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THE INFLUENCE OF ALFALFA SAPONINS AND MUSTARD GLUCOSINOLATES ON THE FERTILITY OF COLORADO POTATO BEETLES (*LEPTINOTARSA DECEMLINEATA* SAY)

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Abstract: The influence of saponins isolated from alfalfa (*Medicago sativa* L.) leaves and glucosinolates isolated from mustard (*Sinapis alba* L.) seeds on the fertility of Colorado potato beetles (*Leptinotarsa decemlineata* Say) reared on potato leaves treated with these substances was tested. It was observed that both compounds restrained the process of egg laying however, the action of saponins was stronger.

Key words: alfalfa saponins, mustard glucosinolates, Colorado potato beetle, fertility

INTRODUCTION

Secondary plant metabolites occurring in plants belonging to different systematic units make an important group of natural substances often showing significant biological activity against many organisms. The aim of our investigation was to find such substances, extract them and then determine the degree of their activity against pests specific and nonspecific for a given crop. This type of compounds often shows defined influence on pest behaviour what may result in changes in some life processes.

For some years we have been working on secondary plant substances such as glucosinolates and saponins (Krzymańska and Waligóra 1983; Krzymańska and Waligóra 1986; Waligóra 1997; Waligóra and Krzymańska 1997; Waligóra 1998a; 1998b; Waligóra and Krzymańska 2000) and the problem of its potential use in plant protection.

Saponins are the group of compounds naturally occurring in many plant species. Their chemical structure is rather complicated and they differ in biological activity and physiological properties. It was found that saponins showed antibiotic or even toxic activity against many organisms (Ishaaya and Birk 1965; Gilpatric 1969; Horber 1972; Krzymańska and Waligóra 1983; Nozzolillo et al.1997) and also allelopathic activity (Oleszek and Jurzysta 1987; Waller et al. 1995). Saponins used in our experiments were isolated from alfalfa (*M. sativa*) leaves.

Glucosinolates are sulphur containing compounds characteristic for family *Cruciferae*. They are synthesized in plant tissues and the level of their content changes during the vegetation season (Drozdowska 1992; 1994; Fieldsend et al. 1991; Kachlicki 1990). It was stated that glucosinolates play some role in mutual interaction between a plant and its pests. For example they play an essential role in finding the host-plant by some insects adapted to feeding on *Cruciferae* and sometimes act as a plant protection agent against insects.

Secondary plant substances are the objects of many surveys and the activity of such substances is tested in relation to different pests. Results obtained to date are interesting, but besides defining the activity of given compound it is also necessary to explain the mechanism of its action and the possibility of using it in plant protection.

MATERIALS AND METHODS

The objects of our investigations were mustard glucosinolates and alfalfa saponins – as secondary plant substances. Our goal was to define their activity against Colorado potato beetle (*Leptinotarsa decemlineata* Say) – particularly the influence of these substances on the insect's fertility. Saponins were extracted from alfalfa (*Medicago sativa* L.) leaves according to the method of Wall (Wall et al. 1952) and glucosinolates from mustard (*Sinapis alba* L.) seeds according to the method described by Jerzmanowska (Jerzmanowska 1967).

Rearing of insects was conducted in spring-summer season (from the beginning of May to the end of July) in the insectarium. First experiments were set up in the very beginning of May. Newly hatched beetles were collected in the field and placed on potato leaves in insulators.

In control combinations leaves stayed untreated while in two other experimental combinations leaves were sprayed with 1% solution of tested substances: saponins or glucosinolates. Next the insects were placed on these leaves (in each insulator 6 females and 4 males). Each combination was set up in 3 replications. Observations were performed everyday during 70 days. Survivals of insects, intensity of feeding, number of egg deposits and number of eggs in each deposit were noted.

RESULTS AND DISCUSSION

The percentage of survival of Colorado potato beetles on treated and untreated plants on particular day of rearing shows figure 1.

The lowest mortality of beetles was recorded in a control combination throughout the whole duration of an experiment. The last records taken on the 70th day revealed only 20% of beetle population dead. In two other experimental combinations a higher mortality was observed, especially in combinations with saponins, where after 50 days of rearing the mortality amounted to almost 60%, and in final stage of experiment 100%. This situation was probably caused by the fact that beetles fed on control leaves much more intensively than on leaves treated with tested compounds. Feeding of beetles on leaves sprayed with saponins was very poor and insects often migrated from the leaves onto the walls of insulator. Leaves in these

