SIMILARITIES AND DIFFERENCES IN PHEROMONAL AND HOST-PLANT RELATED CHEMICAL COMMUNICATION OF FLEA BEETLES *PHYLLOTRETA CRUCIFERAE* GOEZE AND *PH. VITTULA* REDTENBACHER (COLEOPTERA, CHRYSOMELIDAE)

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Phyllotreta vittula Photo: Éva Csonka

INTRODUCTION

Flea beetles (*Phyllotreta* spp.) (Coleoptera, Chrysomelidae) are important pests of mainly cruciferous crops, causing damage in part by feeding on seedlings in early spring, and in part through propagating several plant pathogens **[1-4].** Both *Phyllotreta cruciferae* Goeze and *Ph vittula* Redtenbacher rank among the most important pest flea beetles in Europe **[5]**. We set out to study their pheromonal and host-plant related chemical communication because knowledge gained in these areas may form the basis of the development of new tools and methods useful in their control.

MATERIALS AND METHODS

Field tests were conducted at several sites in Hungary by established methods **[6-7]**, using CSALOMON® VARL funnel traps **[6]** or KLP+ ("hat") traps **[8].** Volatile collections of pheromone were obtained by trapping the volatiles produced by feral insects on charcoal filters (CLSA-Filter, Winterthur, Switzerland) in a closed-loop stripping apparatus **[9].** Charcoal filter washings (by dichloromethane) were analysed by gas chromatography and mass spectrometry. Identification of components were done by matching retention times with those of synthetic standards and comparing mass spectra.

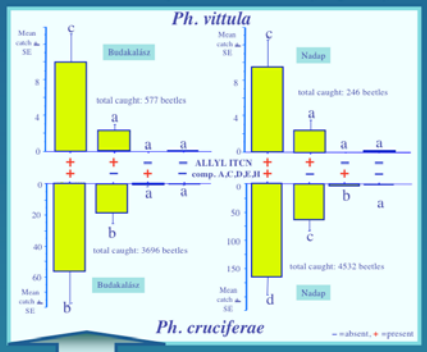


Fig. 1. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with ALLYL ITCN or comp ACDEH, and both baits together in Hungary. Budakalász, Pest county, white mustard, MAR 27 - MAY 1, 2003; Nadap, Fejér county, oilseed rape, APR 1 - MAY 5, 2003. Columns with same letter within one diagram are not significantly different at P=5% by ANOVA, Games-Howell. Data from **[7]**.

RESULTS AND DISCUSSION

We found remarkable similarities in the pheromonal communication of the two species. Recently, male specific pheromone candidate compounds have been identified from a North American population of *Ph. cruciferae* **[10]**, and the blend of synthetic samples showed remarkable field activity in tests in North America **[11].**
Having studied the field activity of these himachalene and cadinene compounds (comp. A, C, D, E, H; labeling according to **[10]**) on European flea beetle populations, we reported that catches of both *Ph. cruciferae* and *Ph. vittula* increased in traps baited with allyl isothiocyanate (ALLYL ITCN) when a mixture of compounds A, C, D, E and H was added (Fig 1) **[7]**.

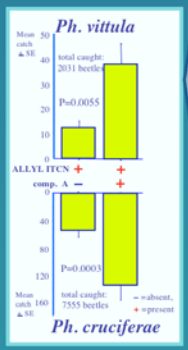
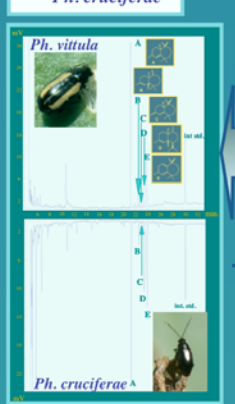


Fig. 2. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with ALLYL ITCN alone, or combined with comp A. Pusztazámor, Pest county, Hungary, white mustard, APR 7 - MAY 23, 2005. P-values result from Student *t* test.

It appeared that for *Ph. cruciferae* only comp. **A** [(5R,5aS)-1,1,5,8- tetramethyl-1,2,3,4,5,6,5a-heptahydrobenzo[1,2-a][7] annulene] was the one for which pheromonal activity could be clearly shown **[7]**. The addition of only comp. **A** to allyl isothiocyanate was capable of increasing catches also in *Ph. vittula*, which suggested that Compound **A** may be the key pheromone component also in this species (Fig 2).

