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Influence of Neem-Azal T/S on feeding activity of Colorado Potato Beetles (*Leptinotarsa decemlineata* Say)

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Abstract. The influence of 0.3% water emulsion of Neem-Azal T/S on the behaviour and feeding activity of Colorado Potato Beetles (*Leptinotarsa decemlineata* Say) has been investigated. In choice test beetles mostly choose clean leaves, but did not avoid Neem-treated leaves entirely. In no-choice test beetles did not refuse to eat the Neem-treated food, although fed reluctantly. Consumption of Neem-treated leaf areas was reduced by 3–5 times in both, as in choice as in no-choice variants in comparison with control. Regarding the Colorado Potato Beetles, Neem-Azal T/S belongs to the category of relative antifeedant: it was not able completely deter beetles from visiting and eating the treated food. The antifeedant activity of preparation, which was expressed by the total coefficient of deterrence, exceeded 100. The interaction of time and treatment had no significant effect on feeding activity. Beetles did not become habituated to Neem-Azal T/S during one week.

Key words: Colorado Potato Beetles, Neem-Azal T/S, placement, food consumption, choice and no-choice test

INTRODUCTION

Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) (CPB) is one of the most dangerous and economically damaging potato pests in Estonia, launching year after year increasingly stronger attacks on potato fields. Unfortunately there are no serious natural enemies of CPB in Estonia. The beetles have developed resistance to all major groups of conventional insecticides used for CPB control worldwide (Pearsall & Hogue, 2000). Because of the high mobility of beetles, Estonia experiences large migrations from more southern regions. Currently, use of chemicals remains the primary method of plant protection against CPB around the world, but conventional synthetic insecticides continue to accelerate the process of increasing resistance of CPB (Wegorek, 2005). High resistance rate of this pest compels the search for alternative control measures. We have studied numerous extracts of local plants, some have insecticidal properties but none is strong enough to control CPB (Ploomi et al., 2005). The idea of using antifeedants and repellents as plant protection products is not novel; there have been many studies concerning the problem. Protecting the crop does not mean only killing the pest. According to Isman (2002), antifeedant is a substance that tastes bad to insects; any substance that reduces feeding by an insect can be considered as an antifeedant. Many different plant extracts have already been tested against various developmental stages of CPB. *Solanum berthaultii* Hawkes extract proved to have antifeedant activity for larvae, reducing the food consumption rate (Yencho et al., 1994). *Heracleum sosnowsky* Manden, *Artemisia absinthium* L., *A. Dracunculus* L., *Tanacetum vulgare* L., *Rheum rhaponticum* L. and *Levisticum officinale* W.D.J. Koch proved to be toxic to the young instars of CPB (Metspalu et al., 2001). Five different plant extracts: *Arctium lappa* L., *Bifora radians* M. Bieb, *Humulus lupulus* L., *Xanthium strumarium* L. and *Verbascum songaricum* Schrenk ex Fisch & Mey reduced the larval feeding of CPB (Gökçe et al., 2006).

Close to two decades ago, antifeedant properties of Neem tree (*Azadirachta indica* L.) products were described after testing with over 220 insect species (Jacobsen, 1989). However, its effect on insects varies greatly between orders and even species (Mordue (Luntz) & Nisbet, 2000). Despite that different effects of Neem-derived substances have already been well investigated, a number of problems may arise in the practical use of these preparations. Specific problem for antifeedants is variable sensitivity of different species and even populations, and habituation to the antifeedant with repeated exposure (Izman, 2002).

The aim of this work was to evaluate the effect of Neem preparation on the feeding activity of Colorado Potato Beetles (*Leptinotarsa decemlineata* Say) and to assess the potential of beetles for habituation during one week exposure to Neem-treated food.

MATERIALS AND METHODS

Commercial Neem preparation Neem-Azal T/S (Celaflor Schädlingsfrei Neem by Scott`s Celaflor CmbH and Co, including 1% azadirachtin of 4% of natural Neem-seed) was obtained from the Trifolio-M company in Germany. Over-wintered Colorado Potato Beetles were captured in July 2006 from the potato fields near Tartu in South Estonia.

