EFFECT OF HYDROXAMIC ACIDS ON WHEAT INFESTING FUNGI (*GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI, FUSARIUM CULMORUM, CEPHALOSPORIUM GRAMINEUM*)

Martyniuk S.[1], Stochmal A.[2], Macias F. A.[3], Marin D.[3], Molinillo J. M. G.[3], Oleszek W.[2]

[1]Department of Agricultural Microbiology and [2]Department of Biochemistry, Institute of Soil Science and Plant Cultivation, 24-100 Pulawy, Poland
[3]Departamento de Quimica Organica, Facultad de Ciencias, Universidad de Cadiz, C/Republica Saharaui s/n, Apartado 40, 11510 Puerto Real, Cadiz, Spain

INTRODUCTION

Hydroxamic acids and related benzoxazolinione compounds commonly occurring in cereals such as corn, rye and wheat are also considered to be an important factor in plant-parasite interactions [1,2,3]. To test antifungal activity of these substances we have chosen three fungal pathogens of cereals differing with respect to a mode of plant infection. *Gaeumannomyces graminis* var. *tritici* is strongly pathogenic to winter wheat and other cereals, except oats. It infects roots, including vascular cylinder, causing blackening and rotting of the root system and stem bases of cereals ("take-all" disease). *Cephalosporium gramineum* infects winter cereals through wounded (broken) roots and colonises water conducting vessels in stems and leaves. Symptoms include striping on leaves and finally premature blighting of the infected plants ("Cephalosporium stripe" disease). *Fusarium culmorum* has a broad range of host plants; in cereals it causes root rot and head blight.

MATERIALS AND METHODS

Allelochemicals. Benzoxazolin-2(3H)-one (BOA) and 6-methoxy-benzoxazolin-2(3H)-one (MBOA) and 2-aminophenol (2-AF) were purchased from Aldrich-Sigma. Other transformation products of these compounds tested in this study (APO, AAPO, HPAA, AAMPO, HPMA, HMBOA and HMPMA) were supplied by Prof. F.A Macias and co-workers.

Fungal cultures, media and testing of antifungal activity. The fungal species used in this study: *Gaeumannomyces graminis* (Sacc.) Arx & Oliv. var. *tritici* Walker, *Cephalopsorium gramineum* Nisikado & Ikata and *Fusarium culmorum* (W.G.Sm.) Sacc. were isolated from diseased winter wheat plants and stored on PDA (potato dextrose agar, Difco) slants at –20^{o} C in a cryoprotectant. Fresh cultures of these fungi were obtained by transferring mycelial plugs to Petri plates containing 20 ml of CMA (corn meal agar, Becton & Dickinson) and incubation for 7-14 days at 20^{o} (*C. gramineum*) or 25^{o} C (other fungi). CMA was also used to test the antifungal activity of all allelochemicals. Portions of the medium were enriched after autoclaving with different volumes of stock solutions (in ethanol) of the tested compounds (96%) to give various concentrations of the compounds in CMA. All treatments contained the same amounts of ethanol, usually 1 ml per 100 ml of the medium, including the control treatment containing pure ethanol. The tested concentrations of the allelochemicals in CMA depended on their availability and antifungal activity. In the case of BOA and MBOA the tested concentrations ranged from 0.125 mM to 4.0 mM while metabolites or derivatives of these hydroxamic acids were examined at markedly lower concentrations, ranging from 0.00125 mM to 0.1mM.

Aliquots of 15 ml of warm CMA containing different concentrations of the tested compounds were poured into Petri plates (90 mm in diam.) and allowed to solidify. The plates were inoculated in the centre with 5 mm discs cut from actively growing colonies of the fungi on CMA without any amendments. There were at least 4 replicated plates for each tested concentration of the compounds. The inoculated plates were incubated in the dark at the optimal temperature for the growth of a particular fungus; 20^{0} C for *C. gramineum* and 25^{0} C for *G. graminis* var. *tritici* and *F. culmorum*.

After different period of incubation, depending on the growth rate of the fungi, colony diameters were measured. For the calculation of the percentage of inhibition the diameter of the inoculum disc (5mm) was subtracted from the measured colony diameters. Results are expressed as percentages of inhibition in relation to the control and 50% effective doses (ED_{50}) were calculated for each compound if the inhibition exceeded 50%.

