ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 53, nr 4: 543-550
1984

Studies on *Medicago lupulina* saponins. 4. Variation in the saponin content of *M. lupulina*

PIOTR M. GÓRSKI, MARIAN JURZYSTA,
STANISŁAW BURDA, WIESŁAW A. OLESZEK, MICHAŁ PŁOSZYŃSKI

Department of Biochemistry and Physiology of Crop Plants,
Institute of Soil Science and Plant Cultivation,
24-100 Puławy, Poland

(Received: February 16, 1984. Accepted: April 16, 1984)

Abstract

The saponin content in the tops of black medic trefoil cv. Renata as well as in some botanical and breeding lines of *M. lupulina* was analysed. It was found that the concentration of biologically active (hemolytically active) saponins in the tops of *M. lupulina* cv. Renata was 2.5% of dry matter. Total saponin content was 3.5% of dry matter. Among 500 individually analysed plants of the Renata variety, the saponin contents ranged from 0.07 to 0.5% in the leaf sap. No saponin-free plant was found. The saponin content was additionally analysed in 300 breeding and 11 botanical lines of *M. lupulina*. Great differences in that material were found but no saponin-free line was present. The saponin content in the particular plant parts as well as in the whole tops of *M. lupulina* throughout the vegetation season was also measured. The possibility and necessity of the selection for a low saponin population of *M. lupulina* is discussed.

Key words: Medicago lupulina, saponin concentration

INTRODUCTION

Alfalfa (*Medicago media* Pers.) and black medic trefoil (*Medicago lupulina* L.) are the only lucerns cultivated in Poland, from among about 100 known species. It is well known that alfalfa, one of the best fodder crops, contains natural compounds — saponins. Alfalfa saponins are known to exhibit different biological activities such as hemolytic and allelopathic. These compounds also cause bloating when fed to ruminants and growth depression when fed to monogastric animals (C h e e c k e 1971). There has been very scarce information on the subject in *M. lupulina* up to date. However, for the first time we have reported the isola-

tion and chemical characterisation of *M. lupulina* saponins (Górski et al. 1984a, b). It was also found that *M. lupulina* meal showed high antinutritional properties (Górski et al. 1984c) but there is still no information about the saponins concentration in *M. lupulina*.

Hence the aim of our present paper is to analyse the saponin content in the Renata variety, some botanical and breeding lines of *M. lupulina* as well as the saponin content in particular plant parts and its variability throughout the season. *M. lupulina* contains two kinds of saponins: medicagenic acid glycosides — toxic to fish and possessing fungistatic, hemolytic and antinutritional activities, and soyasapogenol glycosides showing low or no such biological properties (Górski et al. 1984b). In the present investigation, mainly the concentrations of these biologically active saponins are analysed. In this paper, they are referred to as hemolytic saponins.

MATERIAL AND METHODS

M. lupulina cv. Renata plants were field grown, harvested at the time of flowering, dried at 60°C and ground. *M. lupulina* plants picked at random were divided into leaves, stems, roots and flowers, then dried and ground as above. The seasonal variation in the saponin content was also analysed in plants collected in the field (in 1982). Breeding and botanical lines of *M. lupulina* were grown in the greenhouse in pots (20 plants per line), then collected as above. Seeds of *M. lupulina* were scarified in 96% H_2SO_4 for 5 min. Germinated seeds and seedligns were grown on wet paper in Petri dishes.

Total saponin content was measured gravimetrically according to Van Atta et al. (1961). Hemolytically and fungistatically active saponins were determined by the *Trichoderma viride* biotest (Jurzysta 1979b), hemolytic saponin content in leaf sap was measured with the hemolytic micromethod (Jurzysta 1979a). For quantification, a standard curve was prepared using hemolytic saponins isolated from *M. lupulina* tops (Górski et al. 1984b).

