Flea beetle (Coleoptera: Chrysomelidae) response to alkyl thiocyanates and alkyl isothiocyanates

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Abstract. The attractivity of nine compounds, allyl isothiocyanate (allyl IT), benzyl isothiocyanate (benzyl ITC), 3-butenyl isothiocyanate (butenyl ITC), 3-butenyl thiocyanate (butenyl TC), butyl isothiocyanate (butyl ITC), butyl thiocyanate (butyl TC), 2-phenylethan-1-yl isothiocyanate (phenetyl ITC), 2-phenyleth-1-yl thiocyanate (phenetyl TC), and 2-phenylethan-1-ol, was compared to the beetle genera *Phyllotreta* species. Field tests were performed on fields of wild crucifer plants and on the edge of an oilseed rape field. Test places were at Juuru in Northern Estonia, at Valgeristi in the middle of Estonia and at Matsi in Southern Estonia. In our tests, *Phyllotreta* spp were most attracted to butenyl TC and butenyl ITC; allyl ITC and other tested alkyl-TC, alkyl-ITC, aryl-TC and aryl-ITC had lower attractivity. Cylindrical traps with a large clued area were tested and are recommended for practical usage, capillary polyethylene dispensers are recommended instead of sachet type dispensers. Emission of substances from sachet dispensers is described in the article.

Key words: Flea beetles, *Phyllotreta* spp., kairomone, 3-buten-1-yl isothiocyanate, 3-buten-1-yl thiocyanate, 2-phenyl-1ethyl thiocyanate, sachet dispenser, pest control

INTRODUCTION

Pest control without toxic insecticides is the bottleneck of environmentally friendly agriculture and biodynamic farming. Pheromone and kairomone preparations influencing insect behaviour are effective tools to diminish or avoid the usage of toxic plant protection preparations. Experiences of UK researches confirm this allegation (Blood, 1996). To introduce the environmentally friendly plant protection technology, more information is needed on the status of agroecosystems. In addition to data on climate and biodiversity, the migration possibilities of different species must be considered. Monitoring of ecosystems is expensive. Light traps are usually used and identification of hundreds and thousands of insects is needed. The use of pheromones and kairomones allows trapping certain species only, enabling monitoring with especially high accuracy.

Different alkyl thiocyanates are used for trapping cruciferous plant pests (Peng et al., 1992; Smart et al., 1997). In Estonia, *Phyllotreta undulata* and *P. vittata* have mainly been identified in our collections (unpublished). These two species of *Phyllotreta* spp are significant pests of cruciferous plants in Estonia (Metspalu &

Hiiesaar, 2002). Additionally four other *Phyllotreta* spp have been identified in traps. It is similar to the species spectrum in Sweden, but essentially different from that in England and Canada.

After hibernating adult *Phyllotreta* spp are waiting for cultivated cruciferous seedlings and transplants feeding on wild cruciferous plants. It means that they will have long-distant orientation to plants and good enough migration ability (Pivnick, et al., 1992). Degradation products of glucosinolates are produced when the plant cells are ruptured and the glucosinolates present in vacuoles are hydrolysed by the enzyme myrosinase (β-thioglycosidase glucohydrolase). These degradation products are emitted also in small amounts from intact leaves within the plants of family Brassicaceae via hydrolysis or catabolysis (Fahey, 2001). The amount and structure of substituted isothiocyanates (ITC), nitriles, thiocyanates (TC), and oxazolidinethiones vary depending on the side-chain substitution radical (VanEtten & Tookey, 1977).

As it is seen from research by Bodnaryk et al. (1997), the resistance of mustards to *Phyllotreta* spp is not positively correlated with content of sinigrin. Foliar concentrations of the predominant glucosinolates in the mustards *Brassica juncea* [Indian mustard] (allyl glucosinolate, sinigrin) and *Sinapis alba* (p-hydroxybenzyl glucosinolate, sinalbin) were determined in lines that had been selected in breeding programmes for low concentrations of glucosinolates in their seeds for the oilseed market. The glucosinolate concentrations found in the cotyledons and leaves of the selected lines were also low, often by three or more orders of magnitude, compared with the unselected parent. The chrysomelid *Phyllotreta cruciferae* fed at equal rates on Indian mustard and its low-glucosinolate lines, indicating that *Phyllotreta* species are insensitive to sinigrin, and suggesting that their pest status on low-glucosinolate lines of Indian mustard will likely remain unchanged (Bodnaryk, 1997).