Fresh potato leaves were cut off daily and photocopied by a copying machine before and after each assessment to determine the surface area consumed by beetles. The leaves were dipped into 0.3% water suspension of Neem/Azal (the dose based on firm recommendations for field spraying) for 10 seconds; the control was immersed in pure water for the same period. Before the onset of the experiment, leaf surfaces were let dry at room temperature for about 30 minutes. In choice test, one experimental arena, a round plastic basin with a diameter of 70 cm was used. Treated and untreated leaves were arranged with altering placement on the periphery of the basin. In the no-choice test, two separate identical basins were used, one for the control, with untreated leaves and the other, with Neem-treated leaves. The beetles were placed in the centre of basin. The experiment lasted 7 days and the beetles were subjected to starvation stress throughout the whole trial; a feeding period of 5 hours alternated with a 19-hour starvation period everyday. The tests were replicated three times, N=30 (15♀ and 15♂) for each replication. The same individuals were reused in all replications.

To assess the antifeedant activity of preparation, relative (choice test) and absolute (no-choice test) deterrence coefficients were calculated using formulae: **R**=C-T/C+Tx100; **A**=CC-TT/CC+TTx100 where C and CC are leaf areas consumed in control and T and TT are leaf areas consumed in Neem-treated variant in choice and no-choice tests respectively. Total coefficient of deterrence (**R+A**) can range from **-**200 to +200; the preparation with negligible activity has a total coefficient around zero, and preparation with considerable activity has a total coefficient over 100 (Nawrot et al., 1986). T-test, one-way ANOVA and post-hoc LSD were used for statistical analyses.

RESULTS

Feeding activity in choice test. The results of beetle's food consumption in choice test are presented in figure 1. If the beetles were let choose the food, they preferred untreated leaves, but at the same time they did not reject the Neem-treated food completely. T-test for independent samples indicated a significant difference between the consumed areas of clean and Neem-treated leaves (t=10.07; df=40; P<0.0001). Daily food intake did not change significantly in time within treated ($F_{6;14}$=0.87; P=0.54) or control groups ($F_{6;14}$=0.57; P=0.75) during the whole observation period, though there was one time-point where the difference was significant in control.

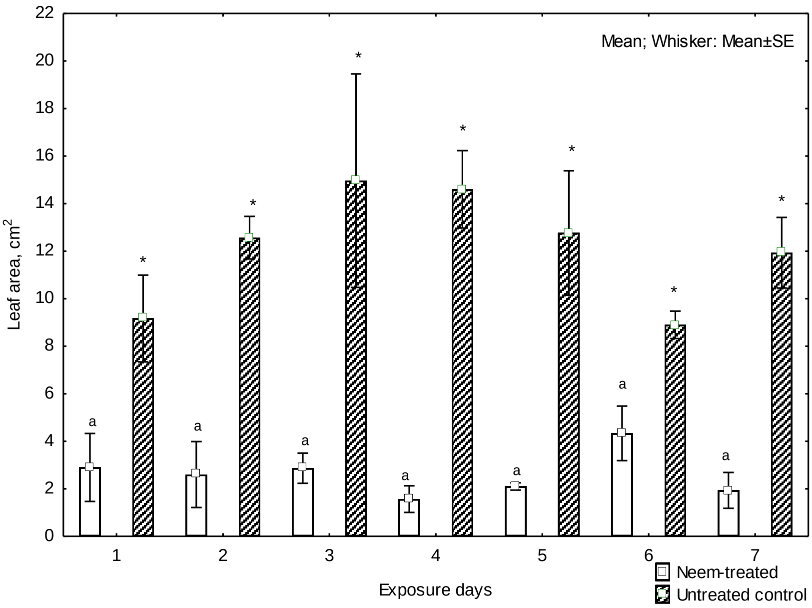

Fig. 1. Mean area (±SE) of potato leaf (cm²) consumed by Colorado Potato Beetles daily under choice condition. The same letters above white columns (a) indicate no significant difference in daily food consumption in treated and asterisk above the striped columns (*) in untreated leaves, P > 0.05.