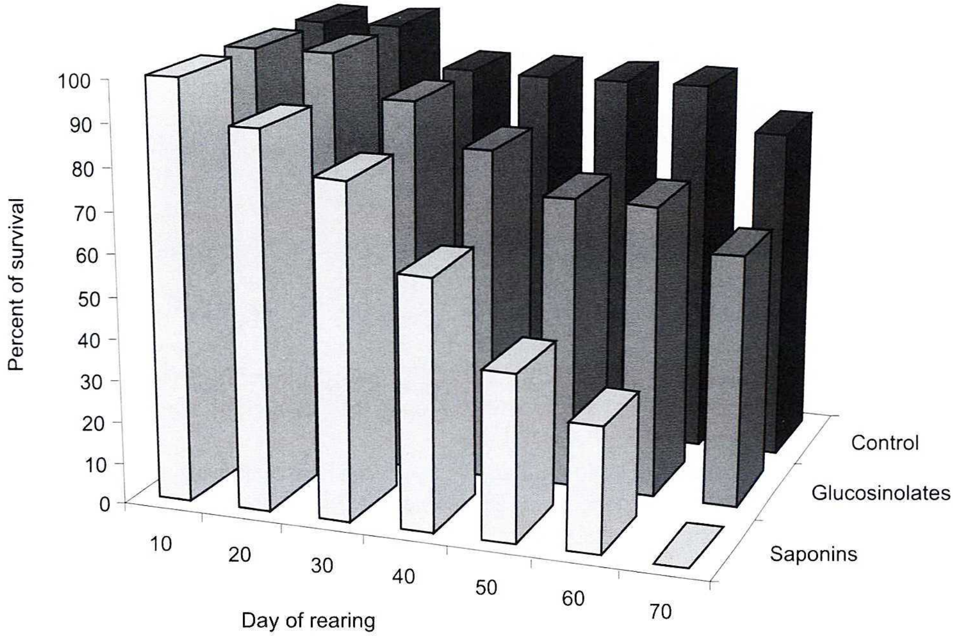

Fig. 1. Percent of survival of beetles on control and treated leaves during the time of experiment

combinations were almost intact as there was no traces of feeding. Meanwhile there were observed traces of feeding attempts on the stems what looked like searching untreated places by beetles. In the glucosinolate combinations the traces of feeding were more distinct, what proved, that beetles fed on these leaves. However, the feeding was not so intensive as on control leaves. This data confirms our previous observations.

The main purpose of these experiments was to obtain the information about the influence of the tested compounds on the process of egg laying by females of Colorado potato beetles. In the control combination insects fed very intensively from the first day of rearing and egg deposits appeared already after 3 days. At first they were single and gradually became more numerous. These egg deposits were most often found on the lower side of leaves – what is normally observed in natural conditions. The intensity of egg laying was also dependent on weather conditions and during cool weather beetles laid much less eggs, what is normal phenomenon. Beetles reared on control leaves were laying eggs practically all the time during the experiment, however the number of deposits differed in particular days in the course of the experiment and in some stages of experiment. In the control combinations the most egg deposits was observed from 19 to 27 day of rearing and then from 40 to 62 day of the experiment.

Laying of eggs in experimental combinations, in which saponins or glucosinolates were applied, looked somehow different. All egg deposits were laid within first few days of rearing. In case of the combination with glucosinolates – eggs were laid during first 10 days, whereas in case of saponins beetles laid eggs only for 4–5 days. Besides, in combinations with glucosinolates egg deposits were laid as normal – on leaves and the number of eggs in deposit was similar to the control combination. In case of saponins deposits consisted of a few (6–10) eggs or most often single eggs were laid on the walls of insulator or on the stem, what is abnormal phenomenon.

The number of eggs deposits obtained from the control leaves was definitely higher than in other experimental combinations: 10 times higher than in the combination with glucosinolates and almost 50 times higher than in the combination with saponins. Egg deposits differed also in the number of eggs – initially deposits were less numerous i.e. from several to a dozen or so eggs and then more numerous reaching even 30–40 eggs per a deposit. Table 1 presents an average (from 3 replications) number of egg deposits and number of eggs laid in each combination during experiment in total and laid by one female.

Table 1 also presents the total number of eggs deposited by beetles in each combination in the course of the experiment. The largest number of eggs was deposited in the control combination (average nearly 1000), while in the glucosinolate combination beetles laid as average about 170 eggs and in saponin combination merely 12.

Results of observations and presented data let us state, that feeding Colorado potato beetles with potato leaves treated with tested secondary plant compounds i.e.: glucosinolates and saponins had significant influence on the fertility of that pest, expressed by the number of eggs laid by females. Results obtained in the control combination differ considerably from those obtained in the experimental combinations. It has been observed, that both tested compounds had strong influence on the process of the reproduction of the insect, especially in the saponin combination where total restraining of egg laying was recorded. It is difficult, at this stage of investigation, to determine the cause of that. Probably the malnutrition of insects caused by reluctant feeding of the pest on leaves treated with tested compounds was of a great importance. As it was mentioned above, especially insects reared on leaves treated with saponins fed very poorly, although no effect of phytotoxicity on leaves was stated. It is common knowledge that nutrition has fundamental influence on the reproductive potential of insects, so malnutrition seems to be important although it can not be the only reason of the phenomenon observed. It seems to be justified to continue this investigation in the aim to determine the real cause of this phenomenon.

