



How can pheromone studies complement taxonomy: a click beetle example (Coleoptera: Elateridae)

Miklós Tóth¹, József Vuts¹, Lorenzo Furlan², Till Tolasch³ Amália Xavier⁴, Teodora Toshova⁵, Mitko Subchev⁵, István Szarukán⁶, Venyamin Yatsynin⁷

¹*Plant Protection Institute, HAS, Budapest, Hungary;* ²*Department of Agronomy, Entomology, Padova University, Agripolis, Italy;* ³*Univ. Hohenheim, Inst. Zool. Fg. Tierökologie, Garbenstr. 30, D-70593 Stuttgart Germany;* ⁴*DRAEDM, Porto, Portugal;* ⁵*Zoology Institute, BAS, Sofia, Bulgaria;* ⁶*Agricultural University, Debrecen, Hungary;* ⁷*Krasnodarskiy NIISKh im. P.P. Lukyanenko, Krasnodar 12, Russia*

Wireworms, the larvae of click beetles (Coleoptera: Elateridae) are important soil-dwelling polyphagous pests all over the world



Traditional forecast and monitoring involves labour-intensive soil sampling methods,





and to obtain wireworms from soil samples collected is time-consuming (several days or more).



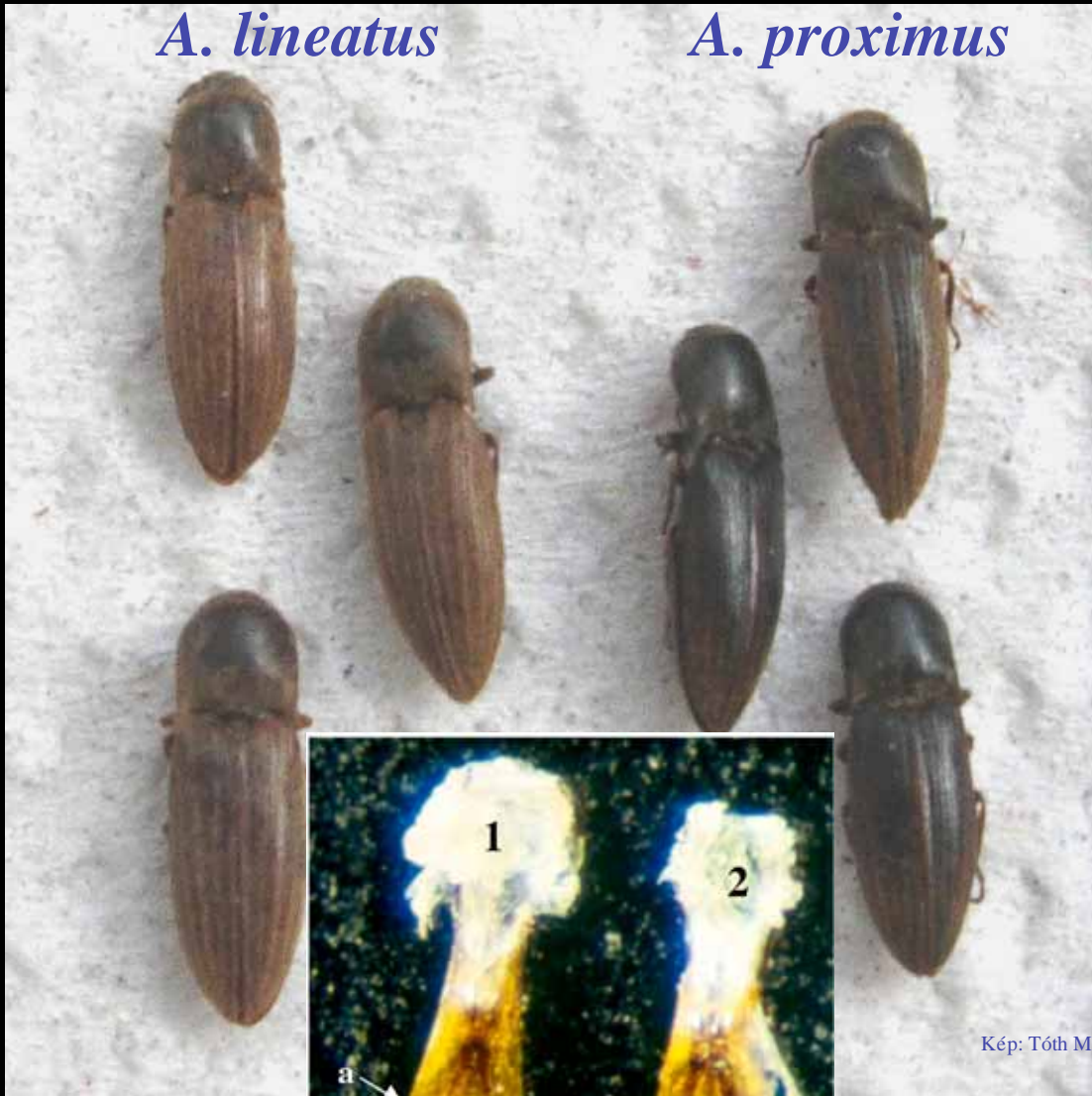
Pheromone-baited traps are much easier and simpler to use: the YF trap design proved to be excellent for several click beetle spp. in Europe and North America.

However, the pheromone composition should be identified!

The two species we worked with:

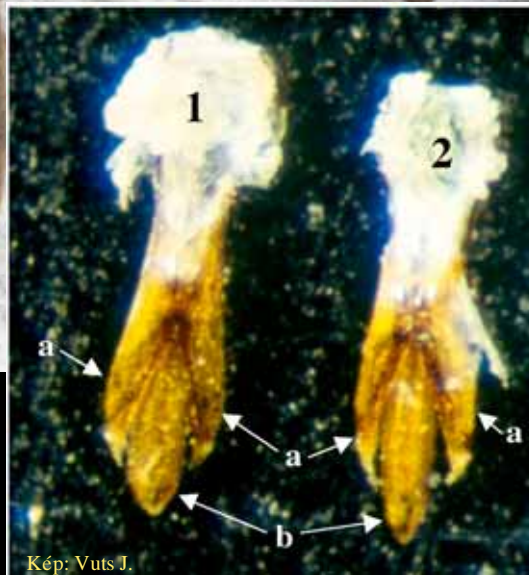
A. lineatus

A. proximus



Determination keys used to tell apart the species:

Ratio of width vs. length of thorax different
&
subtle differences in genital morphology

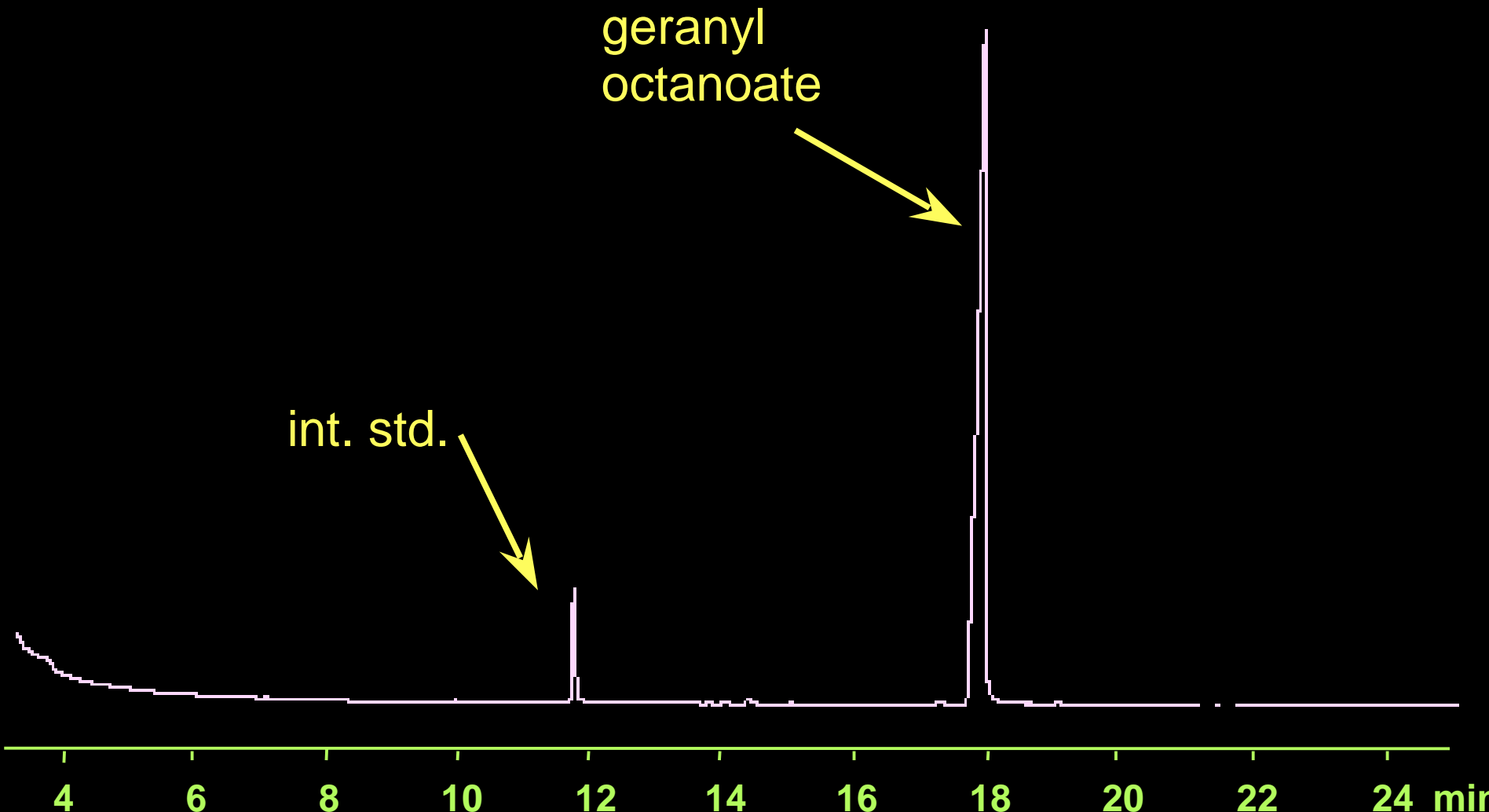


Kép: Tóth M.

