ACTA AGROBOTANICA
Vol. 54, z. 2 – 2001
s. 35–45

Biological activity of saponins from alfalfa tops and roots against Colorado potato beetle larvae

MARYLA SZCZEPANIK[1], KATARZYNA KRYSTKOWIAK[1], MARIAN JURZYSTA[2], ZBIGNIEW BIAŁY[2]

[1]Mikołaj Kopernik University, Department of Invertebrate Zoology, Gagarina 9, 87-100 Toruń, Poland, e-mail: mszczep@biol.uni.torun.pl
[2] Institute of Soil Science and Plant Cultivation, Department of Biochemistry and Plant Quality, Czartoryskich 8, 24-100 Puławy, Poland, e-mail: mjurzyst@iung.pulawy.pl

(Received: 19.06.2001)

Summary

The total saponins of alfalfa, *Medicago sativa* L., included in the diet of Colorado potato beetle larvae reduced their feeding, growth rate and survival. The biological activity of those compounds coming both from the roots and from the aerial parts is closely correlated with the dose. Larvae reared on leaves treated with a 0.5% dose virtually did not feed at all and died after 4–6 days. Lower saponin doses (0.01 and 0.001%) reduced the insects´ feeding to a lesser degree. However, they inhibited their growth, caused an extension of the larval stage and mortality at a level of 76.7– 100%. No major differences have been found in saponin activity depending on its localization in the plant.

Key words: Colorado potato beetle, saponins, alfalfa, *Leptinotarsa decemlineata, Medicago sativa.*

INTRODUCTION

Saponins, especially those of alfalfa, are among secondary plant substances characterized by high biological activity towards a number of organisms. They are known to exhibit toxic properties towards fungi *(Trichoderma viride),* bacteria as well as towards a great many mono- and polyphagous animals (Zimmer et al., 1967, Cheeke et al., 1971, Ameenuddin et al., 1983). They also have a wide range of effect on insects. The insecticidal properties of saponins have been studied in relation

to the flour beetle, *Tenebrio molitor* (Pracros, 1982), the European corn borer, *Ostrinia nubialis* (Nozolillo et al., 1997) the armyworm, *Spodoptera littoralis* (Adel et al., 2000), the spider mite, *Tetranychus urticae* (Puszkar et al., 1994) and a number of other pests.

Substances of natural origin having a negative effect on insects can be used in integrated methods of plant protection. They can be particularly useful in reducing the population of pests whose susceptibility to commonly used chemical insecticides decreases at a relatively quick rate. The Colorado potato beetle (CPB) is one of those pests which are particularly difficult to control due to their increasing resistance to pesticides from various chemical groups (Łąkocy, 1973; Pawińska, Węgorek, 1998; Noronha et al., 1999).

Earlier studies (Waligóra, Krzymańska, 1994; Waligóra, 1998a) on the effect of secondary plant substances on the development of that pest point to a high activity of saponins compared with alkaloids and glucosinolates. However, in those studies only saponins obtained from alfalfa leaves were used, in comparatively large doses. The objective of our studies was a comparison of the activity of total saponins extracted from the roots and from the aerial parts of alfalfa, with different doses of those substances included in the diet of the Colorado beetle larvae.

MATERIAL AND METHODS

Saponins

Total saponins have been isolated from aerial parts and roots of alfalfa *(Medicago sativa* L.) cultivar Radius, according to the known methods (Oleszek et al.,1992b; Biały et al.,1999) as follows: defatted plant material was extracted with boiling methanol. After removing of alcohol the residue was dissolved in water. The solution was placed on LiChroprep RP-18 (25–40 mm) column preconditioned with water. The column was washed with water and 30% methanol successively. Total saponins were eluated with methanol and dried at 60°C.

Insect rearing

Adults of Colorado potato beetle were collected from the field and mass reared in the laboratory. After oviposition the eggs were placed in Petri dishes, transferred into an incubator and kept under standard conditions of 25°C, 50 – 70% RH and 16:8 (light:dark) photoperiod for five days. Newly emerged larvae, about12 h old, were used in the assays.

