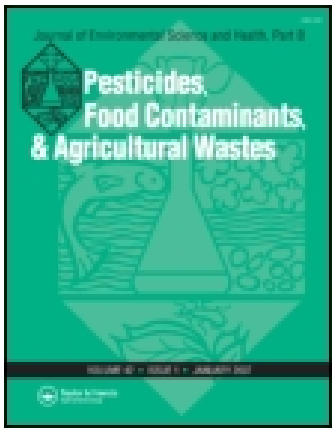

Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lesb20

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Published online: 28 Jul 2014.

To cite this article: Cecilia S. Carranza, Carla L. Barberis, Stella M. Chiacchiera & Carina E. Magnoli (2014) Influence of the pesticides glyphosate, chlorpyrifos and atrazine on growth parameters of nonochratoxigenic Aspergillus section Nigri strains isolated from agricultural soils, Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 49:10, 747-755, DOI: 10.1080/03601234.2014.929860

To link to this article: http://dx.doi.org/10.1080/03601234.2014.929860

Journal of Environmental Science and Health, Part B (2014) **49**, 747–755

ISSN: 0360-1234 (Print); 1532-4109 (Online)
DOI: 10.1080/03601234.2014.929860

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Influence of the pesticides glyphosate, chlorpyrifos and atrazine on growth parameters of nonochratoxigenic *Aspergillus* section *Nigri* strains isolated from agricultural soils

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This investigation was undertake to determine the effect of glyphosate, chlorpyrifos and atrazine on the lag phase and growth rate of nonochratoxigenic *A. niger* aggregate strains growing on soil extract medium at −0.70, −2.78 and −7.06 MPa. Under certain conditions, the glyphosate concentrations used significantly increased micelial growth as compared to control. An increase of about 30% was observed for strain AN 251 using 5 and 20 mg L^{-1} of glyphosate at −2.78 MPa. The strains behaved differently in the presence of the insecticide chlorpyrifos. A significant decrease in growth rate, compared to control, was observed for all strains except AN 251 at −2.78 MPa with 5 mg L^{-1}. This strain showed a significant increase in growth rate. With regard to atrazine, significant differences were observed only under some conditions compared to control. An increase in growth rate was observed for strain AN 251 at −2.78 MPa with 5 and 10 mg L^{-1} of atrazine. By comparison, a reduction of 25% in growth rate was observed at −7.06 MPa and higher atrazine concentrations. This study shows that glyphosate, chlorpyrifos and atrazine affect the growth parameters of nonochratoxigenic *A. niger* aggregate strains under in vitro conditions.

Keywords: Glyphosate, chlorpyrifos, atrazine, *Aspergillus niger* aggregate, soil-based medium.

Introduction

Argentina is an agricultural country that exports raw materials and agricultural inputs. The cropping system adopted widely by the producers requires a great economic investment in agrochemicals to ensure better crops and higher profits.[1] Pesticides are intensively and extensively used in agricultural production to prevent or control pests, diseases, weeds, and pathogens in an effort to reduce or eliminate yield losses and maintain a high quality of products.[2,3]

Glyphosate-tolerant (GT) soybean (*Glycine max* L.) and maize (*Zea mays* L) are among the main crops in the province of Córdoba, Argentina. During the last harvest season (2011/2012), the planted area destined to these crops exceeded 5 and 1 million hectares, respectively.[4] A preplanting application of glyphosate is recommended for production of soybean and maize, followed by one or two applications of this broad-spectrum herbicide during different stages of crop development. Moreover, the insecticide chlorpyrifos, a non-systemic organophosphorus, and the herbicide atrazine are also applied twice or even more times. Chlorpyrifos is used for the control of foliar insects and atrazine is applied to control pre- and post-emergence broadleaf and grassy weeds.[5,6] These compounds are very toxic due to their rapid solubilization in the lipid phase, and act on the central nervous system of cold-blooded animals.[7] With regard to persistence, the reported data indicates that chlorpyrifos remains stable in the soil even after 12 months after application depending on the type of soil and other environmental conditions.[8] Atrazine is one of the most environmentally-prevalent s-triazine-ring herbicides used in major crops and possess an average half-life of over 100 days.[6,9] On the other hand, glyphosate has a moderate persistence in soils and a half-life ranging from 3 to 130 days.[10]