a: lobus lateralis
b: lobus medialis

Kép: Vuts J.

GC analysis of pheromone gland extract of *A. lineatus* was dominated by a huge peak of geranyl octanoate – corresponding to literature data



Composition described for West Ukrainian population attracted no *A. lineatus*. The attraction of *A. ustulatus* is due to the farnesyl compound, which is its known pheromone.

Debrecen, Hungary
Apr 21 - Aug 5,
1994

	Geranyl octanoate	Neryl iso-valerate	E,E-farnesyl acetate	Total caught	
				<i>A. lineatus</i>	<i>A. ustulatus</i>
<p>→</p> <p>Described as pheromone component by Borg-Karlson et al., 1988 (Swedish population), and Siirde et al., 1993 (East-Ukrainian and Russian populations)</p>	+	=	=	30	0
<p>→</p> <p>Described as pheromone components by Siirde et al., 1993 (West Ukrainian population).</p>	=	=	+	0	195
	=	+	+	0	93


Geranyl octanoate showed only low to moderate field activity on *A. lineatus*.

Debrecen, Hungary
Apr 21 - Aug 5,
1994

	Geranyl octanoate	Neryl iso-valerate	E,E-farnesyl acetate	Total caught	
				<i>A. lineatus</i>	<i>A. ustulatus</i>
<p>→</p> <p>Described as pheromone component by Borg-Karlson et al., 1988 (Swedish population), and Siirde et al., 1993 (East-Ukrainian and Russian populations)</p>	+	=	=	30	0
<p>→</p> <p>Described as pheromone components by Siirde et al., 1993 (West Ukrainian population).</p>	=	=	+	0	195
				0	93

Among components found in trace amounts in the gland extract, the addition of geranyl butanoate is highly synergistic in field trapping tests.