Feeding activity in no-choice test. The results of beetle's food consumption in no-choice test are presented in figure 2. The beetles fed reluctantly on Neem-treated leaves, and leaf areas consumed by beetles were significantly smaller compared to the untreated variant (t = 10.0917, df = 40, P < 0.0001). The feeding behaviour of beetles did not change during the whole observation period. There was no significant difference in consumed leaf areas on different evaluation days in the Neem-treated variant ($F_{6;14}$ = 0.57; P = 0.74); although slight fluctuation in feeding activity in untreated variant was observed some day ($F_{6;14}$ = 1.08; P = 0.41).

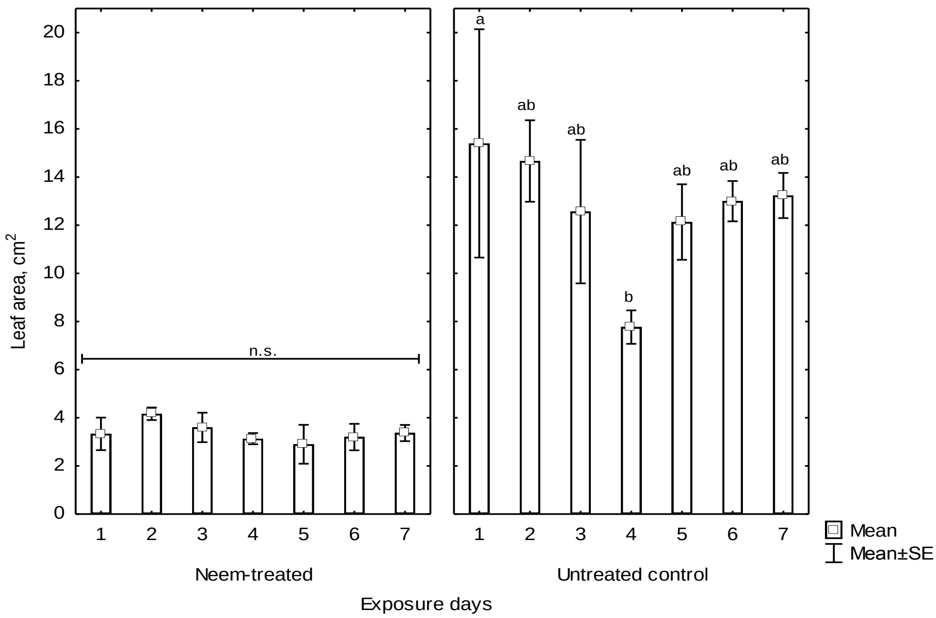

Fig. 2. Mean area (±SE) of potato leaf (cm²) consumed by Colorado Potato Beetles daily under no-choice condition. Left: Neem-treated; right: untreated control. The same letters above the columns indicate no significant difference within the same variant ($P > 0.05$) on different days.

Comparison of feeding activity in choice and no-choice test. The comparison of Neem-treated leaf areas consumed by beetles in choice and no-choice test does not reveal statistically significant differences, still a slight tendency in favour of food consumption in no-choice test was obvious (Table 1). According to ANOVA results, the absolute (A), relative (R) and total (T) coefficient of deterrence did not change significantly during the observation period, 7 days (Table 1).

Table 1. Feeding activity of Colorado Potato Beetles in choice and no-choice test.