RESULTS AND DISSCUSION

SAPONIN CONTENT IN THE TOPS OF *M. LUPULINA* CV. RENATA, BREEDING AND BOTANICAL LINES

It was found that the hemolytic saponin content in the tops of *M. lupulina* cv. Renata at the begining of flowering was very high (2.5% of dry matter). The total saponin content in these tops was also high

(3.5% of dry matter). These findings are in agreement with those of Jurzysta and Nowacki (1979). They found that *M. lupulina* meal caused high growth inhibition in the fungus *Trichoderma viride*. *M. lupulina* was included by the authors in the group of lucernes which caused the highest fungus inhibition. In that group, *M. orbicularis*, *M. polymorpha*, *M. turbida*, *M. falcata* and a few others were listed. The cultivated varieties of *M. media* contained smaller amounts of saponins 0.7—1.1% of dry matter (Jurzysta 1981). However low saponin populations from *M. media* cultivars were obtained through the selection of saponin-free individual plants (Pedersen and Wang 1971, Jurzysta 1979c).

Our next attempt, therefore was to analyse the variability of the saponin content in the population of the Renata variety. Five hundred individual plants were analysed with the fast hemolytic microtest for saponin content in the leaf sap. This method is suitable for screening analyses of fresh samples. The results obtained are presented on Fig. 1. It was found that the hemolytic saponin content ranged from 0.07 up to 0.5% in leaf sap, but no saponin-free plant was found. The distribution of the saponin content in the *M. lupulina* population is similar to the Gauss distribution curve, the distribution of the studied trait in the populations of *M. media* also has a similar pattern. However, in the population of *M. falcata*, the Gauss distribution was not observed, hence 93% are indiwiduals of high saponin content (Jurzysta et al. 1973).

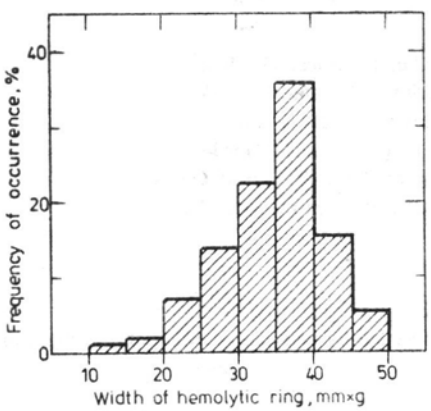

Fig. 1. Frequency of occurrence of individual plants of different hemolytic saponin content in the populations of *M. lupulina* cv. Renata. Saponins were analysed in leaf sap of 500 individuals using the hemolytic micromethod, then divided according to the width of hemolytic ring into 8 classes

The results indicated that there is a selective factor or factors for high saponin content in *M. falcata*, but no such factor is present for high saponin content in *M. media* and *M. lupulina*. It can be due to the

fact that *M. falcata* grows in natural stands while *M. media* and *M. lupulina* are cultivated. Saponins, because of their protective role against plant and animal enemies are more needed in the natural state. On the other hand, the ununiform pattern of saponin content in *M. falcata* may be due to the type of saponin inheritance in that species (J u r z y s t a et al. 1973).

Three hundred breeding and 11 botanical lines of *M. lupulina* were screened for low saponin content. The obtained results are presented in Tables 1 and 2. No saponin-free line, was found, although a few low saponin (0.04—0.07% in leaf sap) breeding lines were selected. It seems that selection for low saponin in *M. lupulina* may be difficult and the question arises if such a selection is necessary.

In spite of strong antinutritional properties of *M. lupulina* meal, the risk of its feeding is not high, since *M. lupulina* is not widely fed to

Table 1

The concentration of hemolytic saponins in leaf sap of *M. lupulina* botanical lines

No	Origin	Saponin concentration, % in leaf sap
I — 2526	Poland (Radzików)	0.25
I — 2513	GDR	0.30
135	GDR (Lipsk, Botanical Garden)	0.36
136	" " " "	0.30
958	" " " "	0.26
602	GDR (Halle, Botanical Garden)	0.24
442	USSR (Crimea, Botanical Garden)	0.35
I — 2511	USSR (Stawropol, Botanical Garden)	0.36
I — 2514	China (Peking, Botanical Garden)	0.26
—	Portugal (Coimbera, Botanical Garden)	0.34
—	Canada (Ottawa, Central Exp. Farm.)	0.30

Table 2

Width of hemolytic ring and hemolytic saponin content in 300 breeding lines

Width of hemolytic ring, mm×9	% of saponin in leaf sap	Frequency of occurrence	
		number of lines	%
5-10	0.04-0.07	5	1.3
10-20	0.07-0.14	131	43
20-30	0.14-0.24	121	40.3
30-40	0.24-0.36	31	11
40-50	0.36-0.50	13	4.4

monogastric animals. However, saponins cause bloating in ruminants and the problem may apear with cattle fed high doses of *M. lupulina*.