RESULTS

Antifungal activity of the tested compounds depended both on their structure and the fungus tested. Comparison of BOA and MBOA (Table 1) shows that the first substance was generally less fungitoxic toward all the fungi tested than the latter one. Accordingly, MBOA effective doses (ED_{50}) for all the fungi were higher than those of BOA. Of the compared fungi, *G. graminis* var. *tritici* was the most sensitive to both compounds and *F. culmorum* the most resistant one. MBOA reduced the mycelial growth of *G. graminis, C. gramineum* and *F. culmorum* by 50% at the concentrations of 77, 134 and 271µg ml^{-1}, respectively, and corresponding values of BOA ED_{50} for the fungi were 111, 189 and 456µg ml^{-1}.

Antifungal activity of the transformation products of BOA and MBOA was examined at markedly lower concentrations, mainly due to higher toxicity of some of these compounds to *G. graminis* var. *tritici* and *C. gramineum*, and partially because of limited amounts of these derivatives. Of the tested compounds APO was found to be most inhibitory to the mycelial growth of *G. graminis* var. *tritici* and *C. gramineum*, with ED_{50} as low as 0.78 and 0.58ug ml^{-1}, respectively (Table 2). 2-aminophenol showed also high antifungal activity with ED_{50} amounted to 0.80µg ml^{-1} in the case of *G. graminis* and 1.4µg ml^{-1} in the case of *C. gramineum.* AAPO reduced growth of *G. graminis* by 50% at the concentration 2.18µg ml^{-1} and that of *C. gramineum* at 4.57µg ml^{-1}. It is interesting to note that none of the above-mentioned transformation products of BOA affected growth of *F. culmorum* at the tested concentrations (Table 2).Other derivatives tested (HPAA, AAMPO, HPMA, HMBOA and HMPMA)

Table 1. Percentages of inhibition (in relation to control) of radial growth of fungi on corn meal agar (CMA) enriched with different concentrations of benzoxazolinone (BOA) and 6-methoxy-benzoxazolinone (MBOA).

Concentration in mM	*Gaeumannomyces graminis* v. *tritici*	*Cephalosporium gramineum*	*Fusarium culmorum*
BOA			
4.0	-	100	59.6
2.0	- ED_{50}=	73.1 LD_{50} =	29.0
1.0	64.7 111ug/ml	34.5 189ug/ml	15.7 ED_{50} =
0.5	22.5	4.9	1.9 456ug/ml
0.25	11.2	-	-
0.125	2.8	-	
MBOA			
4.0	-	-	-
2.0	-	-	62.7
1.0	100 ED_{50} =	63.7 ED_{50} =	27.5 ED_{50} =
0.5	54.9 77ug/ml	21.9 134ug/ml	16.8 271ug/ml
0.25	19.7	8.4	1.9
0.125	19.7	4.7	-

had no substantial effect on the mycelial growth of any of the fungi used in the study (data not shown).

In conclusion, the tested fungal pathogens of cereals responded differently to the tested benzoxazolinone allelochemicals added to corn meal agar; with *F. culmorum* being the most tolerant, both to BOA and MBOA, as well as to their derivatives. APO, AAPO and 2-AF, the transformation products of BOA, showed much stronger antifungal activity against *G. graminis* var. *tritici* and *C. gramineum* than BOA.

Table 2. Percentages of inhibition (in relation to control) of radial growth of the tested fungi on corn meal agar (CMA) enriched with different concentrations of benzoxazolinone (BOA) transformation products

Concentration in mM	*Gaeumannomyces graminis v. tritici*			*Cephalosporium gramineum*			*Fusarium culmorum*		
Metabolites									
	APO	AAPO	2-AF	APO	AAPO	2-AF	APO	AAPO	2-AF
0.1									
0.05									
0.02	100	94	100	100	54	65	11	0	0
0.01	100	89	87	87	34	43	0	0	0
0.005	98	20	50	73	24	17	0	0	0
0.0025	35	16	11	57	20	3	0	0	0
0.00125	18	-	-	32	-	-	-	-	-
	ED_{50} (µg/ml)			ED_{50} (µg/ml)					
	0.78	2.18	0.80	0.58	4.57	1.4			

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