The rate of organophosphorus decomposition in soils depends on several factors such as texture, pH, temperature, moisture, organic carbon content and pesticide

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Received March 3, 2014.

formulation.[11] The exchange of traditional crops by transgenic crops meant a significant advance for agricultural producers. However, it simultaneously incorporated the presence of organochlorine residues in the grain market. The excess of these chemicals in soils causes damages due to long periods of persistence and to the possibility of uptake by other crops because, usually, the water bodies that are adjacent to the agricultural areas are the ultimate recipient of pesticide residues.[12]

The intake of pesticides and their residues through food is the main concern of international organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). These organizations established permissible limits in food for protection of the human population.[13] According to the Argentinean Chamber of Agricultural Health and Fertilizers,[14] the use of pesticides has increased in 858% during the last 22 years while the cultivated area for cereals and oilseeds has only increased in 50% and 30%, respectively.

There is a need to develop safe, convenient and economically feasible methods for pesticide remediation due to environmental concerns associated to the accumulation of pesticides in food products and water supplies.[15] For this reason, several biological techniques involving biodegradation of organic compounds by microorganism have been developed.[16] The microbial metabolism is probably the most important degradative process of pesticides in the soil environment.[17] Thus, it constitutes the basis for bioremediation, since the degrading microorganisms obtain C, N, P or energy from the molecules of pesticides.[18–20] Fungi possess a number of advantages that can be exploited in bioremediation systems because they can degrade insoluble chemical compounds or an extremely diverse range of very persistent toxic environmental pollutants. In addition, the mycelial growth is also advantageous since it allows rapid colonization of substrates, and hyphal extension enables penetration of pollutants.[21] In addition, they can tolerate a wide range of environmental conditions, such as temperature, pH and moisture levels and do not require pre-conditioning to a particular pollutant, because their degradative system is induced by nutrient deprivation.[22]

In a previous study, we evaluated the culturable mycobiota of agricultural soils and found that *Aspergillus* spp. is one of the most prevalent genus being *Aspergillus flavus* and *Aspergillus niger* aggregate the prevalent species. *A. niger* aggregate was isolated in percentages of 50% from maize and soybean soils and of 33% from maize–soybean rotation soils.[23] In addition, we evaluated the effect of glyphosate on the growth rate and on aflatoxin production by *Aspergillus* section *Flavi* strains isolated from these soils.[24] On the other hand, there are reports on *Aspergillus niger* degrading pyrethroid, fenitrothion, carbaryl dimethoate and endosulfan.[25–29] However, there is no information on the influence of other xenobiotic compounds on the development of *Aspergillus* section *Nigri* strains under different water availability conditions. Therefore, the objective of this work was to evaluate the effect of glyphosate, chlorpyrifos and atrazine on the lag phase and growth rates of nonochratoxigenic *Aspergillus* section *Nigri* strains isolated from agricultural soils, under different water potential conditions on soil-based medium.

Materials and methods

Fungal strains

Two nonochratoxigenic *Aspergillus niger* aggregate strains (AN 251 and AN 384) were evaluated. These strains were isolated from fields destined to the production of maize and soybean. The fields are located in the south of the province of Córdoba, Argentina, and have been exposed to successive applications of pesticides. The strains were identified by classic taxonomy according to the methodology proposed by Samson et al.[30] In addition, their ability to produce ochratoxins was evaluated.[31] The strains belong to our culture collection at the Department of Microbiology and Immunology, in the National University of Río Cuarto, Córdoba, Argentina, and were maintained in glycerol (15%, Sigma-Aldrich, St. Louis, MO, USA) at −80°C.

Selected pesticides

Three pesticides, glyphosate, chlorpyrifos and atrazine were chosen given their widespread use and reported association to the agricultural environment. Glyphosate (*N*-phosphonomethylglycine), chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate) and atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] were obtained from commercial formulations: Round-up®, (Monsanto, Buenos Aires, Argentina), Hortal® (Buenos Aires, Argentina) and Icona FW®, (Icona S.A., Buenos Aires, Argentina), respectively. Stock solutions (500 mg L^{-1}) of each of the three pesticides were prepared and then working solutions were done by appropriate dilution in sterile distilled water. These solutions were sterilized with 0.2 μm filter (Whatman International, Ltd. Maidstone, England) and kept at 4°C.