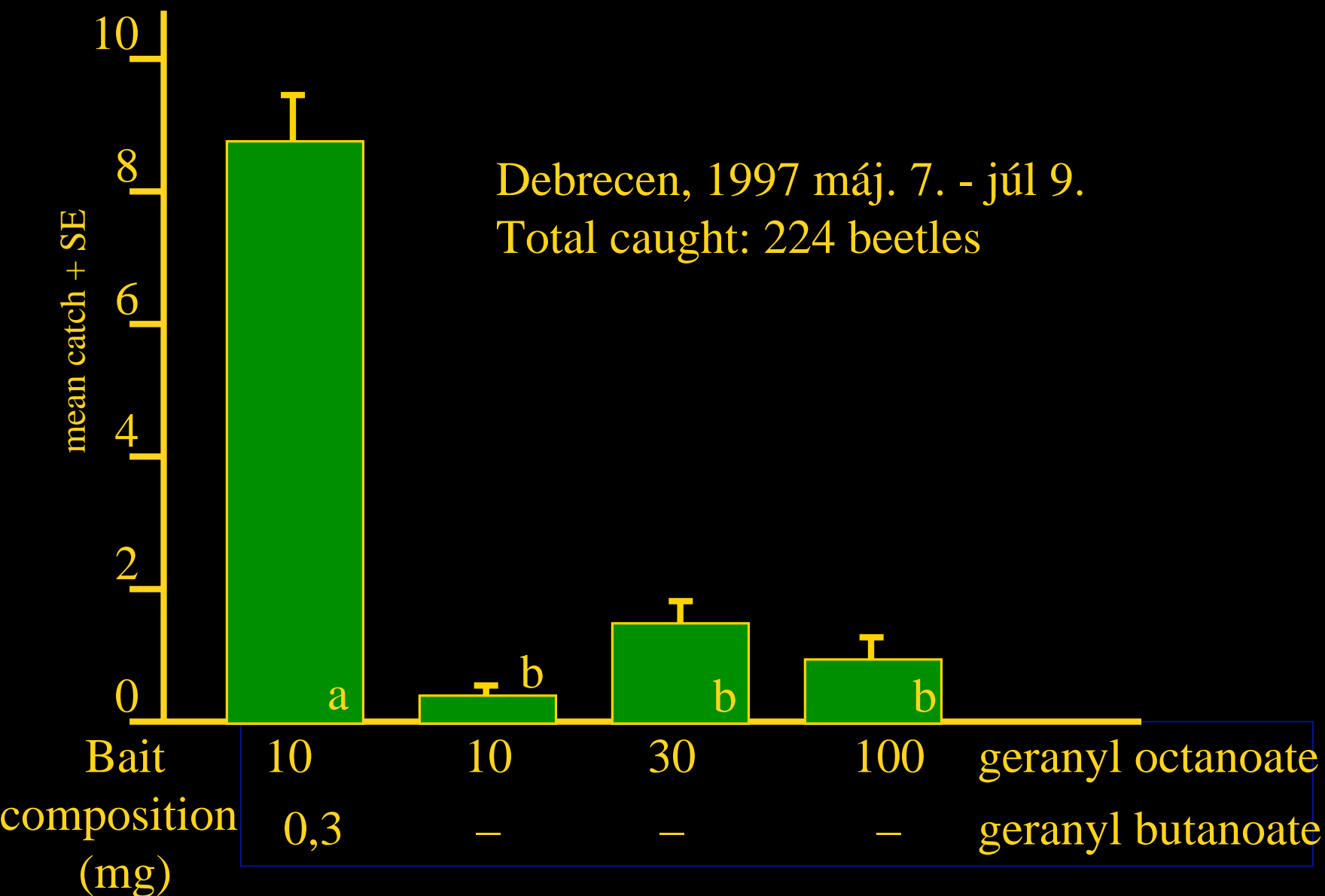
Total caught
in test:
279
beetles



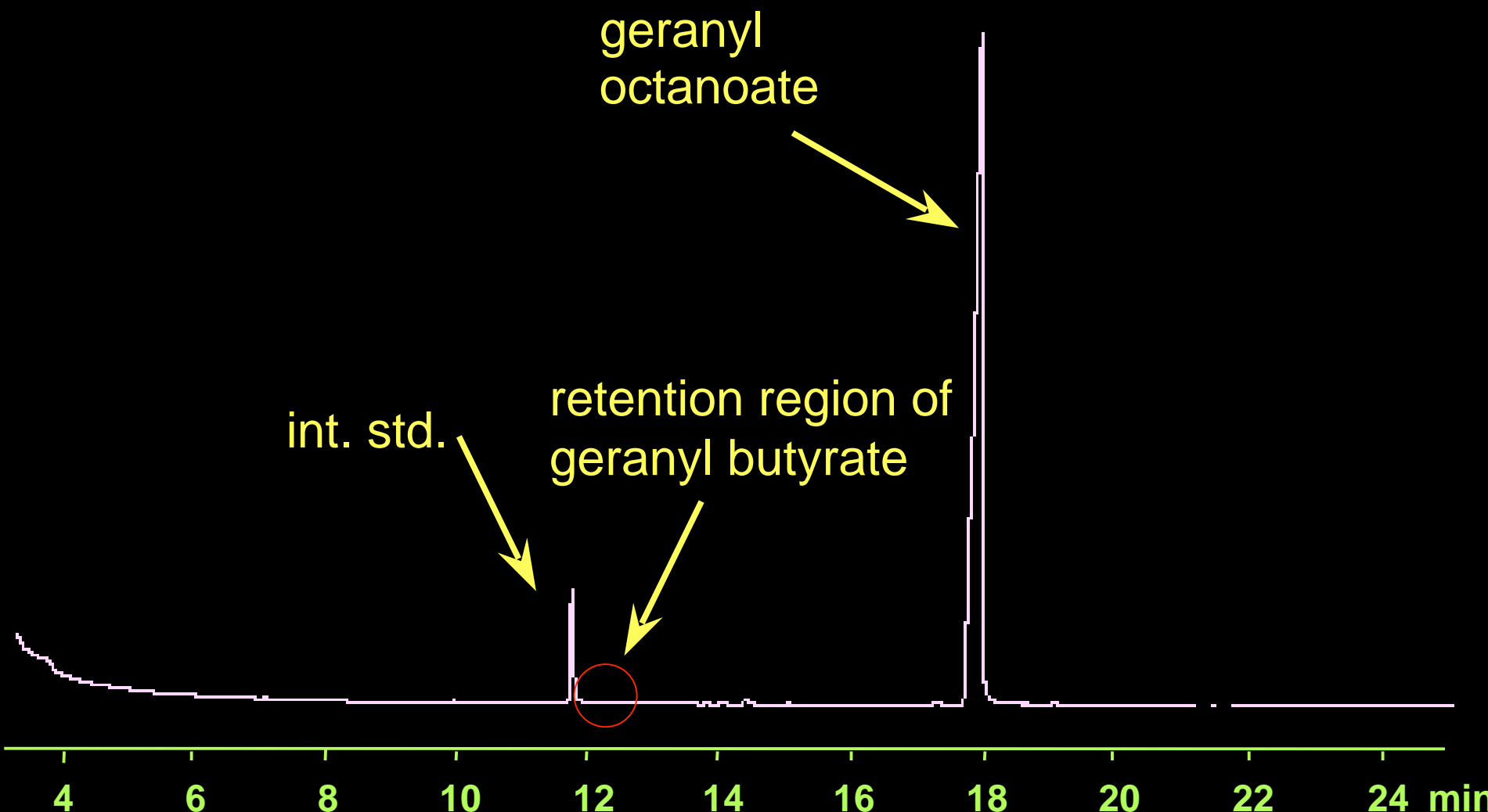
	Geranyl octanoate	Geranyl butanoate	E,E-farnesyl acetate	Geraniol	Geranyl hexanoate	Neryl isovalerate	<i>A.lineatus</i>
10	0
30	2
100	1
10	.	1	0
10	.	.	1	.	.	.	2
10	.	.	.	1	.	.	1
10	1	.	0
10	1	1
							273

Rümlang,
May 23 -
August 14,
1997

This synergistic activity of geranyl butanoate was also confirmed in further field tests.

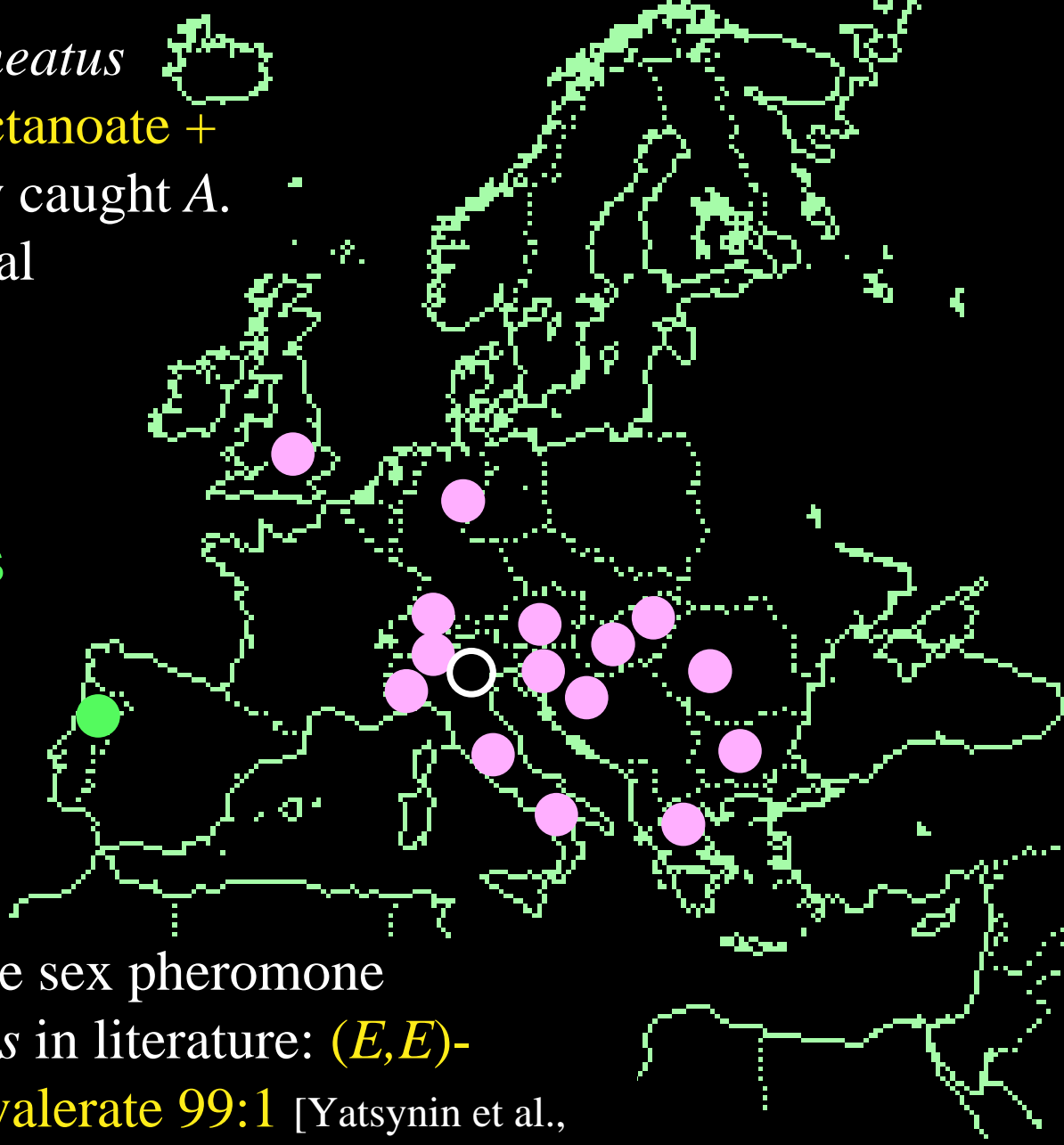


The relatively high ratio of geranyl butanoate in efficient baits for *A. lineatus* is surprising, as in pheromone gland extracts this compound was found only in traces.



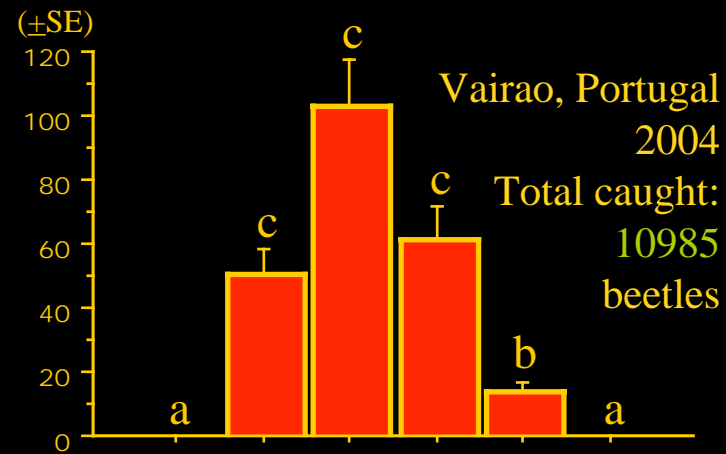
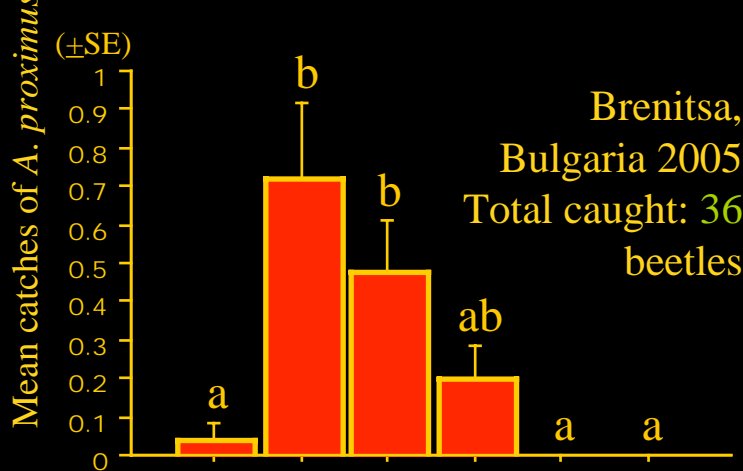
Traps baited with *A. lineatus* pheromone bait (geranyl octanoate + geranyl butanoate) regularly caught *A. proximus* in Portugal

- lineatus catches
- proximus catches
- no catch



This was surprising, because sex pheromone composition for *A. proximus* in literature: (*E,E*)-farnesyl acetate + neryl isovalerate 99:1 [Yatsynin et al., Khim.Sel'sk.Khoz. Moscow, Khimiya, 33-35 (1980)]