Day	Deterrence coefficient			*Mean leaf area (cm^2) ±SE	
	A	R	T	Choice test	No-choice test
1	55.69± 19.58	51,16 ± 23.02	106.85 ± 41.02	2.90 ± 1.30(a)	3.33 ± 0.68(a)
2	54.83± 6.06	68.90 ± 12.82	123.73 ± 16.38	2.60 ± 1.39(a)	4.17 ± 0.26(a)
3	54.30± 5.63	65.63 ± 3.87	119.99 ± 4.25	2.87 ± 0.64(a)	3.60 ± 0.61(a)
4	42.05± 6.05	80.43 ± 7.67	122.49 ± 9.90	1.57 ± 0.56(a)	3.13 ± 0.23(a)
5	61.20± 9.59	69.90 ± 5.73	131.10 ± 15.20	2.10 ± 0.15(a)	2.90 ± 0.81(a)
6	61.09± 3.64	36.48 ± 8.96	97.57 ± 11.56	4.33 ± 1.15(a)	3.20 ± 0.55(a)
7	59.07± 5.21	70.86 ± 12.99	129.94 ± 16.96	1.93 ± 0.75(a)	3.37± 0.34(a)
ANOVA results:	$F_{6;\,14}$=0.48; P=0.81	$F_{6;\,14}$=1.43; P=0.26;	$F_{6;\,14}$=0.38; P=0.87	$F_{6;\,14}$=0.57; P=0.74	$F_{6;\,14}$=0.87; P=0.54

A - Absolute coefficient of deterrence (no-choice test); R - Relative coefficient of deterrence (choice test); T - Total coefficient of deterrence.

* Excerpt from Figs 1 and 2 (Neem-treated leaves)

Mean (±SE) on the same line with same letter (a) indicate no significant difference between choice and no-choice tests (t-test, $P > 0.05$).

DISCUSSION

Preceding results demonstrate that botanical insecticide Neem-Azal T/S reduced significantly the feeding activity of CPB but did not prevent it entirely even in choice test where the beetles have alternative food. The primary antifeedant effects of azadirachtin, the key insecticidal ingredient from the Neem tree seeds, result from stimulation of specific deterrent chemoreceptor on the mouthparts, together with interference of the perception of phagostimulants by another chemoreceptor (Mordue (Luntz), 2000). Although the CPB could discriminate between treated and untreated food, Neem-treated leaves did not remain unharmed neither in choice nor no-choice test. That may be related to the nature of the preparation: azadirahtin is a non-volatile substance and the insect must taste it in order to respond to it (Klocke et al., 1989). According to Danielson (1996), a substance that inhibits insect feeding only for a defined time or rate is referred to as a relative antifeedant; in contrast to absolute antifeedant that is described as substance which the insects refuse to eat in any case. In our test, the feeding activity of CPB was reduced by 3–5 times as in choice as in no-choice tests in comparison with the control, consequently Neem-Azal T/S match well in the category of relative antifeedants. Antifeedant activity of the preparation expressed by the total coefficient of deterrence exceeded 100, thus according to Nawrot`s evaluation scale (Nawrot et al., 1986) we may state that 0.3% Neem-Azal T/S has considerable deterrence activity against CPB adults. For correction we must take into consideration that the biological activity of Neem products is closely related to their azadirachtin content, and the effect is dose-dependant.

One serious problem in pest control is the habituation or desensitization of insects to the botanical antifeedants (deterrents). Some no-choice experiments have documented habituation of insects to different antifeedants, which leads to feeding deterrence decline during increased exposure (Bomford &Isman, 1996; Isman, 2002; Gökçe et al., 2006). Insects that have repeatedly exposed to azadirachtin may adapt to it and become less sensitive; in some insects this happens within as few as only 4-5 hours (Isman, 2002). In our experiment, the Neem-treatment suppressed beetles' feeding for the whole test period, whereas daily consumption of treated potato leaf area remained relatively constant and the total coefficient of deterrence in the first and the last assessment day did not differ significantly. Hereby we may conclude that the Colorado beetles did not become habituated to this preparation. When Neem-treatment is used in the course of agricultural practices, the beetles inevitably get in no-choice conditions. Reduced feeding activity will be supplemented with starvation of adults which leads to decrease of their general fitness for survival.

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