Culture medium, inoculation and incubation

The soil extract solid medium (SESM) was prepared using 200 g of untreated field soil in 400 mL of water. This soil sample (Hapludol with a very fine sandy frank texture) was chosen from a field without previous application of pesticides, in the south of Córdoba, Argentina. The soil/water mixture was autoclaved for 30 min, centrifuged at 2,400 g for 20 min and filtered through filter paper (Whatman no. 1), using a vacuum pump. Then, the water

potential (MPa) of the medium was modified separately to −0.70, −2.78 and −7.06 using the ionic solute KCl.[32] These media were sterilized again at 120°C for 20 min and the solution of each pesticide was applied to the culture media at 45–50°C to obtain the required concentrations (5, 10 and 20 mg L^{-1}). The media were poured into 90-mm sterile Petri dishes. Control plates at each MPa value and without pesticide were also prepared. The culture media were needle-inoculated centrally using a sterile loop with fungal spores, from 7-day-old cultures grown malt extract agar (MEA), suspended in soft agar. Inoculated Petri dishes of the same water potential were sealed together in polyethylene bags. Four replicate plates were used per treatment. Plates were incubated at 25°C for 28 days.

Growth parameters

Two measures of colony radius, at right angles to each other, were taken daily from each replicate. The radius of each colony was plotted against time, and a linear regression was applied in order to obtain the growth rate as the slope of the line to the *X*-axis. The lag phase (h) before growth was also determined.[33]

Growth and lag phase analyses were performed on two *Aspergillus niger* aggregate strains, with three different concentrations of each of three pesticides and three different MPa conditions, with the respective controls. Each analysis was carried out in quadruplicate and all of the experiments were repeated twice.

Statistical analyses

Results obtained in the experiments of effect of glyphosate, chlorpyrifos and atrazine on *A. niger* aggregate growth were analyzed by analysis of variance (ANOVA). All data were transformed to $\log_{10}(x + 1)$ to obtain homogeneity of variance. Means were compared by the Fisher's protected LSD test to determine the influence of the abiotic factors (MPa and pesticide concentration) on the growth rate and lag phase. The analyses were conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA).

Results

Effect of glyphosate, chlorpyrifos and atrazine treatments on lag phase

Table 1 shows the effect of the three pesticides on the lag phase of two *Aspergillus* section *Nigri* strains. In the controls, the lag phase increased significantly as the MPa decreased. With glyphosate, in general, the two strains showed the same behavior pattern. An increase in the duration of their lag phases was observed at −2.78 MPa with increasing concentrations of the pesticide, as compared to controls. By comparison, their lag phases decreased at −7.06 MPa as the concentration of glyphosate increased. The more noticeable decrease was observed with strain AN 251 at 10 and 20 mg L^{-1} ($P < 0.05$).

With regard to chlorpyrifos, a direct relationship between increasing pesticide concentration and increasing lag phase duration was observed at −2.78 and −7.06 MPa. The largest lag phases were observed with 10 and 20 mg L^{-1} of pesticide and an MPa of −7.06. On the other hand, strain AN 251 showed a shorter lag phase than the control at 5 mg L^{-1} and −7.06 MPa.

The two strains also showed a similar behavior pattern in the presence of atrazine. At −0.7 MPa, similar lag phases values were observed between pesticide treatments and controls. On the other hand, a significant increase in lag phase was observed at −2.78 and −7.06 MPa with all of the concentrations of the pesticide, except for strain AN384 at −7.06 MPa, as compared to the corresponding control.

The analysis of variance of the effect of single (MPa, pesticide and pesticide concentration) two- and three-way interaction showed that these factors alone or interacting were statistically significant ($P < 0.05$) in relation to the lag phase for the two *Aspergillus* section *Nigri* strains assayed (Table 2).