SAPONIN CONTENT IN DIFFERENT PLANT PARTS AND SEASONAL VARIABILITY OF SAPONINS IN *M. LUPULINA* TOPS

The hemolytic saponin content in plant organs was analysed using the *T. viride* biotest. This method is not as fast as the hemolytic one but is some more accurate and suitable for analyses of dry-matter samples. The results are shown in Table 3. The highest concentration of saponins was found in the leaves and seeds of *M. lupulina*, a lower one in the stems and flowers. Roots contained even higher amounts of the analysed substances but the quantification of saponin in roots was not made because standard saponins have not been isolated from the roots. However, we found that the growth inhibition of the fungus *T. viride* caused by root samples was almost 10 times higher than growth inhibition caused by the samples of tops. This fact indicated that *M. lupulina* roots, compared to tops, contained higher amounts of saponins or/and these saponins exhibited very high fungistatic properties.

Table 3

Hemolytic saponin content in particular *M. lupulina* parts

Plant organs	Saponin content, % of dry matter
Leaves	3.0
Stems	1.8
Flowers	1.7
Seeds	3.0

The distribution of hemolytic saponins in particular plant parts of *M. media* is different; higher amounts of saponins are present in the roots than flowers, leaves and stems (P e d e r s e n 1975). Seeds of *M. media* contained no hemolytic saponins (J u r z y s t a 1973).

The high concentration of saponins in the seeds of *M. lupulina* and the lack of saponins in the seeds of *M. media* seems to support the view that these two species are distinct in the genus *Medicago*. Changes in the saponin content at the time of seed germination and throughout the season are presented in Table 4. A distinct increase in saponins was observed in germinated seeds. This is probably due to the presence of developing roots which contain a high amount of saponins and/or to the rapid biosynthesis of saponins in germinating seeds. A very high content of hemolytic saponins in germinating seeds may be important because of the allelopathic action of saponins. It was shown earlier that lucerne

saponins inhibited the germination of cereal seeds (Jurzysta 1970). *M. lupulina* is frequently sown in a mixture with grasses and the saponins may effect the germination and growth of grasses, but the problem needs further investigation.

Table 4

Hemolytic saponin content in *M. lupulina* germinating seeds and in tops during the vegetation season

Saponin content in germinating seeds	
Stage	saponin content, % of dry matter
Seeds — dry	3.0
Germinated seeds 2 days	6.8
" " 4 "	7.7
" " 6 "	8.0
Saponin content in the tops during vegetation	
Stage	saponin content, % of dry matter
Before flowering	2.5
Beginning of flowering	2.4
Full blooming	2.2
After blooming	2.1

In the tops of *M. lupulina* the hemolytic saponin content was constant from the seedling stage to the stage of flowering and throughout the vegetation period. The slight decrease in the saponin content at the begining of flowering was probably due to the presence of developing flowers which are low in saponins.

The variation in the saponin content at the time of vegetation of *M. media* is more distinct. The highest amount of hemolytic saponins was found in the tops at the stage of flowering, then the concentration of saponins decreased (Vucurevic et al. 1965).

The saponin content varied also from year to year and from cut to cut (even in the same stand). Differences exceeding 100% between particular years and cuts were observed (Jurzysta 1981). In *M. lupulina* the saponin content is also probably under the great influence of environmental factors (temperature, insolation). But the problem needs further investigation.

Acknowledgments

The authors are thankful to Doc. dr Z. Staszewski of IHAR Radzików for providing the seeds of *M. lupulina* botanical lines and to mgr R. Madajewski of SHR Łagiewniki for providing the seeds of *M. lupulina* breeding lines.

REFERENCES

Cheecke P. R., 1971. Nutritional and physiological implications of saponins. A review. Can. J. Anim. Sci. 51: 621-628.

Górski P. M., Jurzysta M., Burda S., Oleszek W. A., Płoszyński M., 1984a. Studies on *Medicago lupulina* saponins: 1. Isolation and indentification of sapogenins from *Medicago lupulina* tops. Acta Soc. Bot. Pol. 53: 515-525.

