

Institute of Soil Science and Plant Cultivation – State Research Institute,
Puławy, Poland

EFFECTS OF SAPONINS FROM *MEDICAGO ARABICA* ON *IN VITRO* GROWTH OF *CEPHALOSPORIUM GRAMINEUM*

S. Martyniuk and Z. Biały

Abstract

Two glycosides of bayogenin and five glycosides of hederagenin, the triterpenoid saponins dominating in the aboveground parts of *Medicago arabica*, were examined for their inhibitory activity against *Cephalosporium gramineum*. Glycosides of bayogenin tested in this study: 3-*O*- α -L-arabinopyranosyl and 3-*O*- α -L-arabinopyranosyl-28-*O*- β -D-glucopyranosyl with EC₅₀ 3.31 μ g/ml and 5.61 μ g/ml, respectively, inhibited the growth of *C. gramineum* considerably stronger than similar glycosides of hederagenin. Monodesmosides (having one sugar chain linked at the C-3 position) of bayogenin and hederagenin were found much stronger inhibitors of *in vitro* growth of the fungus than bidesmosides (with two sugar chains linked at the C-3 and C-28 positions) of these aglycones.

Key words: *Cephalosporium gramineum*, growth, inhibition, *Medicago arabica*, saponins

Introduction

Cephalosporium gramineum (sporodochial stage: *Hymenula cerealis*), the causal agent of Cephalosporium stripe disease of winter cereals, is an economically damaging vascular pathogen occurring world wide. It infects roots of winter cereals and spreads further through water-conducting vessels into stems and leaves. Symptoms include striping on leaves and finally premature blighting of the infected plants (Ayers et al. 1982, Martyniuk 1993, Douhan and Murray 2001). The pathogen survives in soil as a saprotroph on the previously infected host residues, on which it produces numerous, small conidia serving as the primary inoculum for plant infection during wet and cool seasons of the year (Ayers et al. 1982, Douhan and Murray 2001).

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The pathogen can be controlled by growing winter cereals in rotation with non-host crops, e.g. leguminous plants. Non-host crops reduce inoculum levels of the pathogen in soil, but in the case of legumes also chemical composition of their biomass can be an important controlling factor. For example, medics (*Medicago* spp.) contain saponins (triterpenoid glycosides) which possess a broad spectrum of biological activities, including antifungal activity (Levy et al. 1989 a, b, Gruiz 1996, Jurzysta and Waller 1996, Oleszek 1996, Martyniuk et al. 1999, Adel et al. 2000). In our previous studies (Martyniuk et al. 1999) it was found that mycelial growth of *C. gramineum* on corn meal agar enriched with 100 µg/ml of total saponins (a mixture of various saponins), extracted from aboveground parts of *M. arabica* and *M. murex*, was reduced by 92% and 90% respectively, while saponins from aerial parts of *M. sativa* added at the same rate had no significant effect on the growth of the pathogen on the medium. Similar results were reported by Jurzysta and Waller (1996) for the radial growth of *Trichoderma viride*, the fungus often used as a test organism in this kind of research.

The composition and chemical structures of single moieties of saponins occurring in *M. sativa* tissues, the species most commonly grown in Europe and elsewhere, have been well elucidated. The aerial parts of the species are rich in glycosides of medicagenic acid and zanhic, while in the aboveground parts of *M. arabica* glycosides of bayogenin and hederagenin predominate (Jurzysta and Waller 1996, Biały et al. 2004). The aim of the study was to determine the inhibitory potential of various saponins isolated from *M. arabica* against *in vitro* growth of *C. gramineum*.

Materials and methods

Fungus

The isolate of *C. gramineum* used in this work was obtained from diseased (striped) leaves of winter wheat grown in the field at the Experimental Farm in Osiny, near Puławy. The fungus was stored on potato dextrose agar (PDA) slants in a refrigerator (4°C) and sub-cultured on fresh media as needed.

