9 ^c	11.7 ^b	

* - values marked by the same letters are not differed significantly according to Newman - Kuels test.
 Diameter of colony in mm

Rhizoctonia solani grew worse on media when exposed to 50 and 500 ml/l. The higher concentrations of glyphosate had negatively effect on the growth of the colony of that species and its diameter attained merely 26.3% of the colony diameter of the grown in the control medium (Tab. 1, Fig. 1). Black et al. (1995) also reported fungistatic activity of glyphosate in relation to this species.

It appeared that *Trichoderma hamatum* was sensitive to Roundup at concentration of glyphosate 500 ml/l where the diameter of this colony was smaller by 38% than the control (Tab. 1, Fig. 2). At application rate glyphosate 5 ml/l stimulated significant the mycelium growth of mycelium this species. The diameter of the *T. hamatum* colony grown at low concentration of the herbicide was larger only by 2.7% as compared to the control, which indicated that the species was tolerant of low concentrations of this compound (H a s a n 1994). The stimulation of growth of some fungi species even at higher concentrations of glyphosate was demonstrated by some authors (H a s a n 1994).

After two weeks *T. hamatum* formed conspicuous aggregations of conidial spores in the control medium and on dishes where the concentrations of glyphosate were 5 and 50 ml/l medium. The highest concentration of glyphosate inhibited the sporulation of this species; no aggregations of conidial spores were observed on dishes (Tab. 2). P e t r i e (1995) also reported blocking of sporulation of pathogenic fungi by this compound.

The diameter of the colony of *Microdochium nivale*, which was characterized by slow growth, was significantly reduced only in the case of the highest concentration of the herbicide. It attained 80.3% of the diameter of the control (Tab. 1). After 96 hours sporodochia with a high number of conidial spores were observed (Tab. 2, Fig. 3).

After the same time, the control medium and the medium with the lowest concentration of the herbicide had a small number of spores (Fig. 4). At the glyphosate concentration of 50 ml/l the number of spores constituted

Table 2

Number of spores produced by *Gliocladium roseum* and *Microdochium nivale*.

The average number of spore aggregations formed by *Trichoderma hamatum* depending as the concentration of glyphosate

Species	Control	5 mg glyphosate/ /litre PDA	50 mg glyphosate/ /litre PDA	500 mg glyphosate/ /litre PDA
<i>Gliocladium roseum</i>	++++*	+++	+++	+
<i>Microdochium nivale</i>	+	+	+++	++++
<i>Trichoderma hamatum</i>	22.4	43.0	54.2	0

*according to the scale given in the text

nearly 46% of the spores found at of 500 ml/l (Tab. 2, Figs 3,4). Stimulation of sporulation of this pathogenic species is not advantageous. Perhaps in certain cases it contributes to the concentration of *Fusarium* fungi in the rhizosphere of plants controlled by glyphosate (Kawate 1997).

Gladiolus roseum grew worse at 50 and 500 ml of glyphosate in the medium. At higher concentration, the diameter of its colony constituted 84.4% of that in the control medium (Tab. 1).

G. roseum grew normally on PDA medium with no glyphosate added. It produced conidiophores on which numerous spores were formed (Tab. 2). Similar conidiophores were also seen at the lowest glyphosate concentrations. Conidiophores of the *Verticillium* type were also found and was lower number of spores observed (Tab. 2, Fig. 5). Conidiophores of this type were observed in the treatments which were exposed to 50 and 500 ml/l of glyphosate. In addition, with the highest herbicide concentration in the medium attributed to the numerous deformities of conidiophores (Figs 5, 6). In this treatment a negligible number of conidial spores also appeared (Tab. 2). Changes in the morphology of colonies, abundant sporulation, size and shape of the spores and their germination have been reported as the effects of herbicides (Burgiel 1992; Burgiel and Grabowski 1996; Kornilłowicz 1984; Mikołajska and Fabiszewicz 1992; Petrie 1995).

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Oddziaływanie herbicydu Roundup na wzrost i zarodnikowanie kilku gatunków grzybów w warunkach *in vitro*

Streszczenie

Herbicyd Roundup, testowany w warunkach *in vitro*, wykazywał fungistatyczną aktywność w stosunku do *Trichoderma hamatum*, *Rhizoctonia solani* i *Gliocladium roseum*. Obecność w pożywce najwyższej koncentracji tego herbicydu ograniczała rozwój kolonii *Microdochium nivale*, stymulując jednocześnie jego obfite zarodnikowanie. W tych samych warunkach sporulacja gatunku *Gliocladium roseum* była zahamowana, a jego trzonki konidialne zdeformowane.