Effect of glyphosate, chlorpyrifos and atrazine treatments on growth rate

We only observed a parallelism between reduction of growth rate and decrease in MPa with AN 251 in control treatments (Fig. 1A). On the other hand, with AN 384, the highest growth rate value from controls was observed at −2.78 MPa (Fig. 1B). The different glyphosate concentrations used significantly increased the micelial growth of the two *Aspergillus* section *Nigri* strains respect to control only in some of the evaluated concentrations. This behavior was more evident in AN 251. In this strain, increases of 30% and 34.2% respect to control were observed at −2.78 MPa with 5 and 20 mg L^{-1}. At −7.06 MPa, significant increases of 24.5% and 14% in growth rate were observed with 5 and 20 mg L^{-1}. On the other hand, we observed no significant differences in growth rate of AN 384 among the different treatments and the corresponding control. A slow increment was observed at −0.7 MPa with 10 mg L^{-1} and at −7.06 MPa with 5 and 10 mg L^{-1} of glyphosate ($P < 0.05$) (Fig. 1).

The behavior of the *Aspergillus* strains regarding the insecticide chlorpyrifos was different from that with glyphosate. Significant decreases in growth rate respect to controls were observed in the two strains, with the exception of AN 251 at −2.78 MPa and 5 mg L^{-1}. In this case, a significant increase was observed compared to the control. The most significant reductions in the growth of this strain were observed with 20 mg L^{-1} (71% at −0.7, 37.7% at

Table 1. Effect of glyphosate, chlorpyrifos and atrazine on the lag phase of *A. niger* aggregate strains under different water potential (MPa) conditions on soil-based medium.

		Lag phase (h) ± SD									
		Control	*Glyphosate (mg L^{-1})*			*Chlorpyrifos (mg L^{-1})*			*Atrazine (mg L^{-1})*		
Strains	*MPa*	*0*	*5*	*10*	*20*	*5*	*10*	*20*	*5*	*10*	*20*
AN251	−0.7	32 ± 3.4^{mn}	34 ± 3.4^{lm}	30 ± 5.3^{n}	38 ± 4.3^{kl}	41 ± 4.5^{kl}	39 ± 3.1^{kl}	36 ± 7.7^{l}	35 ± 1.9^{lm}	31 ± 0.8^{m}	30 ± 3.8^{n}
	−2.78	36 ± 2.2^{l}	56 ± 1.8^{i}	45 ± 1.2^{kl}	50 ± 5.7^{jk}	44 ± 5.6^{kl}	53 ± 6.4^{j}	100 ± 7.2^{e}	60 ± 9.5^{i}	51 ± 5.0^{jk}	50 ± 0.9^{k}
	−7.06	121 ± 16.6^{cd}	107 ± 16.3^{de}	68 ± 3.7^{hi}	94 ± 16.7^{f}	107 ± 12.5^{de}	147 ± 23^{b}	140 ± 19^{bc}	127 ± 43.8^{c}	128 ± 8.7^{gh}	168 ± 54.1^{a}
AN384	−0.7	51 ± 5.4^{jk}	49 ± 5.7^{k}	46 ± 6.9^{k}	51 ± 1.7^{k}	47 ± 2.0^{kl}	46 ± 0.4^{kl}	47 ± 7.0^{kl}	49 ± 7.2^{k}	46 ± 3.8^{kl}	51 ± 7.0^{jk}
	−2.78	47 ± 3.2^{kl}	53 ± 6.8^{j}	54 ± 5.5^{ij}	56 ± 2.2^{ij}	50 ± 2.5^{k}	49 ± 6.2^{k}	72 ± 1.7^{gh}	56 ± 6.7^{ij}	56 ± 4.7^{i}	53 ± 1.6^{j}
	−7.06	82 ± 6.2^{fg}	79 ± 10^{fg}	75 ± 6.4^{g}	71 ± 5.0^{h}	97 ± 10.5^{ef}	102 ± 8.2^{e}	114 ± 15.3^{d}	70 ± 4.8^{h}	69 ± 3.3^{h}	79 ± 8.1^{fg}

Values are the mean of four replicates. Means in the same row with the same letter are not significantly different according to the LSD test ($P < 0.05$). SD: standard deviation.

Table 2. Analysis of variance of effect of water potential (MPa), type of pesticide (P), concentration of pesticide (C), strain (I) and their interactions on growth rate and lag phase of *A. niger* aggregate on soil-based medium.