In volatiles collected from male *Ph. vittula*, the presence of all male specific compounds found previously in collections from North American or European populations of *Ph. cruciferae* was verified (Fig 3).

In both species the major component was comp **A**. Of the other components comp. **B** and **D** were present in lower percentages in *Ph. vittula*, than in *Ph. cruciferae* (Table 1). Comp. **E** and probably comp. **C** were observed in similar ratios in the two species.



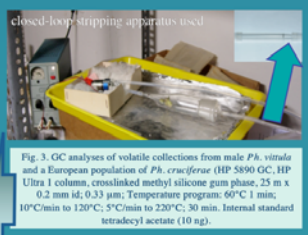


Fig. 3. GC analyses of volatile collections from male *Ph. vittula* and a European population of *Ph. cruciferae* (HP 5890 GC; HP Ultra 1 column, crosslinked methyl silicone gum phase, 25 m x 0.2 mm id; 0.33 µm; Temperature program: 60°C 1 min; 10°C/min to 120°C; 5°C/min to 220°C; 30 min. Internal standard tetradecyl acetate (10 ng).

Table 1. Ratios of male-specific compounds (in percentages of comp. **A**) in volatile collections from *Ph. vittula* and *Ph. cruciferae* Values calculated by comparing FID peak areas. P-values result from Student *t* test. (n = number of samples analysed).

	Ratio in % of comp. A (Mean±SE)			
	comp. B	comp. C	comp. D	comp. E
Ph. vittula	7.9±0.48 (n=6)	2.57±0.3 (n=3)	14.43±1.3 (n=6)	30.6±4.8 (n=6)
Ph. cruciferae	21.9±3.9 (n=4)	1.87 (n=1)	61.03+7.5 (n=4)	46.3±8.1 (n=4)
P-value	0.002	not available	<0.0001	0.12

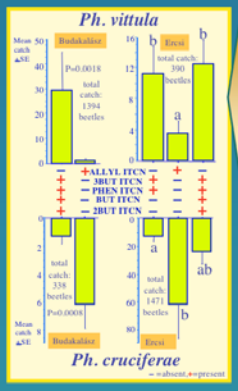
On the other hand, host-plant related chemical communication appeared to show significant differences between the two species.
In preliminary screenings sizeable catches of *Ph. vittula* were recorded in traps baited with 3-butenyl isothiocyanate (= 3BUT ITCN), or an isothiocyanate mixture [= ITCN MIX; this was a gift sample from Prof. E. Mõttus, Tartu Univ., Estonia, consisting of 2-butenyl-, phenethyl-, 3-butenyl- and butyl isothiocyanates), while other compounds (among them also ALLYL ITCN in one of the tests) showed low activity (Fig 4).

Fig. 4. Catches of *Ph. vittula* in traps baited with ITCN MIX (for composition see text), 2-butenyl thiocyanate (2BUT TCN), 3-buteneyl isothiocyanate (3BUT ITCN), allyl isothiocyanate (ALLYL ITCN), butyl isothiocyanate (BUT ITCN), butyl thiocyanate (BUT TCN) or phenethyl thiocyanate (PHEN TCN) in Hungary. Pusztaszabolcs, Fejér county, oilseed rape, MAR 22 - APR 22, 2002; Vecsés, Pest county, cabbage, JUL 19 - SEP 17, 2002. Significance: see Fig 1.

