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EFFECT OF TERPENOID LACTONES AND AZADIRACHTIN ON FOOD CONSUMPTION AND GROWTH RATE OF COLORADO POTATO BEETLE LARVAE, *LEPTINOTARSA DECEMLINEATA* SAY

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Abstract. The effect of some terpenoid lactones (monocyclic δ-hydroxy-γ-lactone; bicyclic δ-hydroxy-γ-spirolactone and bicyclic δ-hydroxy-γ-lactone) and azadirachtin on feeding and growth of *Leptinotarsa decemlineata* Say larvae was studied. Among lactones bicyclic δ-hydroxy-γ-spirolactone showed a strong feeding deterrency. Larvae treated with this compound consumed 0.163 cm^2 (per larva) of potato leaves during 6 days, whereas control larvae ate 0.892 cm^2. When lactones were used, a slight increase in body weight was observed. Azadirachtin, in comparison with lactones, much stronger reduced food consumption and growth rate of insects.

Key words: terpenoid lactones, azadirachtin, antifeedants, Colorado potato beetle

I. INTRODUCTION

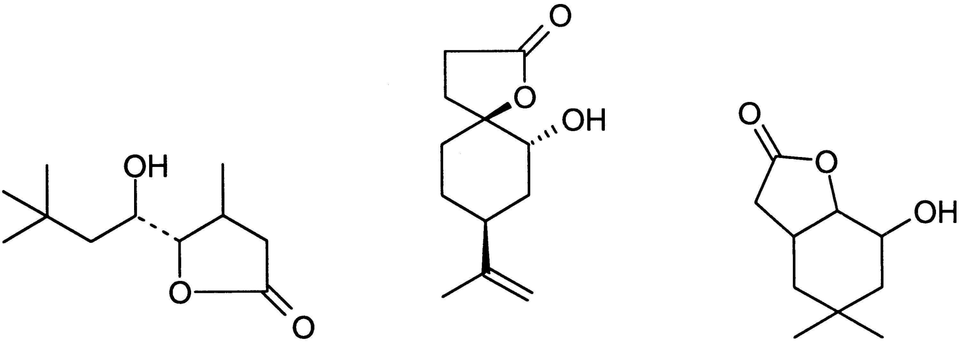
The terpenoid lactones have a broad spectrum of biological activity. Their cytostatic and antiprotozoan as well as fungicidal and bactericidal activity are well known. They are also recognized as antifeedants that impede or stop insect feeding by affecting their taste receptors (Nawrot 1984; Picman 1986). The use of antifeedants might be a supplementing strategy to the classical treatments in the integrated pest management practices in field crops, orchards, or forests. These substances might be useful especially in the control of species that have an ability to develop rapidly insecticide resistance. The results of various studies show that antifeedants are very selective. Therefore, to find an active feeding deterrent it is necessary to investigate the activity of a relatively broad spectrum of compounds against a small group of insects (Nawrot 1983).

The aim of this study was to examine the feeding deterrent activity of selected terpenoid lactones against the Colorado potato beetle. At the same time – for comparison – the antifeedant activity of azadirachtin to these insects was also investigated. The increasing resistance of Colorado potato beetle to the existing insecticides of various chemical groups (Forgash 1985; Pawińska and Węgorek 1998; Noronha et al. 1999) motivates the studies on alternative methods of control of this species.

II. MATERIALS AND METHODS

Lactones, the subject of presented studies, were synthesized in Department of Chemistry of Agricultural University of Wrocław. Monocyclic δ-hydroxy-γ-lactone (**1**) was obtained in four – step synthesis from neopentyl bromide and crotonaldehyde (Obara et al. 1995; Olejniczak et al. 2000a). The last step of this synthesis, acidic lactonization of γ,δ-epoxy esters with p-toluenosulfonic acid monohydrate was carried out in benzene and at room temperature. Bicyclic spirolactone (**2**) was synthesized as a pure (5S,6R,8S)-isomer in five – step synthesis from (-)-(S)-limonene (Paruch et al. 1998). Racemic, bicyclic δ-hydroxy-γ-lactone with condensed rings (**3**) was obtained from dimedone in the six – step synthesis (Wawrzeńczyk et al. 1985; Olejniczak et al. 2000b). Lactonization of ethyl (5,5-dimethyl-2,3-epoxycyclohex-1-yl)acetate was carried out in the tetrahydrofuran – water solution of $HClO_4$ (THF : H_2O : $HClO_4$ = 10 : 5 : 0.1). The structures of studied lactones are presented in Fig.