Laboratory bioassays

Aqueous solutions at four concentrations (0.5, 0.1, 0.001 and 0.0001%) were prepared of the total saponins extracted from the roots or the shoots of alfalfa. Larvae were exposed to potato leaves which had been dipped for 5 s in the appropriate solution, or in water as control, then dried for 1 h at room temperature. Next, the leaf areas were measured using a scanner and special software created by A. Zienkiewicz from the Department of Biophysics, Mikołaj Kopernik University, Toruń. After measure-

ments the leaves were placed in Petri dishes (3 dishes/dose) together with ten Colorado potato beetle larvae. The dishes were kept at 24°C and a photoperiod of 16:8 (L:D) for two days, after which the old leaves were replaced with fresh ones. The remaining uneaten leaf areas were measured by the same method. Consumption was determined by comparing the leaf areas before and after feeding. Early instars (first and second) were reared in small Petri dishes (9 cm diameter), late instars were kept in larger ones (15 x 2 cm). On the twelfth day of experiment, when larvae terminated feeding, they were transferred into 1 l jars containing sand. Measurements of feeding intensity ended with the digging of mature larvae into the sand.

Neonate larvae were weighted before exposure to treated leaves and after each 48 h period to determine the increase in body weight. The weight of larvae and the amount of food consumed were recalculated per one larva. Mortality was recorded daily. The data obtained from the experimental and control larvae were compared using Student´s test.

RESULTS

Effect of saponins on larval feeding

The saponins used in our experiments considerably reduced larval food consumption. Their antinutritional activity depended mainly on the dose applied, and to a lesser degree on their derivation. Only small amounts of potato leaves treated with 0.5 and 0.1% saponin solutions, both from roots and shoots of alfalfa were consumed. In the first 48 hours of observation, the area of leaves consumed was one half of the control. In the following days, the amount of food ingested by the larvae was at a very low level, while the feeding intensity of control larvae increased rapidly. Throughout the larval stage, from emergence until descending into pupation, under local conditions the control larva consumed an average of 28.6 cm^2 of leaves. The total amount of food consumed by larvae reared on treated leaves ranged from 1.10 to 28.74 cm^2. It follows from our experiments that larval feeding was lowest on leaves dipped in 0.5 and 0.1% solutions of saponins from the aerial parts, and only slightly higher if the saponins came from the roots. However, the differences were not significant, and in the experiment with the 0.5% solution the total area consumed constituted only 3.85% (aerial parts) and 4.8% (roots) of the area consumed by control larvae. In the presence of the 0.1% solution of saponins from aerial parts larval feeding was minimally reduced in relation to larvae reared on a diet with root saponins. With lower doses, the strongest antinutritional effect was observed with 0.01% concentration of shoot saponins: the leaf area consumed was one half of the control. Low doses of root saponins reduced feeding by only ca. 20%. Larval feeding level approximating the control one was observed when 0.001% solution of shoot saponins was used. However, even in the presence of the lowest doses very few larvae survived.

The compounds applied affected the duration of the insects´ feeding period. Control larvae terminated feeding and started descending into pupation after 12 days of rearing. Among insects reared on a diet containing 0.01 and 0.001% doses of saponins the feeding period of the surviving larvae was extended by about 4 days (Tab. 1).

