The growth of soil micromycetes in the media containing herbicides Basagran, Labuctril 25 and Oxytril CM

Ovlivnění růstu půdních mikroskopických hub přítomností herbicidů Basagran, Labuctril 25 a Oxytril CM

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The changes of fungal growth in laboratory experiments (estimation of mycelial biomass and colony diameter) were studied by cultivation of 10 species of soil fungi on media with different concentration of the herbicides Basagran, Labuctril 25 and Oxytril CM. The subject fungi responded to increased concentrations of herbicide in different ways. *Cladosporium herbarum* (colony diameter) and *Penicillium janthinellum* (mycelial biomass) were the only investigations to show a similar response to all the herbicides. *Penicillium janthinellum* (mycelial biomass) was the only example of a significant stimulation of fungal growth. Changes in colony pigmentation and sporulation were also recorded.

Při laboratorním pokusu byly sledovány změny růstu 10 druhů mikromycetů při kultivaci v tekutém (stanovení biomasy mycelia) a pevném živném médiu (měření průměru kolonií) s různou koncentrací herbicidů Basagran, Labuctril 25 a Oxytril CM. Sledované druhy mikromycetů reagovaly na vzrůstající koncentraci herbicidů různě, pouze *Cladosporitum herbarum* (průměr kolonie) a *Penicillium janthinellum* (biomasa mycelia) reagovaly stejně ke všem herbicidům. U *P. janthinellum* (biomasa mycelia) byla zaznamenána statisticky významná stimulace růstu mycelia. V práci jsou rovněž popsány změny v pigmentaci a sporulaci kolonií na pevném živném médiu.

Introduction

Soil fungi are an important part of the soil microflora; they not only decompose organic matter and contribute to the development of soil humus but also they decompose organic materials introduced by man such as organic fertilizers and pesticides.

The application of herbicides has become an integral part of modern agrotechnology (Grossbard 1976, Spurrier 1990) and the increasing amounts, and long-term persistence, of these substances causes changes in the composition of the soil microflora and soil chemical processes (Grossbard 1976).

The soil microflora can be suppressed by the presence of herbicides, completely inhibited or stimulated depending on the chemical composition of the herbicide or the physical and chemical conditions of the soil environment or the concentration of the herbicide used (Grossbard 1976, Malkomes 1988). The interaction of herbicides and certain groups of soil microflora is now important in contemporary soil microbiology.

The following is a report of a study of the growth of ten species of soil fungi cultivated in liquid and on solid media containing increasing concentration of the herbicides Basagran, Labuctril 25 and Oxytril CM. This study is subsequent to previous paper (Řepová 1984) studying the effect of herbicides application on micromycetes population of spruce forest soil.

Material and methods

The fungi

The following strains of microscopic fungi isolated from an apple orchard soil that had not received any herbicide, were used in the experiment: *Penicillium chrysogenum* Thom., *P. janthinellum* Biourge, *Aspergillus fumigatus* Fres., *Gliocladium roseum* Bainier, *Myrothecium roridum* Tode ex Steudel, *Cladosporium herbarum* (Pers.) Link ex Gray, *Paecilomyces lilacinus* (Thom) Samson, *Absidia cylindrospora* Hagem, *Zygorhynchus moelleri* Vuill. and *Trichoderma viride* Pers. ex Gray.

Herbicides

Three herbicides were used: Basagran (active component bentazone), Labuctril 25 (active component bromoxynil) and Oxytril CM (active component bromoxynil and ioxynil). These herbicides are commonly used in agriculture for destroying dicotyledonous weeds in cereal crops. In this experiment the herbicides were used in concentration of 48, 96, 144, 192 and 240 ppm.

Methods of estimating fungal growth

Measurements of colony diameter

Three replicate 90 μm Petri dishes containing 25 ml of Sabouraud's agar (Booth 1971) with 1 ml of each herbicide solution, were centrally inoculated. The plates were incubated at room temperature for seven days, and fungal growth was determined by measuring the colony diameter. Changes in the morphology, sporulation and pigmentation were also recorded.