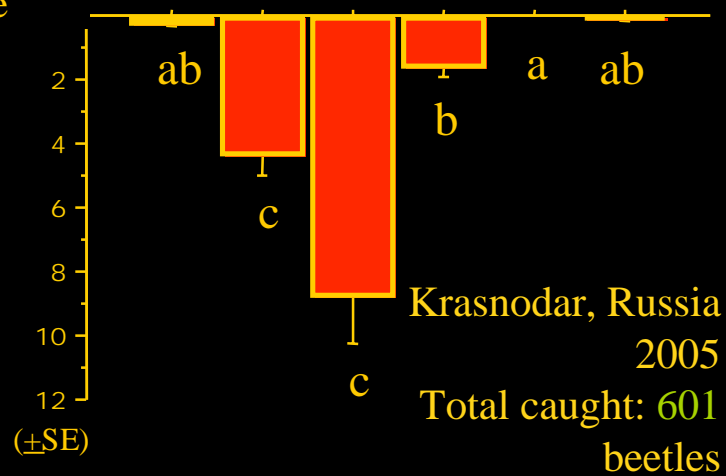
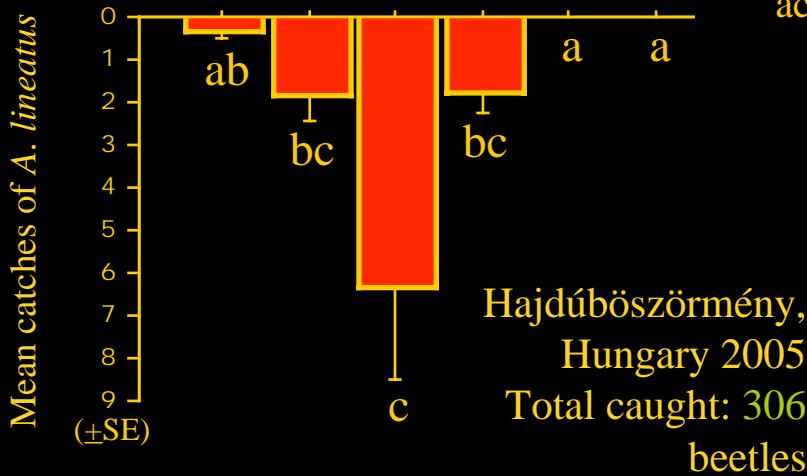
Agriotes proximus



Bait (mg)	30	30	30	3	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30

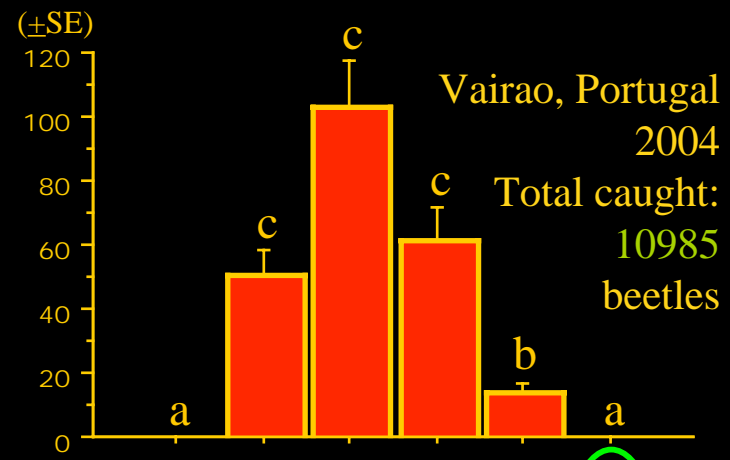
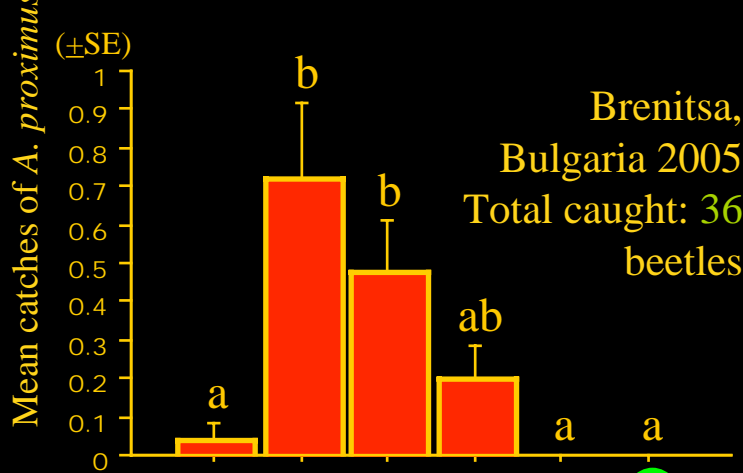
Bait (mg)	30	30	30	3	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30
	30	30	30	30	30	30	30	30	30	30

Agriotes lineatus



A systematic field trapping test was performed (2 sites for each species) comparing a ratio range of the components identified by us and the blend previously described in literature for *A. proximus*.

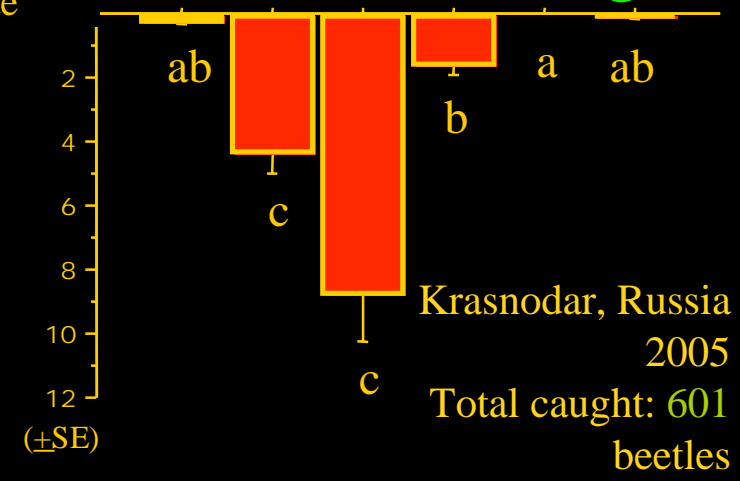
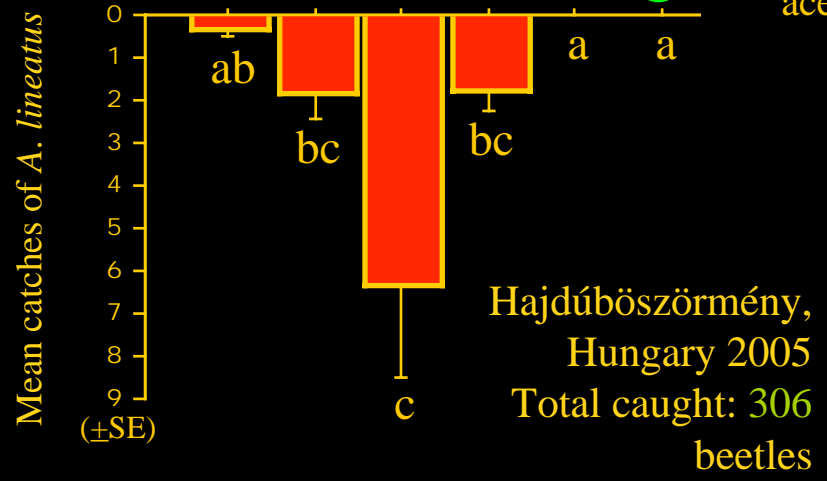
Agriotes proximus



Bait (mg)	30	30	30	3	-	30	geranyl butanoate
	3	30	30	30	-	-	geranyl octanoate
	-	-	-	-	30	-	neryl isovalerate
	-	-	-	-	0.3	-	(<i>E,E</i>)-farnesyl acetate