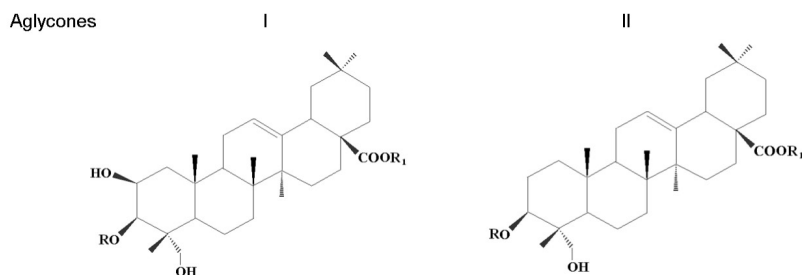
Tested saponins

The following compounds (triterpenoid saponins) were tested for their anti-fungal activity:

- 1 – 3-*O*- α -L-arabinopyranosyl bayogenin,
- 2 – 3-*O*- α -L-arabinopyranosyl-28-*O*- β -D-glucopyranosyl bayogenin,
- 3 – 3-*O*- α -L-arabinopyranosyl hederagenin,
- 4 – 3-*O*-[β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl] hederagenin,
- 5 – 3-*O*- α -L-arabinopyranosyl-28-*O*- β -D-glucopyranosyl hederagenin,
- 6 – 3-*O*-[β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl]-28-*O*- β -D-glucopyranosyl hederagenin,

7 – 3-O-[α -L-arabinopyranosyl(1 \rightarrow 2)- β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl]-28-O- β -D-glucopyranosyl hederagenin.

These compounds were isolated from aboveground parts of *M. arabica*, purified and identified by Biały et al. (2004). Structures of aglycones (I, II) and sugar side-chains (R and R₁) of the isolated saponins are presented in Figure 1.



Saponin number	Aglycone	R	R ₁
1	I	α -L-arabinopyranosyl	H
2	I	α -L-arabinopyranosyl	β -D-glucopyranosyl
3	II	α -L-arabinopyranosyl	H
4	II	β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl	H
5	II	α -L-arabinopyranosyl	β -D-glucopyranosyl
6	II	β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl	β -D-glucopyranosyl
7	II	α -L-arabinopyranosyl(1 \rightarrow 2)- β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl	β -D-glucopyranosyl

Fig. 1. Structures of saponins 1–7 (aglycones: I – bayogenin, II – hederagenin)

Testing *in vitro* growth inhibition of *Cephalosporium gramineum* by saponins

Stock solutions, in 75% MeOH, each containing 10 mg/ml of particular saponins were prepared. From the stock solution appropriate volumes were taken and added to 100 ml portions of autoclaved corn meal agar medium (CMA, Oxide) to achieve saponin concentrations, ranging from 5 to 100 μ g/ml of the medium. All portions of the media, including the control one, contained the same volume (2 ml) of MeOH. The media in aliquots of 20 ml were pored into Petri plates (90 mm diameter) and allowed to solidify. There were five replicated plates per each saponin concentration tested. Next day the plates were inoculated in the centre with a 5 mm disc cut from 21-day-old cultures of *C. gramineum* on CMA. After incubation of the plates for 14 days at $20 \pm 2^\circ\text{C}$ colony diameter was measured. For calculations of the inhibitory effects, the inoculum disc diameter (5 mm) was subtracted from the measured colony diameters. The results presented here are expressed in percentage of *C. gramineum* growth inhibition by particular saponins in relation to the control treatment, which contained equivalent amounts of MeOH.

The 50% effect concentrations (EC_{50} indicates the concentration of a compound at which 50% growth inhibition occurred) were also calculated from dose-response curves using regression analysis (Microsoft Excel).