Estimation of mycelial biomass

Fifty ml of liquid Sabouraud's medium were dispensed into 100 ml flasks. The control was 3 flasks containing only liquid medium and spore suspension. Into three flasks for each herbicide 1ml of spore suspension and 1 ml of each herbicide concentration were added aseptically. After seven days of incubation at room temperature the fungal mycelium was collected by filtration of the culture medium, washing with distilled water, drying at 105°C for five hours, and than weighing.

Statistical analysis of the results

A one-way analysis of the variance at 5% significance was employed for a statistical analysis of the results.

Results

Measurement of colony diameter

The fungi responded in differing ways: inhibition and stimulation, but in some cases no change of fungal growth were found with increasing herbicide concentrations. From Table 1 it is evident that only one species, *Cladosporium herbarum*, reacted similarly to all herbicides, i.e. a gradual decrease in colony growth. Stimulation of colony growth was recorded by *Penicillium chrysogenum* (144 and 192 ppm of Basagran and 240 ppm of Oxytril CM) and by *Aspergillus fumigatus* (48, 96, 192 and 240 ppm of Basagran, 48 and 96 ppm of Oxytril C and Labuctril 25). Almost no effect on colony growth was found with *Trichoderma viride* and *Zygorhynchus moelleri* and the herbicides Basagran and Labuctril 25 - see Table 1. The significant differences between fungal growth with and without herbicide were also recorded.

Some differences in growth habit, pigmentation and sporulation were also found. *Trichoderma viride* growing on medium containing 144 and 240 ppm of Labuctril 25 and 240 ppm of Oxytril CM produced a yellow pigment on the reverse of the colony which diffused into the surrounding agar. Yellow-orange pigmentation was found with *P. janthinellum* at 144 ppm of Labuctril 25 and an intense orange pigmentation at 240 ppm of

the same herbicide. Conversely, less pigmentation was found with *Cladosporium herbarum* at 144, 192 and 240 ppm of Labuctril 25. Increasing sporulation was noted at 144 ppm of Labuctril 25 in both *P. janthinellum* and *Aspergillus fumigatus*.

Estimation of mycelial biomass

The majority of the fungi showed differing responses to different herbicides with the exception of *P. janthinellum* which responded with increasing growth with all three herbicides. Most differences in growth were statistically significant when compared to that of the control (see Table 2).

Discussion

Colony diameter showed differing responses by the fungi to increasing concentrations of the herbicides but generally increasing suppression was noted. Only *C. herbarum* responded in a consistent way to all three herbicides. The overall results are similar to those of Wilkinson et Lucas (1969) when they studied five herbicides. Guillemat et al. (1960) reported suppression of fungal growth. They indicated that half of the fungi had ceased to grow in a medium with 1.5 % simazine added. Pantos et al. (1962) could detect no significant effect on the growth of fungi on solid or in liquid media with simazine or atrazine added; none of the fungi showed any significant inhibition or stimulation. Steinbrenner et al. (1960) in Kaiser et al. (1970) reported two responses when studying mycelial growth on maltose agar with increasing simazine concentration. Firstly, a suppression of mycelial growth in all herbicide concentrations (e.g. *Zygorhynchus, Rhizopus, Alternaria, Trichoderma,* and *Stemphylium*) and secondly growth stimulation (e.g. *Fusarium*). Rudakov et Spiridonov (1979) divided fungi into three groups: 1. Fungi which were suppressed at all herbicide concentrations, 2. fungal growth only suppressed at higher concentrations of herbicides and 3. good fungal growth at middle herbicide concentrations and suppression at low concentrations. This classification appears to be deficient as it does not consider the case of growth stimulation by herbicides as reported by Grossbard (1976), who found that herbicides were particularly stimulatory when they were the source of carbon and nitrogen.

It is also necessary to consider that pH and the composition of the medium will affect the activity of a herbicide as demonstrated by Balická (1969) in Grossbard (1976) where lower pH values were found to be more toxic in most herbicides.

Valášková (1968) and Wilkinson et Lucas (1969) reported that herbicides can inhibit or stimulate spore production. Manturovskaya (1970) described the retardation of the beginning of spore production, whilst a good mycelial growth continued, in the presence of herbicides in the growth medium.