All lactones were used as a 0.1% alcohol solutions. Azadirachtin preparation (NeemAzal-T) was applied as water suspensions (0.1; 0.01; 0.001% a. i.). Potato leaves were dipped for 5s in the appropriate solution and left to air dry for 1h. Next, the leaf areas were measured using scanner and GM1 software (due to permission of MSc. W. Waszak from Department of Sociology, Mikołaj Kopernik University) and placed in Petri dishes (3dishes/dose) together with 10 Colorado potato beetle larvae (neonates). Untreated control leaves were dipped in alcohol or water only. After 48-h old leaves were replaced with fresh ones and remaining uneaten leaf areas were measured by the same method. Neonate larvae were weighed before exposure to treated leaves and every 24-h period to confirm the increase in body weight. Larvae fed untreated leaves were weighed after similar time intervals. Significance of differences between the variants of the experiment was assessed by Student's t-test. Mortality was recorded daily and corrected for control by Abbott's formula (Abbott 1925). The experiments were done in the laboratory.



1 **2** **3**

Fig. Chemical structures of lactones studied

III. RESULTS AND DISCUSSION

The intensity of feeding of larvae reared on potato leaves was different on leaves treated with different lactones (Tab. 1). The highest antifeedant activity was observed in the case of the bicyclic δ-hydroxy-γ-spirolactone (**2**). Its presence caused a low feeding activity in tested insects during the whole period of observation. The amount of consumed food by 6-day old larvae was only slightly higher in relation to 48-hour old individuals. The total area of consumed leaves treated with this lactone was 0.163 cm^2 per one larva during 6-days, which was about 20% of food consumed by control larvae during the same period.

The two other tested lactones had a weaker antifeedant activity. The feeding activity of larvae was similar on leaves treated with both of the lactones. However, the larvae that fed on leaves treated with bicyclic δ-hydroxy-γ-lactone with condensed rings consumed less food. In both cases, the intensity of feeding increased in old larvae as compared to young ones. Nevertheless, the two lactones caused the 50% reduction in consumption of food by larvae as compared with the control.

The comparative study showed that azadirachtin had a stronger antifeedant activity than the tested lactones. The feeding of larvae on azadirachtin-treated leaves was minimal and ranged from 0.011 to 0.052 cm^2, depending on the dose (Tab. 1).

The compounds applied in this study reduced the growth of larvae in different degrees. The body weight of larvae on azadirachtin-treated leaves did not change significantly. At the lowest dose, there was a slight increase in body weight after 48 hours of feeding, and during the following days there was a decline. The larvae treated with δ-hydroxy-γ-spirolactone showed the weakest growth of all larvae. Their body weight was only 52% of the control larvae. The increase of body weight in larvae treated with the two other lactones was larger by 10% (Tab. 2).

Based on the mortality data, it might be stated that the tested lactones did not show a direct insecticidal activity. The mortality that occurred among larvae resulted most likely from starving. There was a clear relation between mortality and the amount of consumed

Table 1

Intensity of feeding of Colorado beetle larvae after lactones or azadirachtin treatment

Compound	Surface of consumed leaves (cm^2/larva) ± SE			
	time after treatment (h)			
	48	96	144	Total
Lactone 1	0.109 (0.0)	0.145 (0.02)**	0.179 (0.01)	0.433
Lactone 2	0.042 (0.0)**	0.062 (0.01)*	0.059 (0.01)*	0.163
Lactone 3	0.090 (0.0)	0.121 (0.02)**	0.177 (0.03)**	0.388
Alkohol. Control	0.139 (0.03)	0.232 (0.01)	0.458 (0.08)	0.892
Azadirachtin 0.1%	0.011(0.0)*	–	–	0.011
Azadirachtin 0.01%	0.024 (0.01)*	0.017 (0.0)*	–	0.041
Azadirachtin 0.001%	0.032 (0.0)*	0.020 (0.0)*	–	0.052
Water control	0.166 (0.02)	0.260 (0.03)	0.566 (0.07)	0.992