Table 1
Feeding activity of Colorado potato beetle larvae after treatment with saponins

Saponin Source	Dose %	Area of consumed leaves (cm^2/larva) (SE)								Total
		Days after treatment								
		2	4	6	8	10	12	14	16	
Roots	0.5	0.46 (0.06)**	0.41 (0.04)**	0.51 (0.04)***	–[1]					1.38
	0.1	0.47 (0.08)**	0.67 (0.12)*	0.83 (0.15)***	0.56 (0.11)***	0.53***	0.88***	–[1]		3.94
	0.01	0.92 (0.15) ns	1.12 (0.17) ns	2.67 (0.88) ns	4.32 (0.94) ns	5.05 (1.33) ns	6.39 (2.50) ns	3.96 (2.03) ns	–[2]	24.43
	0.001	0.64 (0.10) ns	1.15 (0.06) ns	2.21 (0.28) ns	3.82 (0.85) ns	3.56 (1.08) ns	3.27 (0.80) ns	4.68 (0.68) ns	3.29 (0.12) ns	22.62
Shoots	0.5	0.49 (0.04)**	0.61 (0.12)*	–[1]						1.10
	0.1	0.44 (0.08)**	0.42 (0.14)**	0.48 (0.05)***	0.86 (0.14)*	1.13 (0.11)*	–[1]			3.33
	0.01	0.40 (0.05)**	0.75 (0.04)**	0.86 (0.34)**	1.98 (0.14) ns	4.15 (0.51) ns	3.11 (0.53)**	0.48****	2.41****	14.14
	0.001	0.64 (0.10) ns	0.78 (0.21) ns	2.44 (0.49) ns	2.28 (0.43) ns	6.48 (4.14) ns	5.28 (1.15) ns	5.75 (2.37) ns	4.73****	28.74
Control		0.91 (0.05)	1.43 (0.13)	2.54 (0.13)	4.14 (0.75)	6.23 (0.98)	10.09 (0.93)	3.29 (0.77)	–[2]	28.60

Significance of differences from the control values: ***P ≤ 0.001; **P≤ 0.01; *P≤ 0.05; ns-not significant
****Mean of one replicate; [1]100% mortality was recorded; [2] larvae dug into the sand

Table 2
Effect of saponins on the growth rate of Colorado potato beetle larvae

Saponin Source	Dose %	Mean body weight (mg/larva) on successive days after treatment (SE)							
		0	2	4	6	8	10	12	14
Roots	0.5	0.80 (0.15)	1.00 (0.10)**	1.03 (0.15)**	0.95 (0.29)***	– [1]			
	0.1	0.87 (0.09)	2.00 (0.31) ns	3.40 (1.05) ns	4.33 (0.88)**	4.45 (1.85)*	– [1]		
	0.01	0.77 (0.12)	2.33 (0.38) ns	9.80 (1.79) ns	24.70 (5.80) ns	27.03 (5.27) ns	59.10 (19.19) ns	84.97 (23.98) ns	– [2]
	0.001	0.80 (0.10)	2.63 (0.24) ns	7.83 (0.95) ns	20.77 (5.01) ns	29.73 (8.76) ns	65.57 (22.74) ns	86.50 (17.67) ns	108.25 (13.25) ns
Shoots	0.5	0.73 (0.03)	1.20 (0.06)*	1.30 (0.06)**	– [1]				
	0.1	0.60 (0.06)	1.37 (0.18) ns	2.40 (0.72)*	2.60 (0.93)**	3.17 (0.83)**	– [1]		
	0.01	0.63 (0.09)	1.70 (0.21) ns	4.83 (0.79) ns	7.60 (2.62)*	18.30 (7.30) ns	42.25 (6.25)**	49.50 (6.50)**	55.00****
	0.001	0.70 (0.12)	1.30 (0.50) ns	4.60 (1.89) ns	10.57 (5.05) ns	23.60 (8.60) ns	55.77 (20.98) ns	77.57 (43.08) ns	81.30****
Control		0.63 (0.07)	1.90 (0.17)	6.80 (0.75)	22.60 (3.75)	31.97 (6.38)	88.03 (2.03)	126.17 (6.96)	– [2]

Significance of differences from the control values: ***P ≤ 0.001; **P≤ 0.01; *P≤ 0.05; ns-not significant
****Mean of one replicate; [1]100% mortality was recorded; [2] larvae dug into the sand

Effect on weight gain and larval mortality

Growth of the insects under study varied considerably and was correlated with the dose applied, but saponins always inhibited that process. Larvae reared on leaves treated with 0.5% dose showed minimal variations in body weight and died after several days without virtually increasing in body weight. Low gain in weight was also observed after using the 0.1% concentration. The daily weight gains remained at a very low level, and more than 90% of the insects died after eight days of rearing, having reached only 10-14% of the body weight of the controls.