Different cruciferous plants are not equally attractive to *Phyllotreta* spp. Choice tests were conducted in field in North Dakota in 1990 and in laboratory to determine the feeding preference of *Phyllotreta cruciferae* for oilseed rape, *Brassica napus*, and crambe, *Crambe abyssinica*. Chrysomelidae feeding (primarily abaxial) was significantly greater on oilseed rape than on crambe. Although Crysomelidae presence on crambe was not inhibited, feeding pits were often shallow and smaller, suggesting the presence of a gustatory deterrent in crambe tissue. Mechanical tissue damage had no effect on chrysomelid feeding on oilseed rape, but it caused an increase in the incidence of feeding on crambe. Since the preference for damaged crambe was manifest only after a long exposure to the pests, release of volatile attractants by the plants in response to damage was unlikely. The area of feeding in damaged crambe remained slight despite the increased incidence of feeding, suggesting that damage did not notably compromise resistance in crambe (Anderson et al., 1992).

In any case, the behavioural reaction of *Phyllotreta* spp to host plant volatiles is complex. Our research has demonstrated that in the case of flea beetles not only alkyl isothiocyanates are attractive to *Phyllotreta* spp but also alkyl thiocyanates demonstrated high attractivity. Compared to isothiocyanates, alkyl thiocyanates are not only less toxic compounds, they can be prepared using simple nucleophilic substitution reactions (Liblikas et al., 1999a, 1999b, 2001). In this study we present results on behavioural reaction of *Phyllotreta* spp to some alkyl thiocyanates and alkyl isothiocyanates in Estonia.

MATERIALS AND METHODS

Field trapping experiments at Juuru were designed to determine the attractivity of different ITC and TC for *Phyllotreta* spp. Small cups, having a volume of 180 ml and a glued area of 50 cm^2 were used as traps in 2000. Cups of different colour were tested, the possible influence of the colour on trap catch will be discussed elsewhere. In experiments at Juuru white-, yellow- and blue-coloured traps were used and trap colour had no or little influence on catch, as it is demonstrated with experiments at Valgeristi (Liblikas et al., in preparation). Attractivity of all the substances was compared in traps of the same colour. Traps were hung up on sticks at 0.5–1.0 m above the ground 5–20 metres from each other. A sachet dispenser was placed inside the cup (conical trap). During experiments in oilseed rape field, the traps were located on the edge of the field. In experiments at Juuru in 1999, Atrakon A type delta traps (Tartu Flora, Estonia) were used. The Atrakon A has a trapping window sides of 8 x 6 x 6 cm and a length of 16 cm, the bottom size was 9.5 x 15.5 cm, coated with sticky glue (glue was obtained from Tartu Flora, or from Oecos, UK).

Experiments at Valgeristi, nearby Tartu, were designed to establish the possibility to use traps for mass trapping of *Phyllotreta* spp and determine the best dispenser and trap type. In these experiments and in field bioassay tests at Matsi in 2002, large open cylindrical traps with a glued area of 600 cm^2 were used. These cylindrical traps made from 1.5 l plastic bottles (height 20 cm and diameter 9.5 cm) were found to be more suitable than the deltatraps (Atrakon A). The bottom was cut off and a yellow paper sheet was placed inside. The upper ends of the traps were closed, dispensers were placed inside the traps at a distance of 5 cm from the top stopper. In experiments at Matsi, the cylindrical traps were covered with changeable yellow sticky plastic sheets.
Polyethylene sachet dispensers had a size of 9 x 9 x 0.12 mm, Wettex® was used as the substrate for the attractive compounds. Maximum loaded amount was 80 mg of test compound. Evaporation speed from sachet dispensers depended linearly on the evaporation area, in the case of more volatile compounds, such as allyl ITC, evaporation was exponential in time; in the case of compounds with lower vapor pressure, the evaporation was linear (Fig. 1). Evaporation speed was strongly dependent on temperature (Fig. 2).