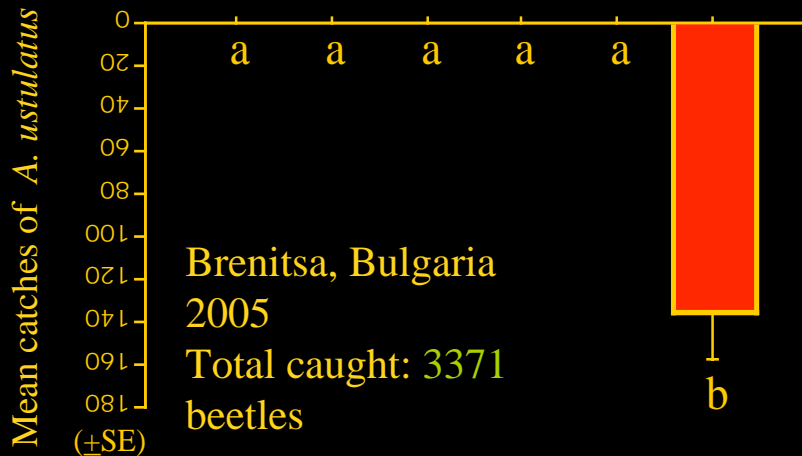
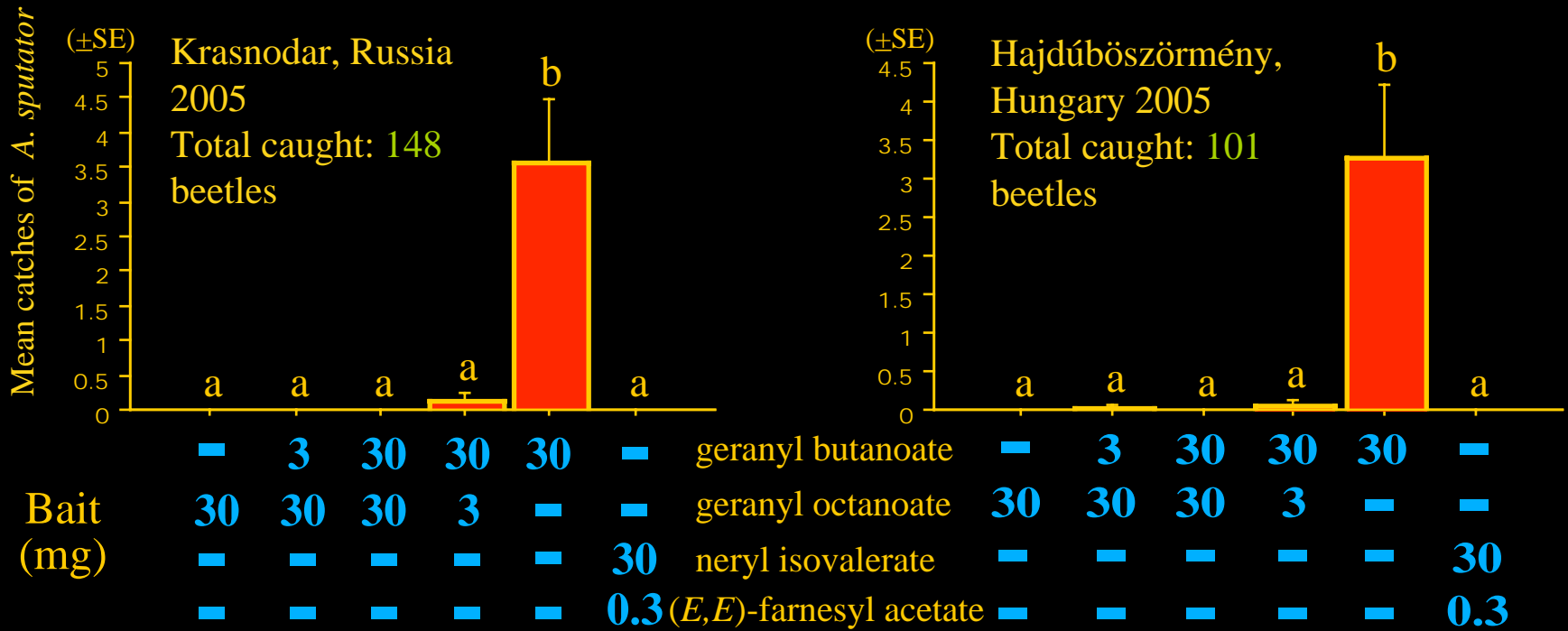
Bait (mg)	3	30	30	30	-	30	geranyl butanoate
	30	30	30	3	-	-	geranyl octanoate
	-	-	-	-	-	30	neryl isovalerate
	-	-	-	-	-	0.3	(<i>E,E</i>)-farnesyl acetate

Agriotes lineatus



1) The blend previously described in literature for *A. proximus* was inactive.

Agriotes sputator



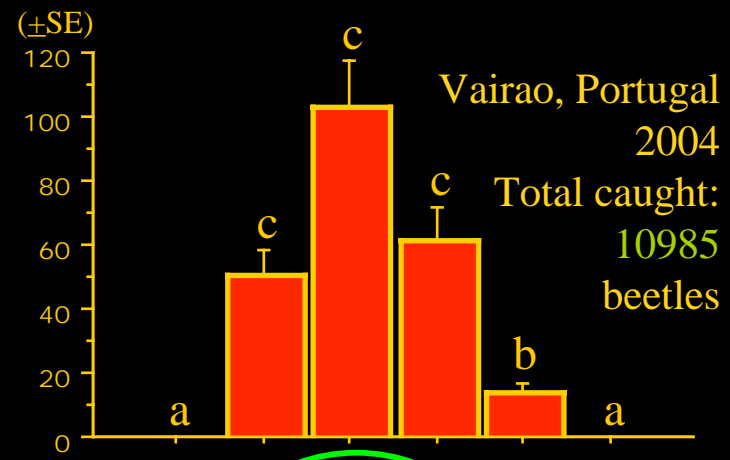
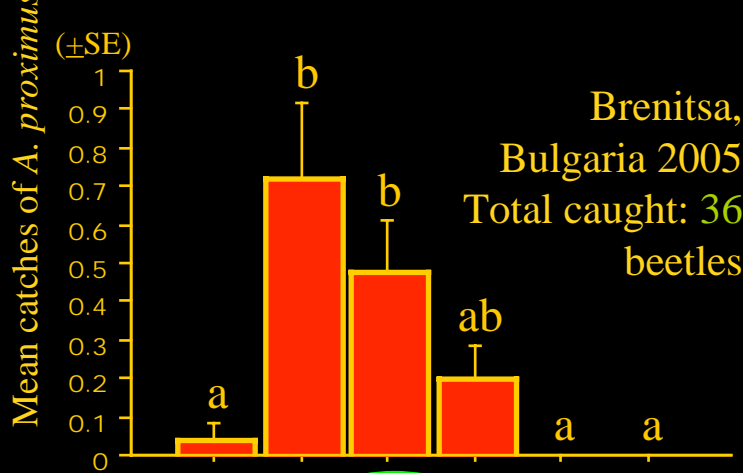
Other spp. captured:

A. sputator: geranyl butanoate is main pheromone component

A. ustulatus: (E,E)-farnesyl acetate is main pheromone component

Agriotes ustulatus

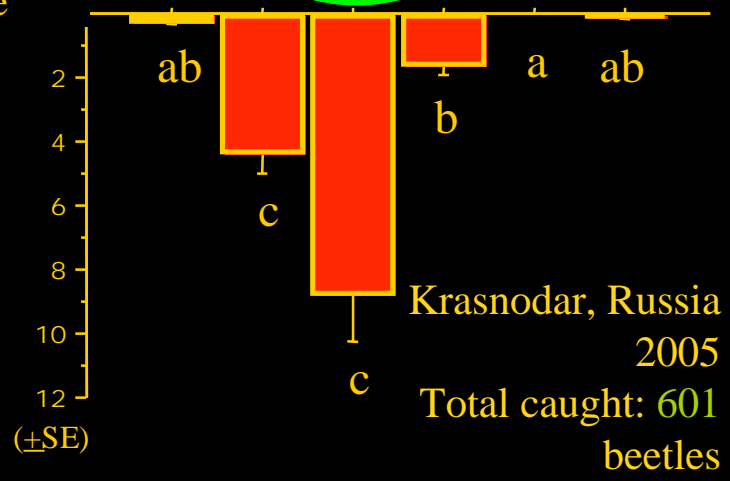
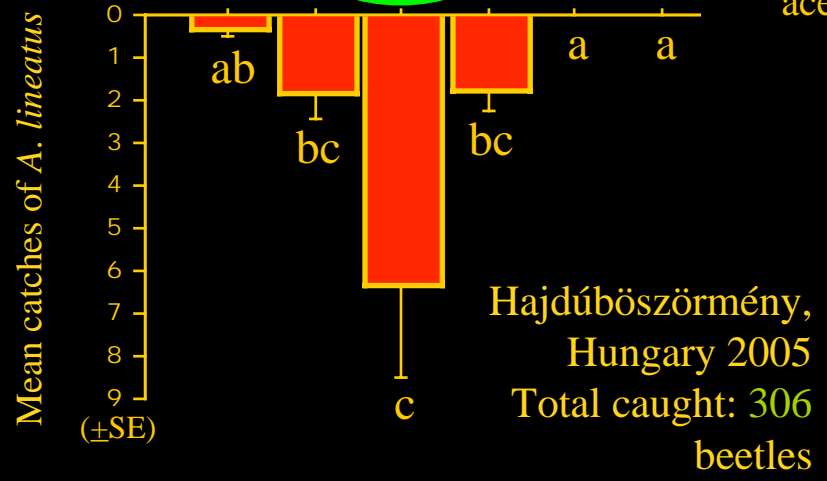
Agriotes proximus



Bait (mg)	30	3	30	30	30	30	geranyl butanoate
	30	30	30	3	30	30	geranyl octanoate
	-	-	-	-	-	30	neryl isovalerate
	-	-	-	-	-	0.3	(E,E)-farnesyl acetate

Bait (mg)	30	3	30	30	30	30	geranyl butanoate
	30	30	30	3	30	30	geranyl octanoate
	-	-	-	-	-	30	neryl isovalerate
	-	-	-	-	-	0.3	(E,E)-farnesyl acetate