Lower saponin doses inhibited larval growth to a much lesser extent. They did not, however, with a few exceptions, let the larvae reach a body weight approximating control in spite of the comparatively high consumption (Tab. 2). E.g. out of 30 individuals reared in the presence of 0.001% solution of root saponins only four had body weights similar to control. The pupae they formed were also similar to control pupae in point of body weight. Larvae with lower body weights (ca. 60–70% of the control) after an extended feeding period often dug into the sand. However, not all of them pupated: some died in the larval stage, and a small percentage formed small pupae, whose body weights were much lower than the control. During the first stage of observations the mortality of the insects increased slowly, particularly among larvae fed on a diet with an addition of root saponins. Generally, however, the mortality of insects reared in the presence of both root and shoot saponins were very similar and amounted to 76.7-100% (Fig. 1 A, B).

DISCUSSION

The saponins included in the diet of Colorado beetle larvae had a highly adverse effect on their development and feeding behaviour. The deterrent properties of saponins reported for many insect species (Sutherland et al., 1975; Meisner, Mitchell, 1983; Adel et al., 2000) have also been found in our studies. Inhibitory effect of saponins from alfalfa (*Medicago sativa* L.) leaves on the feeding of CPB was observed by Waligóra (1998b) also. The saponins applied in her study acted as strong feeding deterrents, especially against larvae, but only at 1 and 0.5% concentrations. The lowest dose of saponins (0.25%) caused the smallest differences in intensity of feeding between control and treated larvae. In our study larvae offered a diet with 0.1% dose of saponins, both from roots and shoots, ingested minimal amount of food, which was not enough to keep them alive. These differences in the activity connected with the compound concentration may have resulted from the different age of the larvae examined. We used very young (L_1) larvae while Waligóra (1998b) carried out experiments with older ones. In the studies by Nozzolillo et al., (1997) a higher susceptibility of the *Ostrinia nubilalis* Hubner neonate larvae to saponins from alfalfa in comparison with older (L_2) ones was observed, too.

Food ingestion by insects is stimulated by a number of factors, viz., the chemical nature of the food recognized by its smell or taste, its digestibility, the extent of the alimentary canal filling and of the passage along the gut. It follows from earlier studies