Górski P. M., Jurzysta M., Burda S., Oleszek W. A., Płoszyński M., 1984b. Studies on *Medicago lupulina* saponins: 2. Isolation, characterization and biological activity of saponins from *Medicago lupulina* tops. Acta Soc. Bot. Pol. 53: 527-533.

Górski P. M., Jurzysta M., Burda S., Oleszek W. A., Płoszyński M., 1984c. Studies on *Medicago lupulina* saponins: 3. Effect of *M. lupulina* saponins on the growth and feed utilization by mice. Acta Soc. Bot. Pol. 53: 535-541.

Jurzysta M., 1970. Effect of saponins isolated from seeds of lucerne on germination and growth of cereal seedlings. Zeszyty Nauk. Univ. M. Kopernika w Toruniu, Zeszyt 23, B XIII: 253-256.

Jurzysta M., 1973. Isolation and chemical characterization of saponins from lucerne seeds (*Medicago media* Pers.). Acta Soc. Bot. Pol. 42: 201-207.

Jurzysta M., 1979a. Haemolytic micromethod for rapid estimation of toxic alfalfa saponins. Acta Agrobot. 32: 5-11.

Jurzysta M., 1979b. Uproszczona metoda oznaczania biologicznie czynnych saponin lucerny za pomocą grzyba *Trichoderma viride*. Biul. Branż. Hod. Roślin 1: 16-18.

Jurzysta M., 1979c. Biochemical characteristic and feeding value of alfalfa selected for saponin content. Biul. IHAR, No 135, Suppl. Int. Congr. Eucarpia. pp. 316-327.

Jurzysta M., 1981. Zawartość saponin biologicznie czynnych w odmianach lucerny mieszańcowej. Biul. Oceny Odmian 9: 13-14.

Jurzysta M., Nowacka D., Nowacki E., 1973. Inheritance of the level of substances inhibiting *Trichoderma viride* development in local populations of alfalfa. Genet. Pol. 14: 365-373.

Jurzysta M., Nowacki E., 1979. Saponins of the genus *Medicago*. Acta Agrobot. 32: 13-17.

Pedersen M. W., 1975. Relative quantity and biological activity of saponins in germinated seeds, roots, and foliage of alfalfa. Crop. Sci. 15: 541-543.

Pedersen M. W., Wang L. L., 1971. Modification of saponin content of alfalfa through selection. Crop. Sci. 11: 833-835.

Van Atta G. R., Guggolz J., Thompson C. R., 1961. Determination of saponins in alfalfa. J. Agr. Food Chem. 9: 77-79.

Vucurevic N., Delic J., Milio B., Turcic M., 1965. Dinamika deputowania saponina u lucerki u zawistnosti od godisujeg doba i stadiuma rozwoja biljke. Veterinaria 14: 315-319.

Badania nad saponinami Medicago lupulina.
4. Zróżnicowanie zawartości saponin w roślinach M. lupulina

Streszczenie

Badano zawartość saponin w częściach nadziemnych lucerny chmielowej (*Medicago lupulina*) odm. Renata oraz w liniach botanicznych i liniach hodowlanych tej rośliny. Stwierdzono dużą zawartość biologicznie czynnych (hemolitycznie czynnych) saponin — 2,5% s.m. Ogólna zawartość saponin w badanym materiale była również duża — 3,5% s.m. Przeprowadzona analiza zawartości saponin w liczącej 500 pojedynków populacji odmiany Renata wykazała, że zawartość hemolitycznie czynnych saponin w badanej populacji waha się od 0,07 do 0,5% w soku liściowym. Nie znaleziono jednak ani jednego osobnika bezsaponinowego. Analizy zawartości saponin dokonano również w 300 liniach hodowlanych i 11 liniach botanicznych *M. lupulina*. W badanym materiale stwierdzono duże zróżnicowanie zawartości saponin, nie znaleziono jednak żadnej linii bezsaponinowej. Zawartość saponin badano także w poszczególnych częściach roślin oraz w całych częściach nadziemnych w okresie wegetacji. Dyskutowana jest możliwość i potrzeba prowadzenia selekcji w kierunku uzyskania niskosaponinowej populacji *M. lupulina*.