Table 2. Total catches of *Ph. vittula* in traps baited with ITCN MIX, 3BUT ITCN or ALLYL ITCN and unbaited traps. Nadap, Fejér county, Hungary., oilseed rape, SEP 14- OCT 8, 2002.

bait	total catch
ITCN MIX	418
3BUT ITCN	247
ALLYL ITCN	2
unbaited	2

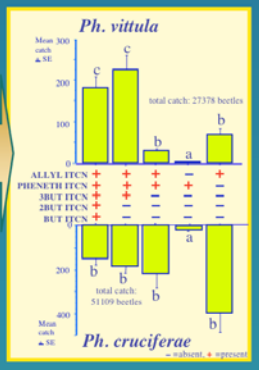
Results of another preliminary unreplicated test also showed high catches of *Ph. vittula* with ITCN MIX and 3BUT ITCN, while almost nothing in traps with ALLYL ITCN and unbaited (Table 2). This suggested that *Ph. vittula* may respond more sensitively to isothiocyanates other than ALLYL ITCN, which compound is a known and potent attractant for *Ph. cruciferae* **[12-16]**. Since numerically most *Ph. vittula* were caught with the ITCN MIX in the above tests, we continued with comparison trials between combinations of components of ITCN MIX and ALLYL ITCN to confirm this hypothesis.



Ensuing tests confirmed that *Ph. vittula* responded better to the mixture of the components of ITCN MIX, than to ALLYL ITCN (Fig 5). It was revealed also that the omission of butyl isothiocyanate and 2-butenyl isothiocyanate from the mixture did not influence catches. On the other hand, *Ph. cruciferae* catches were generally greater in ALLYL ITCN baited traps.

Fig. 5. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with isothiocyanate blends or ALLYL ITCN in Hungary. Budakalász, Pest county, white mustard, MAR 29 - APR 30, 2004; Ercsi, Fejér county, maize, AUG 9 -SEP 17, 2004. Significance: see Fig 1. P-values result from Student *t* test (Budakalász).

Fig 6. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with isothiocyanate blends or single compounds. Pusztazámor, Pest county, Hungary, white mustard, MAR 21 - JUN 1 2005. Significance: see Fig 1.



In further tests catches of *Ph. cruciferae* were similar in all treatments containing ALLYL ITCN alone or in mixtures (Fig 6). On the other hand, highest numbers of *Ph. vittula* were caught with blends containing 3BUT ITCN. This suggested that 3BUT ITCN might predominantly be responsible for attractivity of the mixture in previous tests towards *Ph. vittula*.

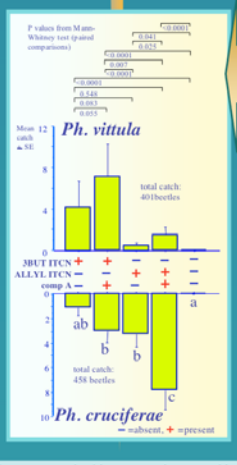


Fig 8. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with 3BUT ITCN or ALLYL ITCN alone and in combination with comp. **A**. Pusztazámor, Pest county, Hungary, white mustard, MAY 4-29, 2006. Significance: see Fig 1.

The importance of 3BUT ITCN was corroborated in a final test where again highest *Ph. vittula* catches were observed in traps with baits containing 3BUT ITCN, while *Ph. cruciferae* responded best to baits containing ALLYL ITCN (Fig 7). The binary blend caught similar numbers as the respective single compounds in both species.

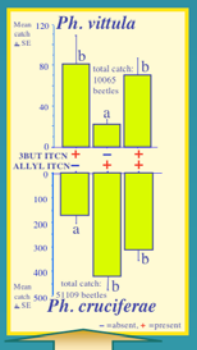


Fig 7. Catches of *Ph. vittula* and *Ph. cruciferae* in traps baited with 3BUT ITCN, ALLYL ITCN or their blend. Pusztazámor, Pest county, Hungary, white mustard, MAR 31- MAY 23, 2006. Significance: see Fig 1.

When comp. **A** of the pheromone was added to 3BUT ITCN or ALLYL ITCN (as host-plant compounds), in *Ph. vittula* 3BUT ITCN combined with comp. **A** appeared to be most active (Fig 8). In *Ph. cruciferae* highest catches were observed in ALLYL ITCN combined with comp. **A**, supporting results of earlier studies **[7]**.

CONCLUSIONS

In the present study we demonstrated that the pheromone composition of the two species is very similar. In their responses towards host-related isothiocyanates however, the species showed differing preferences. Best field activity was observed with optimal combinations of pheromonal and host-plant-related stimuli. For practical applications, the use of a bait containing the above isothiocyanates plus the common pheromone component may be advantageous as it would attract both important pest *Phyllotreta* spp. in high numbers.

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