In this study two fungi showed changes in sporulation - *P. janthinellum* and *A. fumigatus*, in both cases spore production was stimulated. *T. viride* and *P. janthinellum* showed

a significant pigmentation of the colony reverse whereas *C. herbarum* showed reduced pigmentation. Wilkinson et Lucas (1969) also reported a reduction in colony pigmentation.

The fungal biomass in liquid media were reduced in most cases with the exception of *P. janthinellum* which showed a significant increase with all herbicide concentrations.

Sikka et al. (1965) in Kaiser et al. (1970) recorded the stimulation of mycelial growth of common soil fungi in the presence of triazine herbicides in liquid nutrient medium even when not limited for nutrients. Bakalivanov (1972) described both increased and decreased growth of mycelium in liquid media with herbicides. This author recorded significant stimulation of the growth of *Aspergillus niger* and *A. tamarii* in a medium with prometryne, but inhibition of *A. tamarii* in a medium with dikotex. Abdel-Fattah et al. (1983) found suppression of *Alternaria alternata* growth at 78, 313 and 626 ppm of atrazine whilst *Trichoderma viride, Gliocladium roseum, Myrothecium verrucaria* and *Cunninghamella echinulata* were suppressed at the middle and upper concentrations and *Penicillium vermiculatum* was suppressed at highest concentrations only.

Grossbard (1976) states that microscopic fungi are more sensitive to a considerable number of herbicides than are bacteria. Similarly fungi appear to be more tolerant of herbicides in pure culture than in the soil environment. Rudakov et Spiridonov (1979) found, for example, that the same herbicide concentration which evoked only a slight fungal inhibition in laboratory conditions, suppressed the growth of a whole population of fungi in a soil environment. These authors suppose that herbicides do not act on the fungi directly but change the whole relationship of the mycocenose.

Microscopic fungi also participate in the processes of detoxication and degradation of herbicides (Hsu et Capmer 1979, Chahal et al. 1977, Tweedy et al. 1970), by using them as a source of carbon and nitrogen (Kaufman et al. 1963, Kaufman 1970). It would seem that soil microscopic fungi are an essential part of the soil microflora and participate in the breakdown of herbicides in the soil environment.

Table 1 — Growth rates of fungi on solid nutrient medium containing herbicides (Values were expressed as a percentage of the control). Asterisks indicate statistical conclusive results on the significant level $\alpha = 0.05$(*), $\alpha = 0.01$(**) and $\alpha = 0.001$(***).

	Concentration of Basagran (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	85.5	90.3	93.0	96.7	85.9	
Penicillium janthinellum	100	79.9	100.0	91.0	87.3	91.0	
Myrothecium roridum	100	98.3	101.7	93.0	91.2	96.4	
Zygorhynchus moelleri	100	94.4	100.0	100.0	100.0	100.0	
Trichoderma viride	100	100.0	100.0	100.0	100.0	100.0	
Cladosporium herbarum	100	91.4	85.1	91.0	86.6	82.1	
Penicillium chrysogenum	100	102.9	90.5	112.4	108.2	100.0	
Paecilomyces lilacinus	100	86.5	92.8	91.0	80.7	100.0	**
Gliocladium roseum	100	96.8	95.7	91.4	92.5	93.5	
Aspergillus fumigatus	100	102.0	106.6	100.0	107.1	102.0	

	Concentration of Oxytril CM (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	80.5	74.4	73.0	71.4	57.0	***
Penicillium janthinellum	100	85.0	81.3	78.7	78.7	88.7	
Myrothecium roridum	100	91.2	80.7	75.4	64.9	68.4	***
Zygorhynchus moelleri	100	96.3	85.2	84.1	75.9	74.8	***
Trichoderma viride	100	100.0	98.9	86.7	84.4	98.5	***
Cladosporium herbarum	100	97.8	85.1	83.6	82.1	77.6	
Penicillium chrysogenum	100	83.5	98.8	100.0	95.9	107.8	
Paecilomyces lilacinus	100	74.9	70.4	79.4	73.0	70.4	***
Gliocladium roseum	100	77.9	86.0	73.1	74.2	73.1	*
Aspergillus fumigatus	100	104.1	104.1	91.8	91.8	92.8	**