Table 1. Average number of eggs and egg deposits noted in each experimental combination in the course of experiment

	Control	Glucosinolates	Saponins
Average number of egg deposits in each combination	90	9	2
Average total number of eggs laid in each combination	979	166	12
Average number of egg deposits laid by 1 female in each combination	15	1.5	0.3
Average number of eggs laid by 1 female in each combination	163	28	2

In general, the results given above are very interesting and they prove that saponins are the group of secondary compounds, which is worth to be taken into consideration in further investigations – in relation not only to Colorado potato beetle but other pests as well.

Although saponins as plant substances have become lately the object of interest of many scientists, in the context of the problem of their influence on the behavior and physiology of many various insects, but so far none investigation concerning their influence on Colorado potato beetle fertility was found in literature.

REFERENCES

Drozdowska L. 1992. Glukozynolany i ich metabolizm u rzepaku w kulturach *in vitro* i *in vivo*. Rozprawy 55, ATR Bydgoszcz, 71pp.

Drozdowska L. 1994. Rola biologiczna glukozynolanów. Post. Nauk. Roln., 5: 61–67.

Fieldsend J.K., Murray F.E., Bilsborrow P.E., Milford G.F.J. 1991. Glucosinolate accumulation during seed development in winter sown oilseed rape. Proc. 8th Int. Rapeseed congress. Saskatoon, Canada: 698–694.

Gilpatrick J.D.1969. Effect of soil amendments upon inoculum survival and function in *Phytophtora* root rot of avocado. Phytopathology 59: 979–985.

Horber E. 1972. Alfalfa saponins significant in resistance to insects. p. 611–628. In "Insect and Mite Nutrition" (J.G. Rodriguez ed.). North Holland Co., Amsterdam.

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

Jerzmanowska Z. 1967. Substancje roślinne – metody wyodrębniania. Wydawnictwo PWN. Warszawa: 278–285.

Kachlicki P. 1990. Glukozynolany i inne związki niskocząsteczkowe specyficzne dla rozwoju *Brassica*. Występowanie, właściwości i rola w metaboliźmie roślin. Rośliny Oleiste – Wyniki Badań 1989. IHAR: 65–74.

Krzymańska J.,Waligóra D. 1983. Znaczenie saponin w odporności lucerny na mszycę grochową (*Acyrthosiphon pisum* L.). Prace Nauk IOR 24 (2): 153–160.

Krzymańska J.,Waligóra D. 1986. Badanie saponin lucerny i ich wpływu na mszycę grochową (*Acyrthosiuphon pisum* H.). Materiały 26. Sesji Nauk. IOR, cz.2: 268–274.

Nozzolillo C., Arnson J.T., Campos F., Donskov N., Jurzysta M. 1997. Alfalfa saponins and insect resistance. J. Chem. Ecology 23 (4): 995–1002.

Oleszek W., Jurzysta M. 1987. The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environment. Plant and Soil 98: 67–80.

Wall M.E., Krider M., Rothman E.S., Eddy C.R. 1952. Steroidal sapogenins. I. Extraction, isolation and identification. J. Biol. Chem., 98: 533–543.

Waller G.R., Jurzysta M., Thorne R.L.Z. 1995. Root saponins from alfalfa (*Medicago sativa* L.) and their allelopathic activity on weeds and wheat. Allelopathy Journal 2 (1): 21–30.

Waligóra D. 1997. Rape glucosinolates and alfalfa saponins as allelopathic factors for lettuce seed's germination. J. Plant Protection Res., 37 (1/2): 109–112.

Waligóra D. 1998a. 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 Protection Res., 38 (1): 93–104.

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

Waligóra D., Krzymańska J. 1997. Bioactivity of saponins – secondary plant substances in relation to the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Insects: Chemical, physiological and environmental aspects. Wydawnictwo Uniwersytetu Wrocławskiego, Wrocław 1998: 237–240.
Waligóra D., Krzymańska J. 2000. Glucosinolates occurring in green parts of mustard (*Sinapis alba* L.) and their biological activity. J. Plant Protection Res., 40 (1): 7–11.

POLISH SUMMARY

WPŁYW SAPONIN LUCERNY I GLUKOZYNOLANÓW GORCZYCY NA PŁODNOŚĆ CHRZĄSZCZY STONKI ZIEMNIACZANEJ (*LEPTINOTARSA DECEMLINEATA* SAY)

Prowadzono badania mające na celu ustalenie wpływu saponin wyizolowanych z liści lucerny siewnej (*Medicago sativa* L.) i glukozynolanów wyizolowanych z nasion gorczycy białej (*Sinapis alba* L.) na płodność chrząszczy stonki ziemniaczanej (*Leptinotarsa decemlineata* Say). W hodowlach tego szkodnika na liściach ziemniaka traktowanych testowanymi związkami zaobserwowano silne hamowanie procesu składania jaj przez chrząszcze stonki. Prawdopodobną przyczyną tego zjawiska jest niedożywienie chrząszczy spowodowane ich ograniczonym żerowaniem na liściach traktowanych testowanymi związkami.