Miniket Si substrate dispensers were used for phenetyl TC and phenetyl ITC in experiments at Matsi. Capillary dispensers for butenyl TC dispensers had as a minimum ten times smaller evaporation speed compared with a 10 x 10 mm sachet dispensers (unpublished).

Species identification. The glue in the traps was dissolved in light petroleum and the beetles were dried before identification of species, using a binocular microscope. Species were identified using species specific yellow strips on wings, size and other morphological characteristics (Freude et al., 1966).

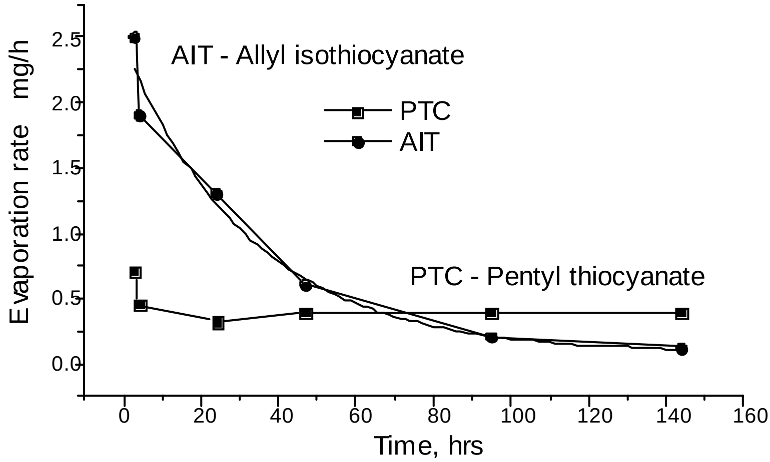

Fig. 1. Emission rate of AIT and PTC from polyethylene sachet dispensers (15x15 mm) at laboratory. Temperature 16–20°C, film thickness 26 µm. In case of PTC the wall thickness was 170 µm. As it is seen, the evaporation of AIT obeys the exponential equation (curve through AIT points).

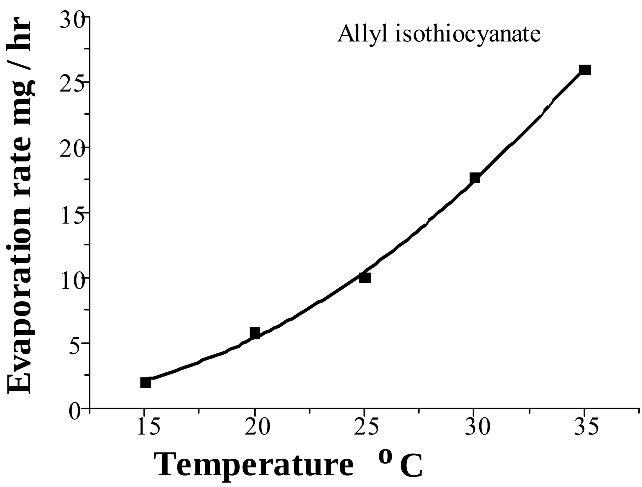

Fig. 2. Temperature dependence on allyl ITC emission rate. Polyethylene sachet dispensers, 10 x 10 mm, wall thickness 26 microns.