	Concentration of Labuctril 25 (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	86.7	77.8	65.0	69.2	62.8	***
Penicillium janthinellum	100	86.1	94.7	79.9	81.3	78.7	
Myrothecium roridum	100	100.0	89.4	94.7	82.4	89.4	*
Zygorhynchus moelleri	100	100.0	100.0	85.9	100.0	60.0	**
Trichoderma viride	100	100.0	100.0	100.0	100.0	71.4	**
Cladosporium herbarum	100	82.1	85.1	79.1	52.3	50.7	***
Penicillium chrysogenum	100	97.5	97.5	82.3	87.6	68.7	**
Paecilomyces lilacinus	100	86.5	88.3	80.7	61.9	68.6	***
Gliocladium roseum	100	80.6	88.2	73.1	74.7	81.6	
Aspergillus fumigatus	100	102.0	110.2	82.6	62.2	55.1	**

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Table 2 — Mycelial growth in liquid nutrient medium containing herbicides (Values were expressed as a percentage of the control). Asterisks indicate statistical conclusive results on the significant level $\alpha = 0.05(*)$, $= 0.01(**)$ and $\alpha = 0.001(***)$.

	Concentration of Basagran (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	95.8	89.6	87.5	77.1	81.2	
Penicillium janthinellum	100	544.4	533.3	422.2	377.7	411.1	***
Myrothecium roridum	100	87.5	90.6	65.6	62.5	68.7	*
Zygorhynchus moelleri	100	100.0	85.7	95.2	123.8	114.2	
Trichoderma viride	100	91.6	87.5	91.6	87.5	83.3	*
Cladosporium herbarum	100	107.7	57.7	80.7	84.6	88.5	***
Penicillium chrysogenum	100	75.5	53.3	48.9	35.5	33.3	***
Paecilomyces lilacinus	100	81.1	66.0	69.2	101.9	88.7	**
Gliocladium roseum	100	71.4	85.7	85.7	71.4	85.7	**
Aspergillus fumigatus	100	80.0	47.5	60.0	67.5	60.0	**

	Concentration of Oxytril CM (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	66.6	54.2	68.7	60.4	20.8	*
Penicillium janthinellum	100	466.6	444.4	355.5	377.7	366.6	***
Myrothecium roridum	100	43.7	28.8	50.0	40.6	34.4	***
Zygorhynchus moelleri	100	104.8	100.0	100.0	80.9	80.9	
Trichoderma viride	100	87.5	66.6	83.3	79.2	70.8	*
Cladosporium herbarum	100	65.4	57.7	53.8	50.0	46.1	***
Penicillium chrysogenum	100	56.7	53.3	44.4	44.4	55.5	***
Paecilomyces lilacinus	100	45.3	32.1	45.3	18.7	22.6	***
Gliocladium roseum	100	114.3	85.7	78.6	78.6	85.7	
Aspergillus fumigatus	100	87.5	77.5	65.0	57.5	60.0	***

	Concentration of Labuctril 25 (ppm)						
Species	0	48	96	144	192	240	
Absidia cylindrospora	100	81.2	58.3	58.3	43.7	33.3	***
Penicillium janthinellum	100	433.3	388.8	366.6	300.0	266.6	*
Myrothecium roridum	100	75.0	50.0	43.7	28.1	18.7	***
Zygorhynchus moelleri	100	123.8	61.9	57.1	90.5	80.9	**
Trichoderma viride	100	91.6	79.2	79.2	70.8	75.0	**
Cladosporium herbarum	100	80.7	69.2	69.2	57.7	50.0	***
Penicillium chrysogenum	100	91.1	60.0	64.4	80.0	64.4	*
Paecilomyces lilacinus	100	58.5	39.6	45.3	37.3	39.6	***
Gliocladium roseum	100	71.4	78.6	71.4	71.4	71.4	
Aspergillus fumigatus	100	67.5	67.5	62.5	57.5	60.0	***

Conclusions

Responses of fungi to increased concentrations of herbicides in nutrient medium were found to differ not only between the species but also between herbicides. Only *Cladosporium herbarum* (colony diameter) and *Penicillium janthinellum* (mycelial biomass) showed a consistent response. A significant stimulation of fungal growth was recorded in one case: mycelial biomass of *Penicillium janthinellum*. In some cases changes of pigmentation, sporulation and growth habit in fungal colonies on medium with herbicide were found.

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