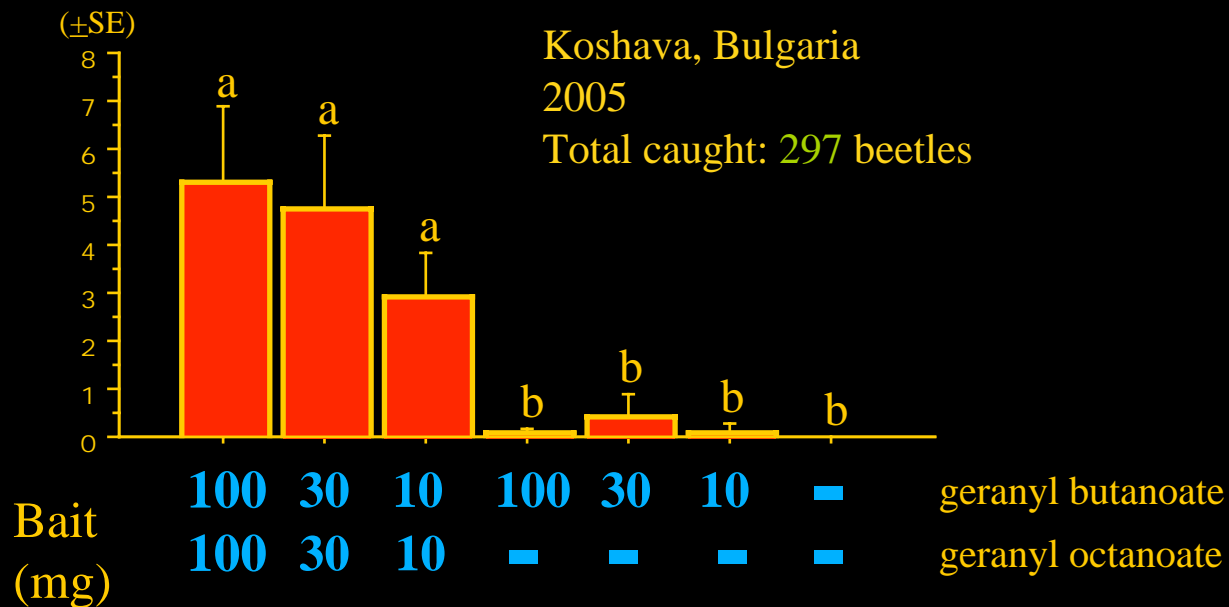
Agriotes lineatus



2) For best attraction in both species both geranyl butanoate and octanoate was necessary, in about equal amounts – no difference between the two species.

Dose test: confirmed the necessity of both components for *A. proximus*

Agriotes proximus



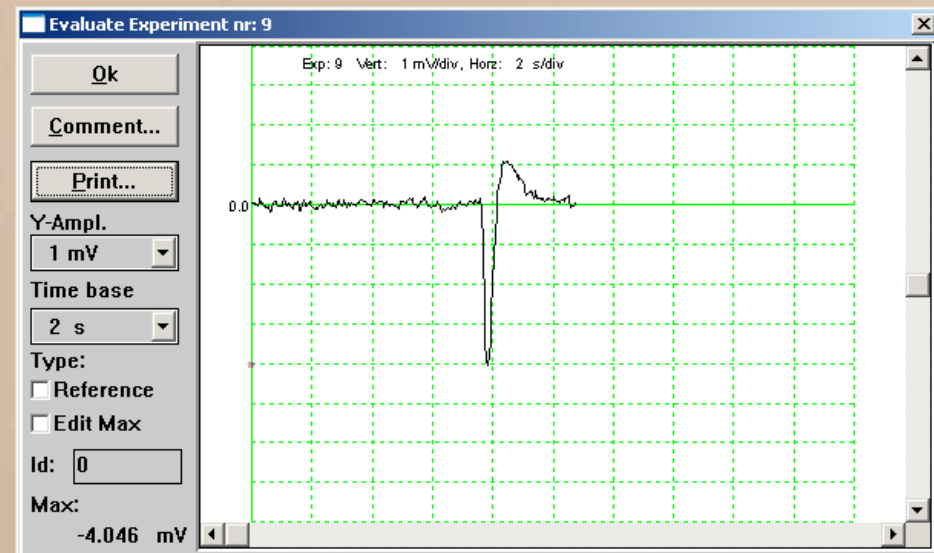
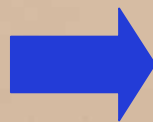
Antennal responses for both *A. proximus* and *A. lineatus* were recorded by an EAG apparatus

air flow with stimulus

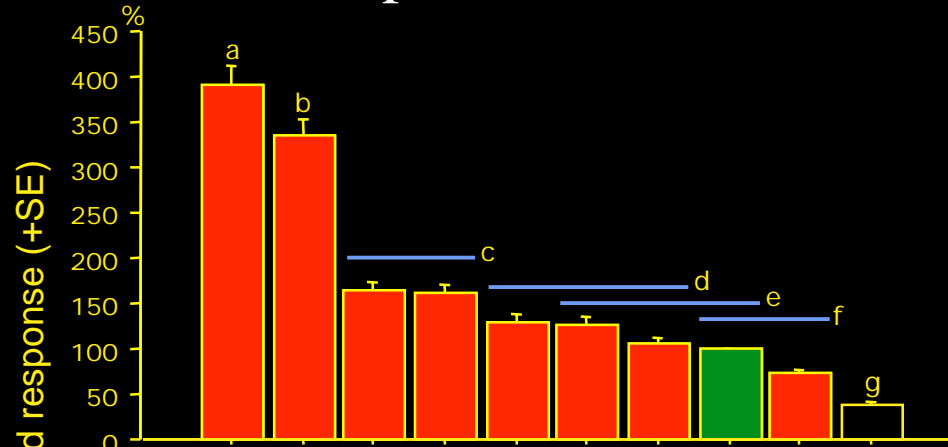
antenna of *A. proximus*

glass capillary electrodes

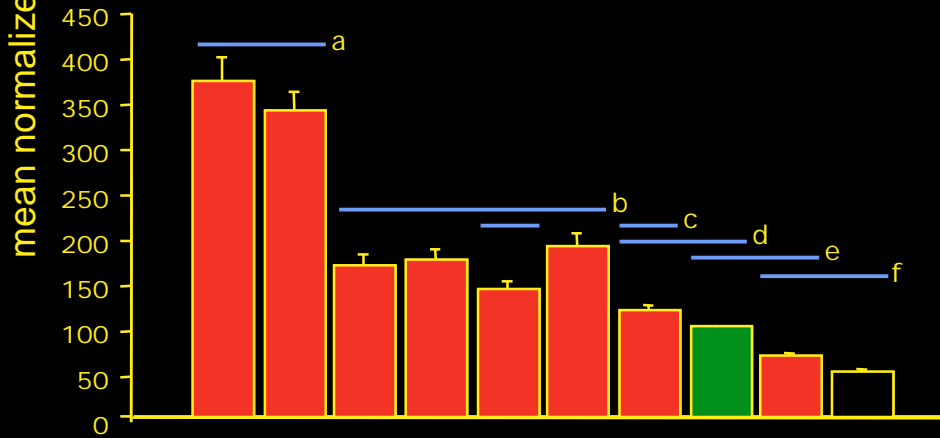
EAG-peak elicited by geranyl butanoate



EAG response spectra of male antennae to a number of synthetic click beetle pheromone components - no difference between the two species