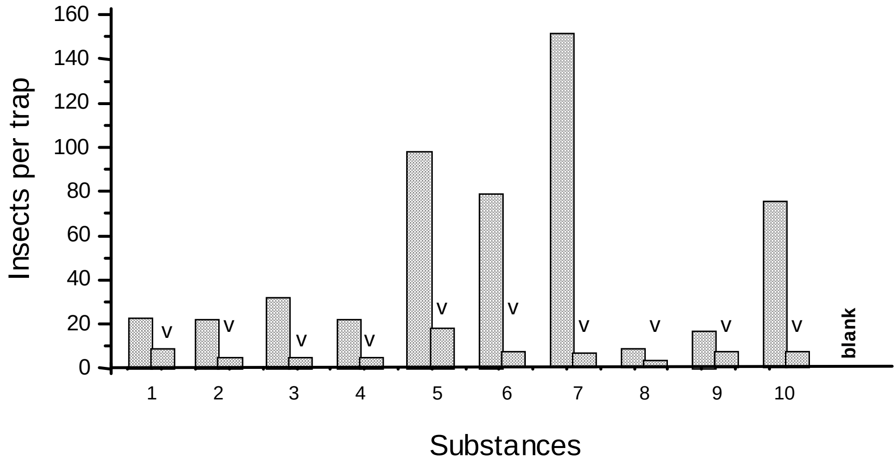

Fig. 3. Attractivity of compounds for *P. vittula* and *P. undulata*. Juuru, old greenhouse, 6–13 June 2000. Conical traps (cups) were used. Tested substances: 1 – phenylethyl TC; 2 – phenylethyl ITC; 3 – phenyl ITC; 4 – benzyl TC ; 5 – butyl TC; 6 – butyl ITC ; 7– butenyl ITC; 8– 2-phenylethanol; 9– pentenyl ITC +2-phenylethanol; 10 – butenyl ITC +2-phenylethanol; blank – no bait. "v" notes *P. vittula*, without letter - *P. undulata.*

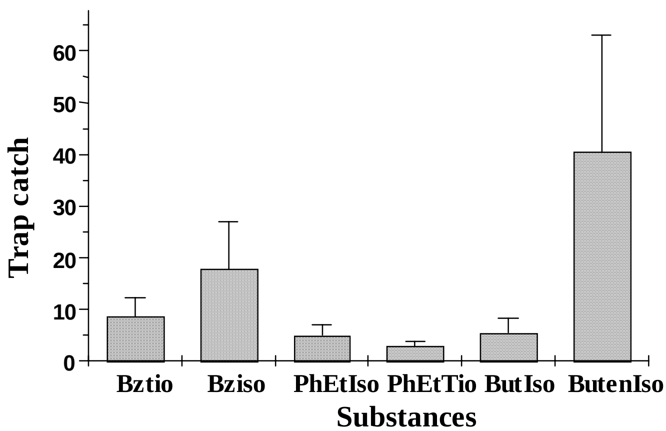

Fig. 4. Attractivity of thiocyanates and isothiocyanates at Valgeristi, nearby Tartu. 5–8 June 2000.Yellow cylindrical traps were used. Substances are marked as next: Bztio – benzyl TC, Bziso – bensyl ITC, PhEtIso – phenethyl ITC, PhEtTio – phenethyl TC, ButIso – butyl ITC, ButenIso – butenyl ITC. Butenyl isothiocyanate was of higher attractivity than other tested substances at level of $P < 0.05$.

RESULTS

In field experiments at Juuru in June 1999, the trap catches were (insects/trap/5 days): butyl isothiocyanate (butyl-ITC) 2.6; butyl thiocyanate (butyl-TC) 8.2; allyl isothiocyanate (allyl-ITC) 9.4; 3-butenyl-1-thiocyanate (butenyl TC) 149. In July–August catches were higher: butyl-ITC 5; butyl TC 43; allyl-ITC 44; butenyl TC 240 insects/trap/5 days. The highest attractivity had butenyl ITC and butenyl TC, butyl ITC and butyl TC demonstrated elevated trap catches as well. Deltatraps Atrakon A were used. As seen, in August the highest insect attraction was released by butenyl TC. Results of both experiments are summarised in Table 1.

Experiments at Mardi in 31.05–11.06.1999 on the edge of an oil rapeseed field demonstrated similar results. Green Atrakon A traps were used (white clued bottoms), Table 2.

In 2000, experiments were carried out using small cups. Results are in Table 3. In 2000, the *Phyllotreta* species were attracted to a different type of traps, similar to those used in 1999, see Fig. 3. Mainly two species were found in traps, *P. undulata* and *P. vittata*.

The same two *Phyllotreta* spp were attracted to isothiocyanates at Valgeristi nearby Tartu. The use of large cylindrical traps enabled us to collect more insects. Fig. 4 summarises trap catches, baited with four isothiocyanates and two thiocyanates. The highest amount of Chrysomelidae was trapped using butenyl ITC. Butenyl TC was not tested in this experiment.

Large transparent cylindrical traps were used to test the influence of basic colours (yellow, green, and white) on attractivity of alkyl ITC to *Phyllotreta* spp. Results confirmed the lack of influence of tested colours on attractivity. (Fig. 5).