Source of variation	*Growth rate*			*Lag phase*		
	Df[#]	*MS*[†]	*F*[‡]	*Df*[*]	*MS*[†]	*F*[‡]
P	2	35.45	125.67*	2	2273.94	17.79*
C	3	9.79	34.72*	3	1496.65	11.71*
MPa	2	42.32	150.02*	2	68253.26	534.09*
I	1	7.02	24.89	1	1649.84	12.91*
P × C	6	9.49	33.66*	6	800.87	6.27*
P × MPa	4	0.63	2.23*	4	1083.36	8.48*
P × C × MPa	12	1.64	5.83*	12	793.43	6.21*
P × C × MPa × I	12	0.41	1.44	12	410.34	3.21*

[#]Degrees of freedom.
[†]Mean square.
[‡]F-Snedecor.
*Significant $P < 0.05$.

−2.78 and 22.7% at −7.06 MPa, respectively) (Fig. 2A). In AN 384, significant decreases in growth rate were observed at the MPa conditions. The highest decreases were recorded at 20 mg L^{-1} (53.5%, 54.4% and 43% at −0.7, −2.78 and −7.06 MPa, respectively) ($P < 0.05$) (Fig. 2B).

With regard to atrazine, significant differences respect to control were only observed in some pesticide concentrations and MPa conditions. An increase in growth rate of AN 251 was observed at −2.78 MPa with 5 and 10 mg L^{-1}. This increase was only significant at 5 mg L^{-1} ($P < 0.05$). By comparison, a 25% decrease in this parameter was observed at −7.06 MPa and 20 mg L^{-1} atrazine (Fig. 3A). In general, similar values between treatments and controls were observed for AN 384. Different results were obtained at −0.7 MPa with 10 mg L^{-1} of pesticide, where a significant reduction (13%) in growth rate was observed. A similar reduction in growth rate was observed in of the all treatments at −2.78 MPa (Fig. 3B).

The analysis of variance of the effect of single (MPa, type of pesticide and pesticide concentration) two- and three-way interaction showed that these factors alone or combined were statistically significant ($P < 0.05$) in relation to the growth rates of the two *Aspergillus* section *Nigri* strains assayed (Table 2).

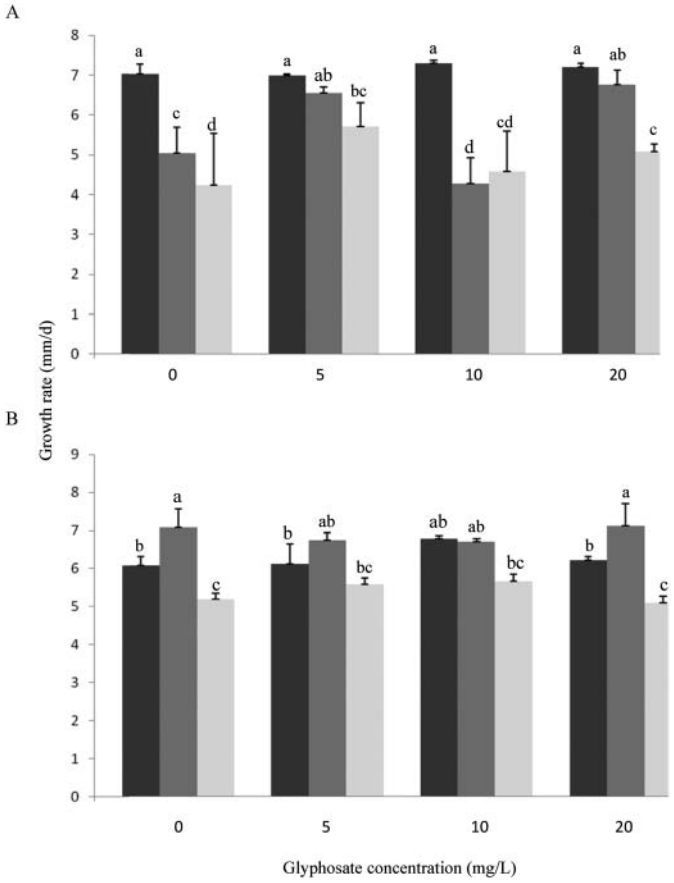

Fig. 1. Effect of glyphosate on growth rate of *Aspergillus niger* aggregate strains AN 251 (A) and AN 384 (B) under different water potential conditions on soil-based medium. −.70 MPa (■), −2.78 MPa (▓) and −7.06 MPa (▒). Mean values are based on quadruplicated data. Means with the same letter are not significantly different according to the LSD test ($P < 0.05$).