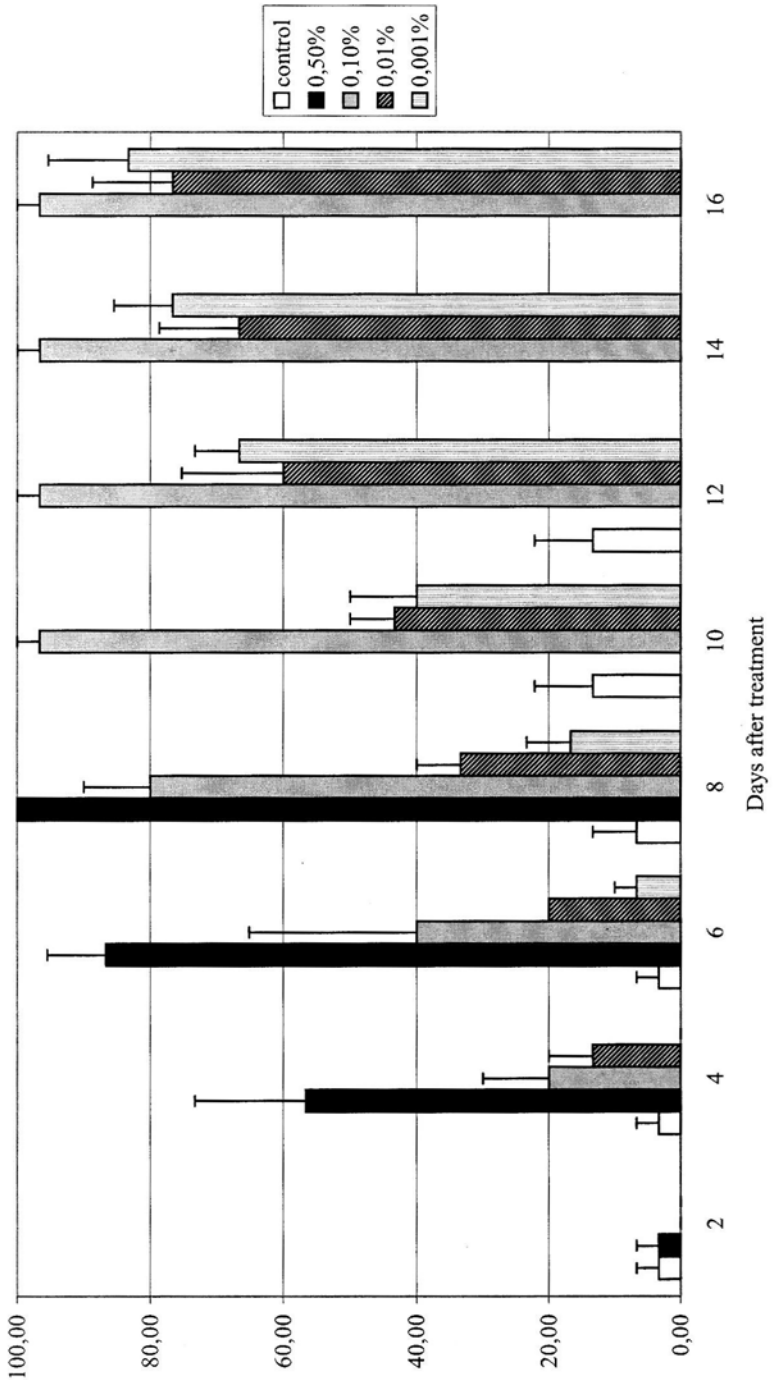
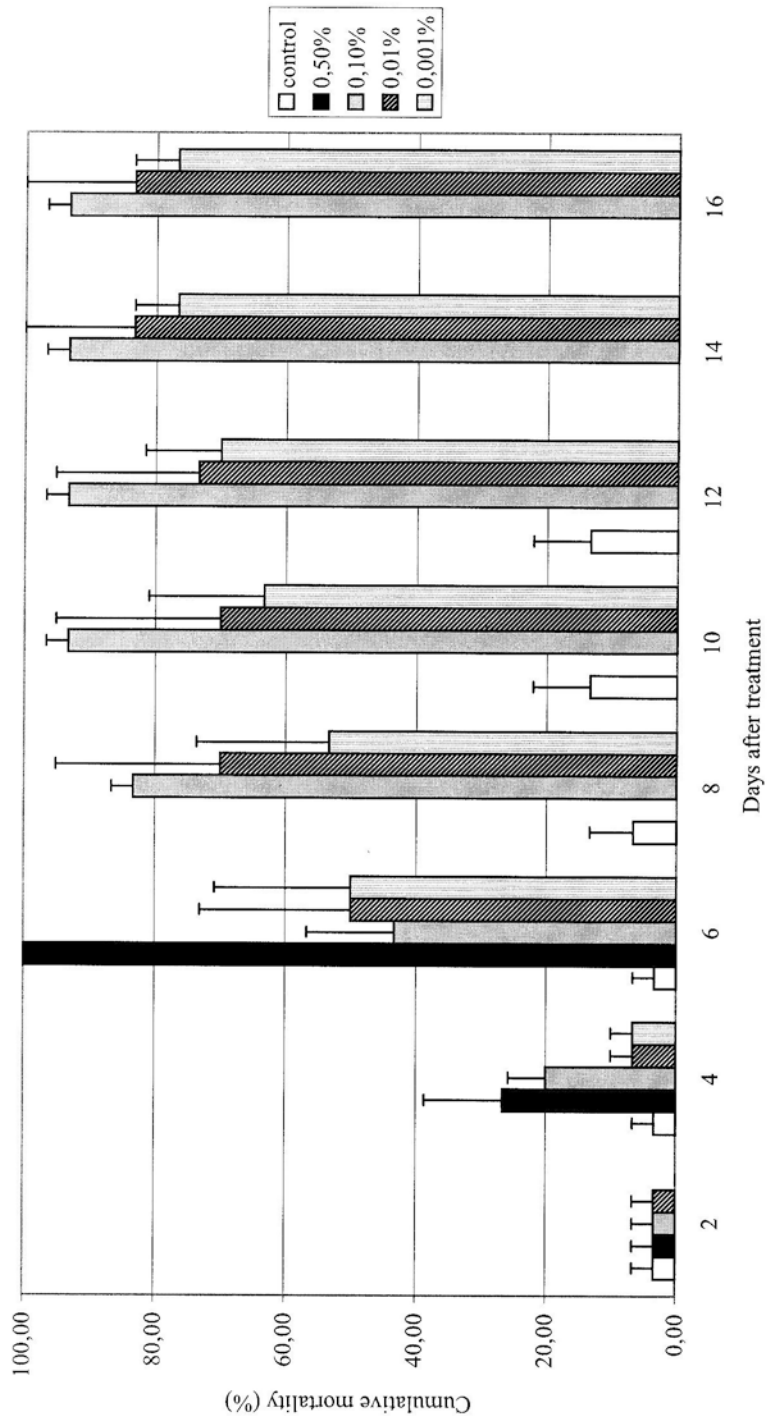