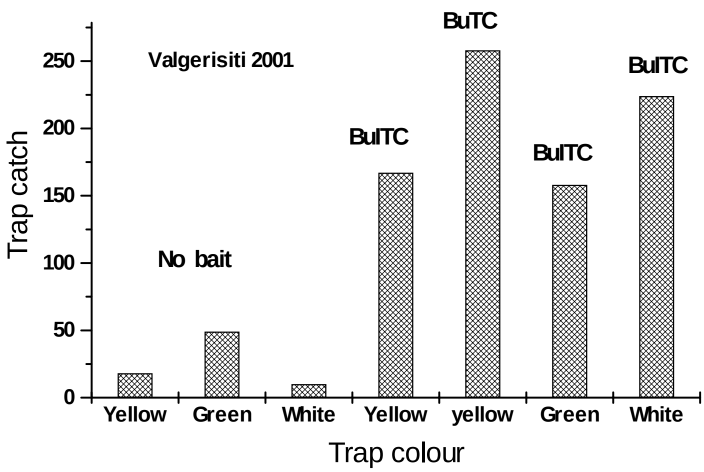

Fig. 5. Influence of trap colour on trap catch. Experiments at Valgeristi, 2001. Tested substances: BuITC - butyl isothiocyanate, BuTC - butyl thiocyanate. All the differences in baited trap catches are not significant at 95% level, all the non-baited trap catches are different from baited trap catches at 99% level.

Table 1. Attractivity of some ITC and TC to *Phyllotreta* spp in experiments at Juuru. Results of experiments in July, 1999. Sum of trapped *Phyllotreta* spp is given.

Substance	*P. undulata*	*P. vittata*	*P.vittula*	*P. nemorum*	*unidentified*	Sum
Phenyl ITC	1	1	0	0	0	2
Butyl TC	186	25	16	13	0	240
Butenyl TC	870	108	0	26	4	1,008
Benzyl ITC	86	2	0	0	1	89
Allyl ITC	103	13	3	7	25	151
No bait	0	0	0	0	6	6
sum	1,246	149	19	46	36	1,496
%	83.29	9.96	1.27	3.07	2.41	100

Table 2. Attracted to butenyl ITC insects at Mardi, May–June 1999.

Substance	*P. undulata*	*P. vittata*	*P. vittula*	*P. nigripes*	Sum
Butenyl IT (%)	278 (72.6)	75 (19.6)	15 (3.9)	15 (3.9)	383 (100.0)
No bait	43 (43.9)	26 (26.5)	13 (13.3)	16 (16.3)	98 (100.0)

Table 3. Attractivity of substances in open cups at Juuru in 2000.

Tested compound	Test place					
	old greenhouses[1]		homegarden[2]		oilseed rape field[3]	
	insects per trap	± (St. error)	insects per trap	± (St. error)	insects per trap	± (St. error)
But-3-enyl ITC	31.6	10.7	5.8a	2.3	43,4	34,1
Butyl TC	23.4	6.7	2.6b,a	1.1	10.0	11.7
Butyl ITC	17.6	12.4	1.2b,c	0.74	1.2	1.3
But-3-enyl TC	16.6	7.5	10.8 a	3.8	69,8	21,8
Phenethyl ITC	8.2	2.5	3.8a,b	1.4	0.2	0.4
Phenyl IT	---	---	2.2b	1.020	---	---
Allyl IT	---	---	2b,c	1.140	---	---
Benzyl IT	---	---	1b,c	0.632	16.8	5.4
Phenetyl TC	---	---	---	---	0.8	0.8
No bait	0.9	1,2	0.2c	0.20	3.4	2.5

[1]All *Phyllotreta* species, 2001, 6–13 June, green small (conical) traps
[2]Only *P. undulata,* 11 June 2001, White small (conical) traps.
[3]Mainly P. vittata 31 July–03 August 2001 data 31.07–3.08, white cups (small conical traps.

Table 4. Trapping test at Matsi in July 2002. Trapped amount of insects per day is given

Dispenser type	Tested compound	Test period and mean number of insects per trap per day	
		20–22 July	22–27 July
Polyethylene capillaries	Butenyl TC	7.5 ± 3.4	0.7 ± 0.7
Miniket (silicone)	Phenethyl ITC	3.0 ± 2.4	2.8 ± 2.9

Table 5. Trapping test at Matsi in July 2002. Identifyed Phyllotreta spp, % from identified species.