Discussion

The results showed that the selected nonochratoxigenic *A. niger* aggregate strains are able to grow effectively in a soil-based medium containing low nutrient status and a range of different concentrations of glyphosate, chlorpyrifos and atrazine, under different MPa conditions. The strains showed resistance to the three pesticides evaluated, which suggests that these strains present tolerance to the tested doses. Each evaluated pesticide showed a different effect on the growth parameters and this effect depended on the concentration and the MPa condition. The growth rates with glyphosate (5–20 mg L^{-1}) were always higher or similar to controls (media without pesticide) depending on the MPa conditions, while the length of the lag phases varied with MPa and glyphosate concentration. These growth rate results are similar to those found previously with aflatoxigenic *Aspergillus* section *Flavi* strains isolated from soils and grown on maize meal-based media.[24] and nontoxigenic *A. flavus* grown on soil-based medium.[23] The lag phase results obtained in the present study partially agree with those informed by these authors because, in general, this parameter decreased as the concentration of glyphosate increased.

In other study, Reddy et al.[34] observed inhibition in radial growth of two toxigenic *A. flavus* strains only when these strains were grown on rich media with 10 mM of glyphosate. However, on a medium devoid of nutrients, radial growth was reduced by approximately 50% and 80% with 5 and 10 mM of glyphosate, respectively.

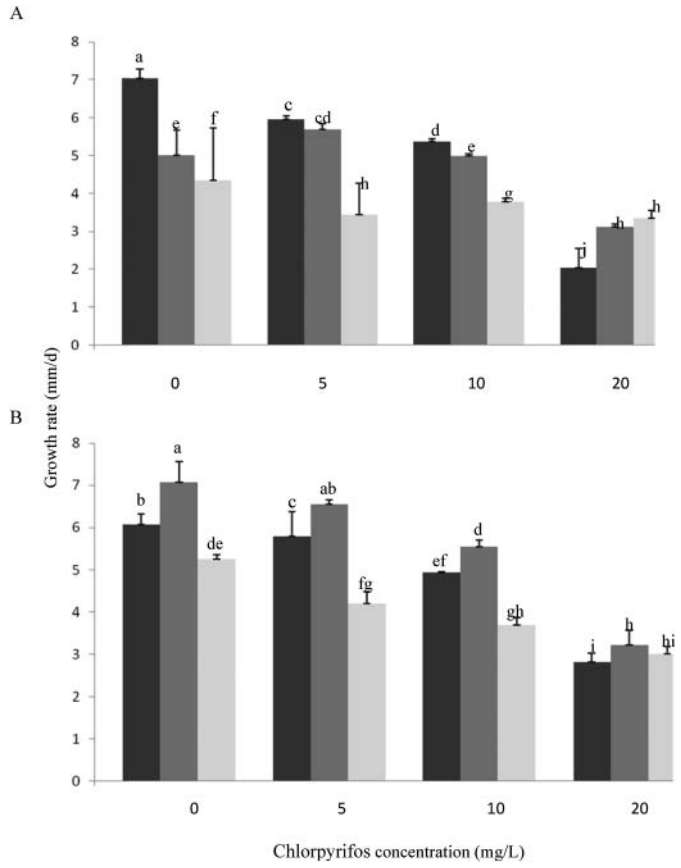

Fig. 2. Effect of chlorpyrifos on growth rate of *Aspergillus niger* aggregate strains AN 251 (A) and AN 384 (B) under different water potential conditions on soil-based medium. −0.70 MPa (■), −2.78 MPa (▩) and −7.06 MPa (▩). Mean values are based on quadruplicated data. Means with the same letter are not significantly different according to the LSD test ($P < 0.05$).