Fig. 1 Effect of saponin dose on mortality of the Colorado potato beetle larvae
A – saponins from roots; B – saponins from shoots. Vertical bars represent ∓ SE of the mean.

A


B
control
0,50%
0,10%
0,01%
0,001%
Cumulative mortality (%)
100,00
80,00
60,00
40,00
20,00
0,00
2
4
6
8
10
12
14
16
Days after treatment

on other insect species (I s h a a y a, B i r k, 1965; A d e l et al., 2000) that saponins reduce the digestibility of food, probably by inhibiting the secretion of digestive enzymes. The resulting lingering of the alimentary substance in the gut has no doubt a limiting or completely inhibiting effect on feeding. Starvation, possibly due to loss of an attractive taste in the food with saponins, as well as disturbance of the digestion and assimilation processes undoubtedly caused a decrease or stoppage in the insects´ growth rate. The clearly lower increase in body weight in relation to the amount of food ingested by larvae reared on a diet containing lower saponin doses suggests that the assimilation of the food ingested is lower than in the control.

The quantity and quality of the food ingested, besides other abiotic factors, affect the insects` growth and development. The growth rate determines the succession of developmental stages. Reaching the critical body weight is a signal stimulating the release of steroid hormones, responsible for the process of metamorphosis (M i g u l a, 1990). Thus the low growth of body weight in the Colorado beetle larvae could have been one of the main causes of the extension of the larval stage and of the small number of pupae evolved. However, the adverse effect of saponins, particularly of their aglycones, on the production of ecdysteroid hormones must also be taken into account. According to H a r m a t h a et al. (1987), A d e l et al. (2000) and other researchers, the extension of developmental stages in insects poisoned with saponins was due to the fall in the production of ecdysteroids. It can therefore be assumed that the same mechanisms of saponins action occurred in the case of the Colorado potato beetle.

In our studies no significant differences in activity have been found between root and shoot saponins. Similar results have been obtained by T a v a and O d o a r d i (1996) in their studies on the insecticidal activity of saponins from the roots and from the leaves of alfalfa towards the European grape moth, *Lobesia botrana*. On the other hand, the results of studies carried out by N o z z o l l i l o et al. (1996) and A d e l et al. (2000) point to a higher activity of root saponins compared with shoot ones. Similarly, S a n i e w s k a et al. (in press) found that antifungal activity of root saponins is higher than those from aerial parts.