Results

The tested compounds were predominating saponins in *M. arabica* shoots (Biały et al. 2004). Chemically, they were glycosides of triterpenoid aglycones: bayogenin (compounds 1 and 2) and hederagenin (compounds 3, 4, 5, 6 and 7) (Fig. 1). The majority of these compounds exerted an inhibitory effect on *in vitro* growth of *C. gramineum* but the effects of particular compounds differed substantially (Table 1), indicating that their antifungal activity was structure-dependent.

Table 1

Inhibition of mycelial growth of *Cephalosporium gramineum* on corn meal agar (CMA) enriched with different concentrations of saponins (% of control) and the estimated EC_{50} values

Saponin concentration in CMA ($\mu\text{g/ml}$)	Tested saponins						
	1	2	3	4	5	6	7
1.0	4	0	0	0	0	0	0
2.5	45	20	0	0	0	0	0
5	75	63	15	0	0	0	0
10	78	78	47	3	4	0.5	0
25	82	93	80	25	6	1	0
50	93	100	86	57	23	32	2
EC_{50} ($\mu\text{g/ml}$)	3.31	5.61	14.58	44.45	–	–	–

1 – 3-*O*- α -L-arabinopyranosyl bayogenin,
 2 – 3-*O*- α -L-arabinopyranosyl-28-*O*- β -D-glucopyranosyl bayogenin,
 3 – 3-*O*- α -L-arabinopyranosyl hederagenin,
 4 – 3-*O*-[β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl] hederagenin,
 5 – 3-*O*- α -L-arabinopyranosyl-28-*O*- β -D-glucopyranosyl hederagenin,
 6 – 3-*O*-[β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl]-28-*O*- β -D-glucopyranosyl hederagenin,
 7 – 3-*O*-[α -L-arabinopyranosyl(1 \rightarrow 2)- β -D-glucopyranosyl(1 \rightarrow 2)- α -L-arabinopyranosyl]-28-*O*- β -D-glucopyranosyl hederagenin.

Glycosides of bayogenin (compounds 1 and 2), with EC_{50} 3.31 $\mu\text{g/ml}$ and 5.61 $\mu\text{g/ml}$, respectively, were markedly more inhibitory to *C. gramineum* than similar glycosides of hederagenin (compounds 3 and 5).

Monodesmosides of bayogenin and hederagenin (compounds No. 1, 3 and 4, which have sugars at the C-3 position only) had higher antifungal activities than bidesmosides of these aglycones, having sugars (either mono- or polysaccharide chains) linked to the aglycones at the C-3 and C-28 positions (compounds 2, 5–7).

From among all saponins tested, bayogenin monodesmoside had the highest inhibitory activity, as indicated by the lowest EC_{50} value amounted to 3.31 $\mu\text{g/ml}$ (Table 1), against *C. gramineum*, the fungal pathogen used in this study. Comparing two monodesmosides of hederagenin it is worth noticing that the compound having a sugar chain composed of two sugars: glucose and arabinose, linked at the C-3 position (compound No. 4), had lower antifungal activity than hederagenin with one sugar (arabinose) only at the same carbon (compound No. 3).

Discussion

Most of the saponins obtained from the aboveground parts of *M. arabica* and tested for their biological activity against *C. gramineum* were glycosides of hederagenin (four compounds) and two other saponins were glycosides of bayogenin (Fig. 1). However, the latter two compounds were markedly more inhibitory to the tested fungus than respective saponins of hederagenin (Table 1). These structurally very similar triterpenoid compounds differ only with respect to the additional hydroxyl group at the C-2 in bayogenin skeleton (Fig. 1). This hydroxyl group, which increases the polarity and thus solubility of the bayogenin glycosides, may be responsible for the higher biological activity of these compounds as compared to the glycosides of hederagenin (Levy et al. 1989 a, b, Oleszek 1996).