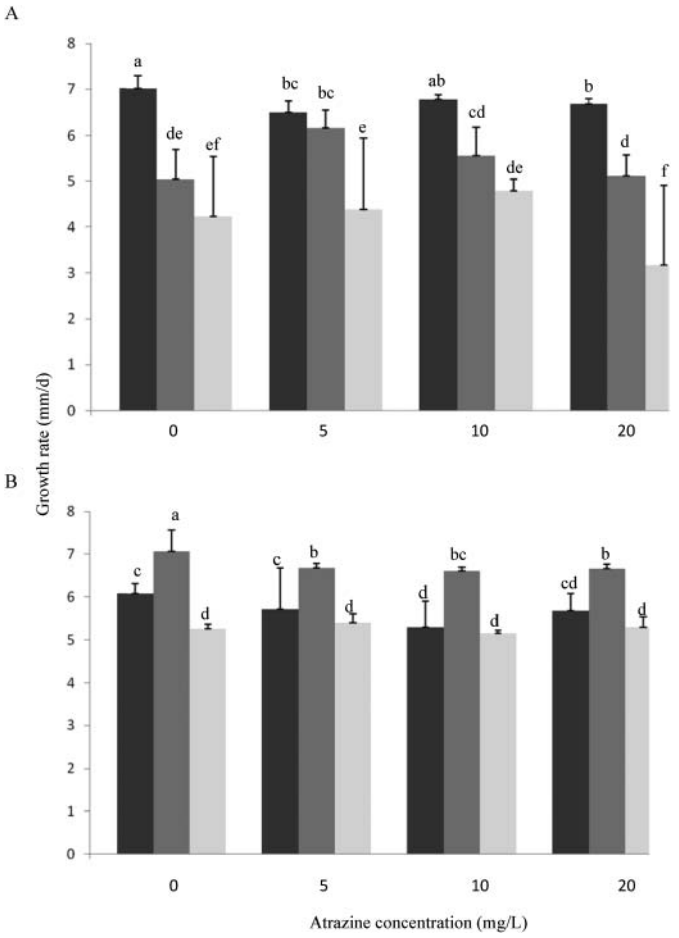

Fig. 3. Effect of atrazine on growth rate of *Aspergillus niger* aggregate strains AN 251 (A) and AN 384 (B) under different water potential conditions on soil-based medium. −0.70 MPa (■), −2.78 MPa (▩) and −7.06 MPa (▩). Mean values are based on quadruplicated data. Means with the same letter are not significantly different according to the LSD test ($P < 0.05$).

Similarly, Hasan[35] reported a significant decrease in the dry mycelium mass of a toxigenic *A. parasiticus* strain when growing on a medium containing 20% sucrose, 2% yeast extract and a high glyphosate content (50 to 1,000 ppm). These results do not match with those informed in the present study since, in general, no inhibition in growth rate was observed in any of the assayed conditions.

Responses of other fungal species to glyphosate or its formulations can vary depending on susceptibility to the herbicide.[36,37] Glyphosate-tolerant fungi may metabolize glyphosate, if they are able to use the available phosphate or the amine structures. Several fungal species have been evaluated for their response to this pesticide in assays in vitro. Some authors showed enhanced growth of *Fusarium* spp., *Trichoderma harzianum* and *Rhizoctonia* spp. on glyphosate supplied culture media, depending on the concentration of the pesticide.[38–40] Although these results were obtained with other fungal species, they are comparable to those found in the present study because the concentration of glyphosate conditioned the growth of the fungi under certain MPa conditions.

With regard to chlorpyrifos, the observed results were different compared to those with glyphosate and atrazine. In general, a decrease in growth rate with increasing pesticide concentrations was observed at the same MPa condition. A significant increase was only observed in AN 251 at −2.78 MPa with 5 mg L^{-1}. Some studies indicate that different fungal species present different capacity to degrade chlorpyrifos efficiently in liquid media and soil.[41] Omar[42] observed that *Aspergillus terreus* showed the greatest potential to mineralize organic phosphorus and sulfur from chlorpyrifos (10, 50 and 100 ppm active ingredient) in liquid media followed by *A. tamari* and *A. niger*, among other fungal species. In other study with *Phanerochaete chrysosporium*, Bumpus et al.[43] reported a 27.5% degradation after 18 days of incubation. Al-Mihanna et al.[44] observed a rapid degradation of chlorpyrifos with a mixture of pathogenic fungi compared to the medium inoculated with a single strain. Xu et al.[45] reported a complete mineralization after 5 days using a co-culture of