Saponins occurring in alfalfa *(Medicago sativa)* are a complicated mixture of triterpenoid glycosides which are derivatives of medicagenic acid, oleanolic acid, zanhic acid, hederagenin bayogenin and soyasapogenols. It is widely known that the high activity of alfalfa saponins is due to glycosides of medicagenic acid, and hederagenin (O l e s z e k et al., 1992a) which occur in higher concentration in alfalfa roots than in shoots. The similar activity of root and shoot saponins in our experiments let us presume that the Colorado potato beetle larvae could also be susceptible to other glycosides than those of medicagenic acid and hederagenin. The results of these studies call for further research on the susceptibility of the Colorado potato beetle to individual saponins and their aglycones.

REFERENCES

Adel M. M., Sehnal F., Jurzysta M. 2000. Effects of alfalfa saponins on the moth *Spodoptera littoralis*. J. Chem. Ecol., 26 (4): 1065–1078.

Ameenuddin S., Bird H. R., Pringle D. J., Sunde M. L. 1983. Studies on the utilization of leaf protein concentrates as a protein source in poultry nutrition. Poultry Sci., 62: 505.

Biały Z., Jurzysta M., Oleszek W., Piacente S., Pizza C. 1999. Saponins in alfalfa (*Medicago sativa* L.) root and their structural elucidation. J. Agric. Food Chem., 47 (8): 3185–3192.

Cheeke P. R. 1971. Nutritional and physiological implications of saponins: a review. Can. J. Anim. Sci., 51: 621.

Harmatha J., Mauchampe B., Arnault C., Slama K. 1987. Identification of a spirostanetype saponin in the flowers of leek with inhibitory effects on growth of leek-moth larvae. Biochem. Syst. Ecol., 15: 113–116.

Ishaaya I., Birk Y. 1965. Soybean saponins IV. The effect of proteins on the inhibitory activity of soybean saponins on certain enzymes. J. Food Sci., 30: 118–120.

Łąkocy A., 1973. Teoretyczne i praktyczne aspekty odporności szkodników roślin rolniczych na pestycydy. Prace Nauk. Inst. Ochr. Raślin. 14 (2): 5–84.

Meisner J., Mitchell B. K. 1983. Phagodeterrency induced by two cruciferous plants in adults of the flea beetle, *Phyllotreta striolata* (*Coleoptera, Chrysomelidae*). Can. Entomol., 115: 1209–1214.

Migula P. 1990. Podstawy fizjologii owadów. Skrypty Uniwersytetu Śląskiego No. 453.

Noronha Ch., Goettel M., Butts R. 1999. Colorado potato beetle gaining insecticide resistance across praires. Pest Manag. News 10(4): 4

Nozzolillo C., Arnason J. T., Campos F., Donskov N., Jurzysta M. 1997. Alfalfa leaf saponins and insect resistance. J. Chem.Ecol., 23: 995–1002.

Oleszek W., Jurzysta M., Gorski P. 1992a Alfalfa saponins - the allelopathic agents. In „Frontiers of allelochemical research" (ed. Rizvi. S. J. H. and Rizvi V.), Chapman and Hall, London, pp. 151–164.

Oleszek W., Jurzysta M., Ploszynski M., Coloquhoun I. J., Price K. R., Fenwick G. R. 1992b. Zahnic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. J. Agric. Food Chem., 40: 191–196

Pawińska M., Węgorek P. 1998. Monitoring wrażliwości stonki ziemniaczanej na wybrane insektycydy. Materiały 38 Sesji Nauk. Inst. Ochr. Roślin. Streszczenia, str. 54.