Monodesmosides of both aglycones (bayogenin and hederagenin) were found to be more potent inhibitors of *in vitro* growth of *C. gramineum* than bidesmosides of these aglycones. With respect to medicagenic acid mono- and bidesmosides, dominating in the aboveground parts of *M. sativa*, similar results were obtained (Martyniuk and Jurzysta 2005). It was suggested that biological (antifungal and insecticidal) activity of saponins, including glycosylated triterpenoids, results from the ability of these compounds to form complexes with sterols and probably also with other constituents, e.g. proteins and phospholipids, of cell membranes, disturbing functioning of these cell structures (Levy et al. 1989 b, Gruiz 1996, Tava and Odoardi 1996, Adel et al. 2000). It was also shown that saponins with free carboxylic group at C-28 of triterpenoid moiety were more fungitoxic than when these groups were esterified or glycosylated (Levy et al. 1989 b, Oleszek 1996, Tava and Odoardi 1996). In the case of bidesmosides we examined, the second sugar chain was linked to aglycones (bayogenin and hederagenin) through the carboxyl group at the C-28 position and this was probably the main reason for the lower antifungal activity of these compounds as compared to monodesmosides.

In the present studies the most inhibitory saponin against *C. gramineum* appeared to be monodesmoside of bayogenin (compound 1 with 3.31 $\mu\text{g/ml}$). In our earlier studies this compound was substantially less inhibitory (EC_{50} 13.7 $\mu\text{g/ml}$) against *in vitro* growth of *Gaeumannomyces graminis* var. *tritici*, another fungal pathogen of cereals (Martyniuk and Biały 2008). These results are in accordance with reports of Levy et al. (1989 a, b) and Saniewska et al. (2003) indicating that fungal plant pathogens differ substantially with respect to their sensitivity to triterpenoid saponins.

Comparison of two monodesmosides of hederagenin has shown that the compound having a sugar chain composed of two sugars: glucose and arabinose, linked at the C-3 position (compound No. 4), was less inhibitory to *C. gramineum* than hederagenin with one sugar (arabinose) only at the same carbon (compound No. 3). Similar results were obtained by Levy et al. (1989 a) who reported that monodesmoside of medicagenic acid with two sugars at C-3 was also less inhibitory to *Trichoderma viridis* than monodesmoside with one sugar only. It was suggested by these authors that the composition of the sugar component might influence the hydrophilicity of the compared compounds, thus affecting their transport to the site of action.

Streszczenie

ODDZIAŁYWANIE SAPONIN *MEDICAGO ARABICA* NA WZROST *IN VITRO* *CEPHALOSPORIUM GRAMINEUM*

Badano wpływ siedmiu saponin (glukozydy trójterpenów aromatycznych: bajogeniny i hederageniny) wyodrębnionych z części nadziemnych *Medicago arabica* na wzrost grzyba *Cephalosporium gramineum*, powodującego naczyniową pasiastą złączy. Hamowanie wzrostu badanego grzyba testowano na agarowej pożywce z wyciągiem kukurydzianym (corn meal agar), z dodatkiem różnych ilości (od 1 do 50 µg/ml) badanych związków saponinowych. Wykazano, że glukozydy bajogeniny: 3-O-α-L-arabinopyranosyl (EC₅₀ 3.31 µg/ml) i 3-O-α-L-arabinopyranosyl-28-O-β-D-glukopyranosyl (EC₅₀ 5.61 µg/ml) były silniejszymi inhibitorami wzrostu *C. gramineum* niż takie same glukozydy hederageniny. Monodesmozydy (cukry przyłączone do węgla C-3 aglikonu) bajogeniny i hederageniny hamowały intensywniej wzrost badanego grzyba niż bidesmozydy tych związków aromatycznych (z cukrami przy węglu C-3 i C-28 aglikonu).

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Authors' address:

Prof. Dr. hab. Stefan Martyniuk, Dr. Zbigniew Biały, Institute of Soil Science and Plant Cultivation – State Research Institute, ul. Czarторыskich 8, 24-100 Puławy, Poland, e-mail: sm@iung.pulawy.pl

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