bacteria and *Trichosporum* spp., which were exposed to 50 mg L^{-1} of chlorpyrifos. In addition, Fang et al.[46] found a similar result with *Verticillium* sp. More recently, Kulshrestha and Kumari[47] reported a high degradation (83.9%) of chlorpyrifos (300 mg L^{-1}) by a strain of *Acremonium* spp. grown in a nutritive medium. Finally, Gao et al.[48] obtained similar results with *Cladosporium cladosporioides* strain Hu-01. Although the biodegradation of chlorpyrifos by *A. niger* aggregate strains was not evaluated in the present study, the obtained results are promissory because the strains showed tolerance and capacity of development in the presence of this insecticide in concentrations between 5 and 20 mg L^{-1}, at different MPa conditions.

In general, the results obtained with regard to atrazine are comparable with those obtained with glyphosate due to that, with the same MPa condition, the growth rate values were similar to those of controls. An increase in growth rate was only observed in AN 251 with 5 and 10 mg L^{-1} at −2.78 MPa, whereas a significant reduction in this parameter was observed in the two *A. niger* aggregate strains with certain MPa conditions and atrazine concentrations. Of the three pesticides evaluated, atrazine has been reported as the most difficult to degrade by microbial metabolism. There is no information on the influence of triazinic compounds on *Aspergillus* growth rate in assays in vitro under different environmental conditions. In addition, few reports have demonstrated the ability of some soil fungi (*Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp and *Penicillium* spp.) to partially degrade atrazine.[49] Bending et al.[50] informed that the fungus *Coriolus versicolor* takes 42 days to reach the maximum degradation 86% of atrazine in liquid culture. Levanon [51] and Modin et al.[9] showed the importance of combining fungi and bacteria for atrazine mineralization. Partial degradation was demonstrated with the white-rot fungus *Phanerochaete chrysosporium* in culture medium [52] and in non-sterile soil samples in the presence of wood chips.[53] *Pleurotus pulmonarius* is another lignocellulolytic fungus that has been found to degrade atrazine in liquid culture.[54] Hai et al.[55] reported that a mixed fungus–bacteria culture (white-rot fungus and activated sludge) removed 98% of atrazine from the liquid medium in 2 weeks. Atrazine is generally considered as one of the most persistent pesticides.[9] Since the conditions of these studies are different from those of the present work, a strict direct comparison is not possible. On the other hand, the tolerance to atrazine (at applied doses of 5–20 mg L^{-1}) observed in non-acclimated *A. niger* aggregate strains is especially interesting. Considering that some authors have reported that the concentrations and forms of C and N are major determinants of atrazine biodegradation,[56] further works will allow to determinate the most adequate nitrogen and/or carbon supplementation in the culture medium to improve the metabolization of atrazine.

There are few reports on the degradation potential of organophosphate pesticides such as glyphosate, chlorpyrifos and atrazine or their derivatives by fungi like *Aspergillus* section *Nigri* strains. All these reports emphasize the great potential of soil fungi for bioremediation but only few fungi with organophosphate compounds-hydrolyzing enzymes have been reported.[41] An organophosphorus hydrolase (67 kDa) with the ability to hydrolyze a range of P–S bonds was isolated from *Aspergillus niger* ZHY256.[25] In addition, certain conditions such as temperature, moisture, nutrient status, pH and aeration that favor microbial growth and enzymatic activity in soil would also generally promote metabolic degradation of pesticides.[57,58] An overview of the obtained results and the aspects given above reveals that the effect of glyphosate, chlorpyrifos and atrazine on native *A. niger* aggregate strains requires further investigation in a microcosm system to determine the possible participation of nonochratoxigenic black Aspergilli in the biodegradation of these xenobiotic compounds in agricultural soils.

Acknowledgments

C.S. Carranza is a fellow from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), C.L. Barberis, S.M. Chiacchiera and C.E. Magnoli are members of the research career of CONICET.

Funding

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT-PICT-2482/10) and Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto (SECYT-UNRC- 18/C391).

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