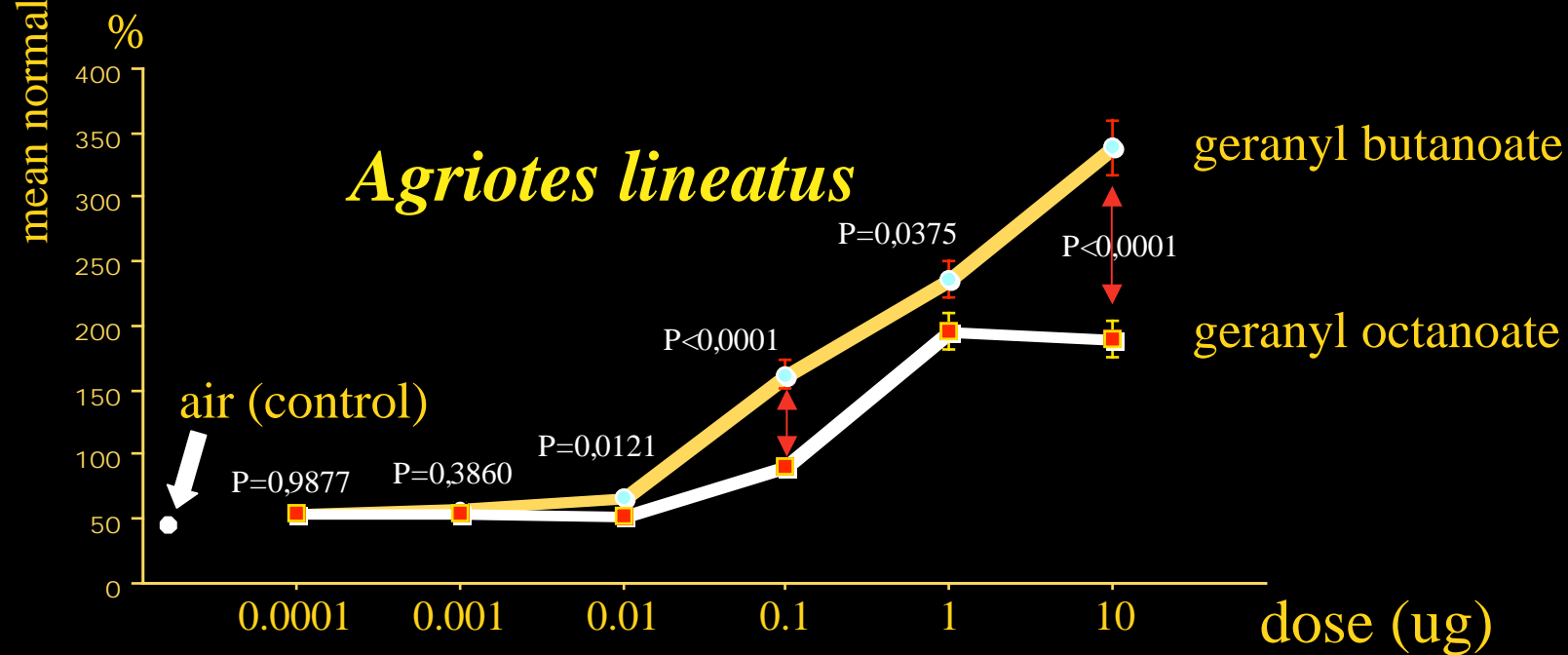
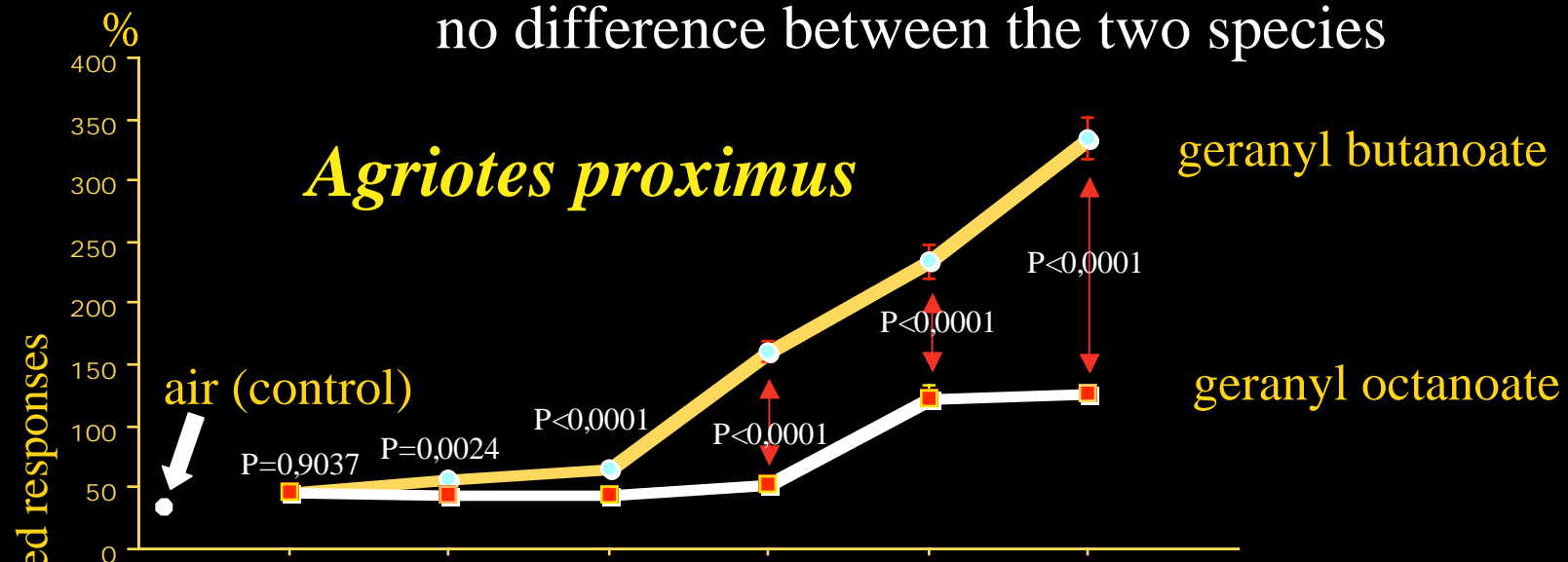
Agriotes proximus



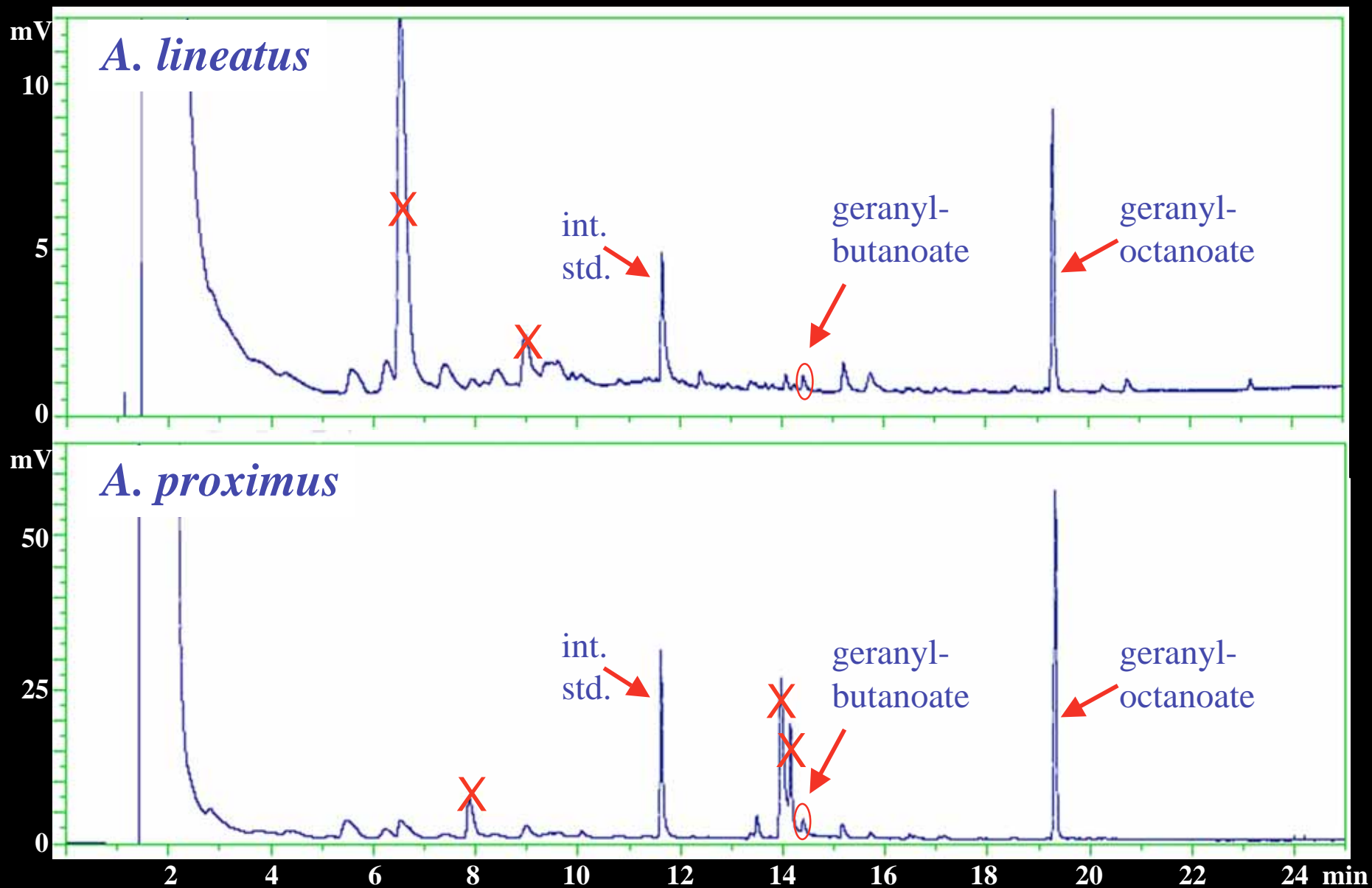
Agriotes lineatus

geranyl propionate
geranyl butanoate
geraniol
geranyl hexanoate
geranyl isovalerate
geranyl octanoate
(E,E)-farnesyl acetate
(E,E)-farnesyl butyrate
(E,E)-farnesol
air
stimulus

Antennae of both species responded better to geranyl butanoate – no difference between the two species