* differences statistically significant at $P< 0.01$

** differences statistically significant at $P< 0.05$

Table 2

Growth rate of Colorado beetle larvae after lactones or azadirachtin treatment

Compound	Mean larval weight (mg) ± SE			
	time after treatment (h)			
	0	48	96	144
Lactone 1	0.49 (0.05)	1.18 (0.07)	1.92 (0.08)**	2.97 (0.41)**
Lactone 2	0.51 (0.07)	0.81 (0.06)**	1.64 (0.10)*	2.59 (0.13)*
Lactone 3	0.51 (0.07)	1.20 (0.16)	1.86 (0.28)**	3.12 (0.24)*
Alkohol control	0.56 (0.60)	1.31 (0.10)	2.83 (0.34)	4.94 (0.29)
Azadirachtin 0.1%	0.64 (0.00)	0.61 (0.11)*	–	–
Azadirachtin 0.01%	0.64 (0.08)	0.84 (0.06)*	–	–
Azadirachtin 0.001%	0.60 (0.06)	1.04 (0.08)*	0.76 (0.08)*	–
Water control	0.57 (0.13)	1.73 (0.18)	3.03 (0.67)	5.2 (1.55)

* differences statistically significant at P< 0.01

** differences statistically significant at P< 0.05

food. The larvae treated with the bicyclic spirolactone, which had the highest antifeedant activity, died the soonest, and their mortality was the highest. The two remaining lactones of weaker antifeedant activity also caused mortality among larvae but only after 4 days of feeding. The larval survival was slightly higher in the case of lactone **1**, which was the weakest antifeedant. The variation in mortality of insects after application of antifeedants has frequently been reported in literature (Mordue et al. 1985; Ley and Toogood 1990).

In the azadirachtin experiments, all larvae died after 5 days of observation. The almost non-existing feeding was probably the cause of their high mortality rate, but the toxic effect of this compound could not be excluded. According to Schmutterer (1990) larvae of *Coleoptera*, especially those of phytophagous *Coccinellidae* (*Epilachna*) and *Chrysomelidae* are rather sensitive to neem products. There is not only an antifeedant and growth effect in these groups, but also a contact effect as well. In the studies by Schrod et al. (1996), azadirachtin caused a high mortality among the Colorado potato beetle too.

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WPŁYW LAKTONÓW TERPENOIDOWYCH I AZADIRACHTYNY NA ŻEROWANIE I WZROST LARW STONKI ZIEMNIACZANEJ *LEPTINOTARSA DECEMLINEATA* SAY

STRESZCZENIE

Zbadano w warunkach laboratoryjnych wpływ wybranych laktonów terpenoidowych i azadirachtyny na żerowanie, wzrost i przeżywalość larw L_1 stonki ziemniaczanej, *Leptinotarsa decemlineata* Say. W badaniach zastosowano monocykliczny δ-hydroksy-γ-lakton, bicykliczny δ-hydroksy-γ-spirolakton i bicykliczny δ-hydroksy-γ-lakton o skondensowanych pierścieniach – wszystkie w postaci 0,1% roztworów alkoholowych. Preparat NeemAzal-T oparty na azadirachtynie A podano w postaci wodnej zawiesiny (0,1; 0,01 i 0,001% sbcz). Użyto metody ekspozycji na traktowanych liściach ziemniaka.

Stwierdzono, że spośród laktonów najsilniejsze właściwości deterentne wykazywał bicykliczny δ-hydroksy-γ-spirolakton. W jego obecności powierzchnia zjadanych liści była redukowana o 80% w porównaniu z kontrolą. Pozostałe dwa laktony ograniczały żerowanie owadów o około 50% (nieco silniej bicykliczny δ-hydroksy-γ-lakton). Przy obniżonym żerowaniu obserwowano słabszy przyrost masy ciała larw oraz postępującą śmiertelność. Po 6 dniach hodowli na liściach traktowanych laktonami około 50% owadów było martwych; w próbie kontrolnej śmiertelność wynosiła 6,6%.

Azadirachtyna w porównaniu z laktonami była silniejszym antyfidantem. Wśród traktowanych larw obserwowano prawie całkowite zaprzestanie żerowania, brak przyrostu masy ciała oraz wysoką śmiertelność.