Phyllotreta spp	Attractants and percentage of caught insect spp	
	Phenethyl ITC	Butenyl TC
P. undulata	23.0	32.0
P. vittata	64.0	59.0
P. vittula	13.0	9.0

To test the influence of lower doses of attractant thiocyanates and isothiocyanates Miniket Si type substrate dispenser (Mõttus et al., in preparation) loaded with phenethyl ITC and polyethylene capillary dispensers loaded with butenyl ITC were tested in oilseed rape fields at Matsi in July 2002. Field experiments were carried out during 10–27 July in ten replicates. Due to the extraordinary hot and dry summer, the flight of flea beetles ended earlier. The results are summarised in Table 4. During 20–22 July, butenyl TC had higher attractivity compared with phenethyl ITC, mainly three species were trapped (Table 5).

DISCUSSION

We have studied the attraction capacity of alkyl thiocyanates (TC) and alkyl isothiocyanates (ITC) for the *Phyllotreta* spp in different vegetation periods and in different biotopes. Results at Juuru demonstrated that both butenyl ITC and butenyl TC had the highest attractivity compared with other tested alkyl ITC and alkyl TC (Tables 1 and 3). On the basis of three years of field experiments it is not possible to conclude the preference of butenyl TC or butenyl ITC. Both compounds had the highest but somewhat variable attractivity to *Phyllotreta* spp (Tables 1 & 3). It may be due to an influence of differences in the composition of plant volatiles, caused by differences in age of plants; differences in concentrations of TC and ITC in effluvia from dispensers, etc. For instance, the typical green leaf volatiles Z-3-hexenol and 2-phenylethanol had a strong inhibitory effect in combination with butenyl TC (unpublished). At the same time, volatility of substances from sachet dispensers strongly depends on temperature. In the case of allyl ITC, a 10ºC rise in temperature elevates the emission rate by about two and a half times (Fig. 2).

The low attractivity of allyl ITC to *Phyllotreta* spp in Estonia was not expected. In Saskatoon, Canada, allyl ITC is used as an attractant for insects cued to cruciferous and ten Phyllotreta spp have been attracted to traps baited with ally ITC (Pivnick et al., 1992; Cho et al., 1995). In our experiments at Juuru, allyl ITC had low attractivity and was excluded from further experiments.

Chrysomelidae captured in different places in Estonia were essentially the same, (Tables 1, 2 & 5, Fig. 3). Altogether five *Phyllotreta* species were identified in trap catches of our experiments, *P. undulata* and *P. vittata* were always the most abundant species. At the end of flight of Crysomelidae, *P. vittata* was more abundant compared with summer-time collections (Tables 3 & 5). An analysis of the experiment data demonstrates that it is due to the shorter flight time of *P. undulata* prevailing in Estonia. Our research was not aimed at identifying all the captured insects, some species were damaged and usually about 60–70% of captured insects were identified. Experiments on using of kairomonal attractants for indication of *Phyllotreta* spp is in progress.

The construction of the trap is of significance. Deltatraps are common by need for moths' pheromones and they may be recommended if insects are orientating to the source of attractant. In the case of kairomones insects are looking for a region with an elevated content of attractive substances in air rather than for the source. This may be the reason why the closed delta type Atrakon A had not high enough trap catches. For the same reason plastic "bucket" style traps had low attractivity at Matsi (unpublished). Large cylindrical traps, having an outer glued surface of about 600 cm^2 and a volume of 1.5 l, demonstrated the best results in experiments at Valgeristi and Matsi. They had 12-fold larger trapping area compared with conical traps, made from plastic cups having a volume of 180 ml. Effluvium of dispensers creates a region inside the cylindrical traps where the air is more or less saturated by kairomone. We assume that the effluvium from kairomone dispensers can disperse through the open bottom of the trap and through the walls. In any case, the closed cylindrical traps limit the evaporation speed of attractants, compared with traps where the dispenser is placed in open air, such as bucket traps and water traps.

Our research indicates that the inexpensive and convenient trapping technique designed for the pest *Phyllotreta* spp will be an effective tool for diminishing or avoiding the usage of toxic plant protection chemicals.

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