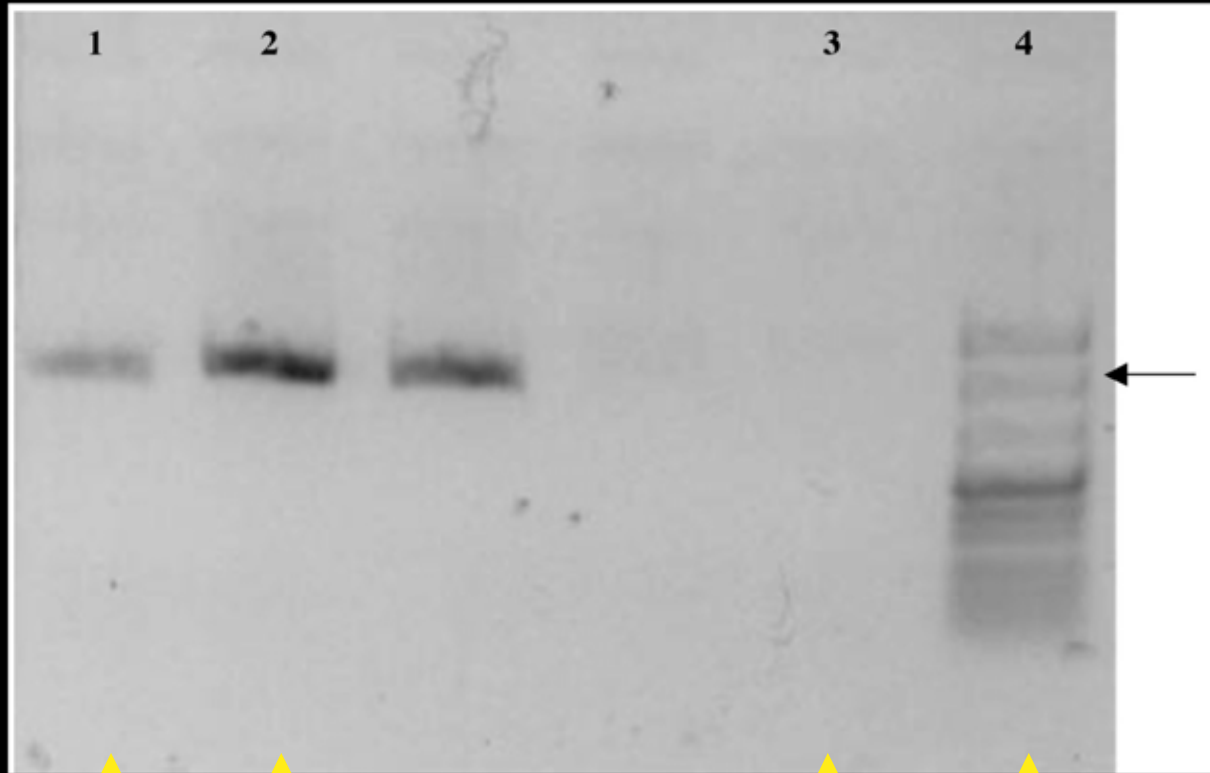


GC analysis of volatile collections from alive feral female beetles



Special thanks are due to Éva Bálintné Csonka (PPI HAS, Budapest) for performing the collections.

Preliminary molecular comparisons on feral specimens collected in Bulgaria (*A. proximus*) or Italy (*A. lineatus*) [Special thanks are due to Tamás Felföldi (ELTE, Budapest) for advice and directions in performing molecular studies]



Gel electrophoresis of PCR products of cytochrome oxidase gene

arrow shows 880 base pair-long DNA fraction

Agriotes
lineatus

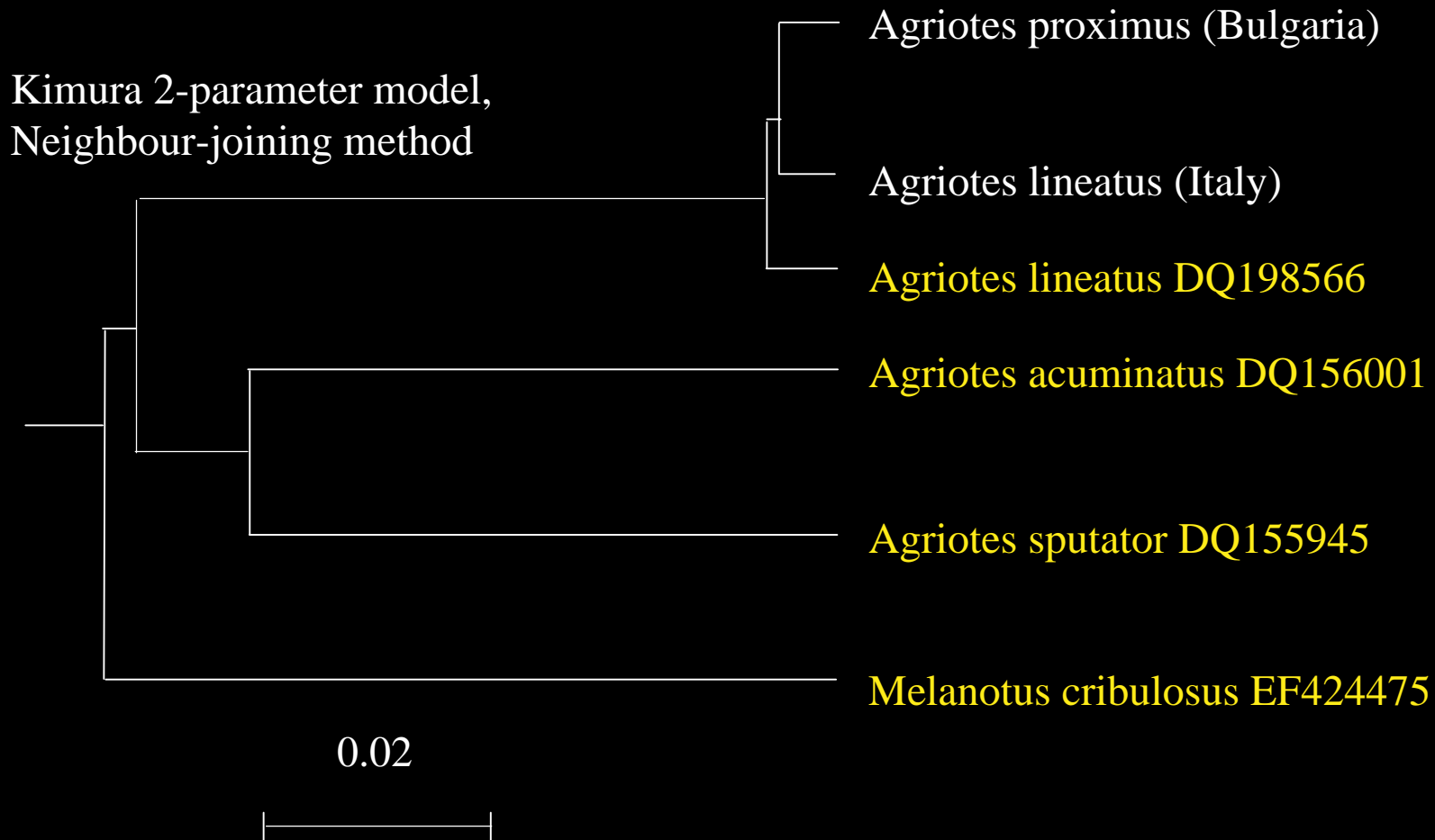
Agriotes
proximus

negative
control

marker

Preliminary molecular comparisons on feral specimens collected in Bulgaria (*A. proximus*) or Italy (*A. lineatus*) [Special thanks are due to Tamás Felföldi (ELTE, Budapest) for advice and directions in performing molecular studies]

Comparison of DNA sequences coding cytochrome oxidase: feral insects collected by us, or data from literature database



Preliminary molecular comparisons on feral specimens collected in Bulgaria (*A. proximus*) or Italy (*A. lineatus*) [Special thanks are due to Tamás Felföldi (ELTE, Budapest) for advice and directions in performing molecular studies]

Comparison of DNA sequences coding cytochrome oxidase: feral insects collected by us, or data from literature database

	A. proximus (Bulgaria)	A. lineatus (Italy)	A. lineatus DQ198566	A. acuminat. DQ156001	A. sputator DQ155945
A. proximus (Bulgaria)					
A. lineatus (Italy)	0.002				
A. lineatus DQ198566	0.005	0.003			
A. acuminatus DQ156001	0.154	0.154	0.154		
A. sputator DQ155945	0.122	0.122	0.122	0.150	
M. cribulosus EF424475	0.156	0.156	0.158	0.199	0.173

Figures give ratio of differing vs. similar base pairs

Summary

We found no significant difference between *A. lineatus* and *A. proximus* in:

- composition of female-produced pheromone (both gland extracts and volatile collections)
- electrophysiological responses of males to synthetic pheromone components
- optimal field composition of synthetic pheromone
- preliminarily in some molecular aspects

Therefore a taxonomic revision of the two taxa may become worthwhile.