Pracros P. 1982. Interet compare de l'estimation biologique de la valeur nutritionnelle des sources proteiniques par des vertebres (rats et poulets) et par un insecte (*Tenebrio molitor* L). C. R. Acad. Agric. Fr.: 1279–1285.

Puszkar L., Jastrzębski A., Jurzysta M., Biały Z. 1994. Saponiny lucerny jako szansa w integrowanej ochronie chmielu. Materiały 34 Sesji Nauk. Inst. Ochr. Roślin. Część II-Postery: 255–259.

Saniewska A., Jurzysta M., Biały Z. 2001. Differential antifungal activity of alfalfa (*Medicago sativa* L.) saponins originated from roots and aerial parts for some ornamental plants pathogens. Acta Agrobotanica, 54 (1) : 31–43

Sutherland O. R. W., Hood N. D., Hillier J. R. 1975. Lucerne root saponins a feeding deterent for the grass grub, *Costelytra zealandica* (*Coleoptera: Scarabaeidae*). N. Z. J. Zool., 2: 93–100.

Tava A., Odoardi M. 1996. Saponins from *Medicago spp.*: Chemical characterization and biological activity against insects. In G. R. Waller and K. Yamasaki (eds.). Saponins Used in Food and Agriculture. Plenum Press, New York: 97–109.

Waligóra D., Krzymańska J., 1994. Wpływ roślinnych związków wtórnych – glukozynolanów, saponin i alkaloidów na żerowanie stonki ziemniaczanej (*Leptinotarsa decemlineata* Say). Materiały 34 Sesji Nauk. Inst. Ochr. Roślin. Część II – Postery: 9–12.

Waligóra D., 1998a. Biological activity of secondary plant substances glucosinolates, alkaloids and saponins, expressed by their effects on development of Colorado potato beetle, *Leptinotarsa decemlineata* Say. J. Plant Prot. Res., 38 (2): 158–173.

Waligóra D., 1998b. The influence of different secondary plant substances – glucosinolates, alkaloids and saponins on the food choice by larvae and beetles of Colorado potato beetle (*Leptinotarsa decemlineata* Say). J. Plant Prot. Res., 38 (1): 93–104.

Zimmer D. E., Pedersen M. W., McGuire C. F., 1967. A bioassay for alfalfa saponins using the fungus *Trichoderma viride* Pers., Crop Sci., 7: 223.

Aktywność biologiczna saponin z korzeni i części nadziemnych lucerny siewnej (*Medicago sativa* L.) wobec larw stonki ziemniaczanej, *Leptinotarsa decemlineata* Say

Streszczenie

Zbadano w warunkach laboratoryjnych różne aspekty oddziaływania saponin z lucerny siewnej, *Medicago sativa* L. na larwy stonki ziemniaczanej. W badaniach zastosowano wodne roztwory (0,5; 0,1; 0,01 i 0,001%) sumy saponin wyodrębnionych zarówno z korzeni jak i z części nadziemnych lucerny, które podawano 12-godzinnym larwom L_1, metodą ekspozycji na traktowanych liściach ziemniaka.

Przeprowadzone badania wykazały, że saponiny lucerny włączone do diety larw stonki ziemniaczanej obniżały żerowanie, wzrost i przeżywalność badanych owadów. Aktywność biologiczna tych związków była ściśle skorelowana z dawką. Larwy hodowane na liściach traktowanych 0,5% roztworem praktycznie nie żerowały i ginęły po 4-6 dniach. Niższe dawki saponin (0,01 i 0,001%) w mniejszym stopniu ograniczały żerowanie owadów, wpływały jednak hamująco na ich wzrost. Obserwowany, zdecydowanie mniejszy przyrost masy ciała w porównaniu z ilością pobranego pokarmu sugeruje, że saponiny zakłócały procesy trawienia i przyswajania. Stosowane związki powodowały również przedłużenie stadium larwalnego oraz śmiertelność na poziomie 76,7 – 100 %. Nie stwierdzono większych różnic w aktywności saponin związanych z miejscem ich występowania w roślinie.