Click beetles and pheromones – an overview

Miklós Tóth

Plant Protection Institute, HAS, Budapest, Hungary
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. However, the pheromone composition should be identified first!

On the picture: the YF trap design specifically developed for pheromone trapping of click beetles (Furlan Inform. Fitopat. 10:49. (2004))
The very first chemical structures elucidated from click beetles (female-produced pheromone) were organic acids.

- Valeric acid (pentanoic acid) for *Limonius californicus*.
  - Jacobson Science 159:208 (1968)

- Caproic acid (hexanoic acid) for *Limonius canus*.
  - Butler Environ. Entomol. 4:229 (1975)
Starting from the eighties, a number of geranyl and farnesyl esters were identified mainly by scientists from the Soviet Union.

**Example structures:**

- Geranyl butyrate
  - $((E)-3,7)$-dimethyl-2,6-octadienyl butyrate
  - i.e. *A. sputator*

- (E,E)-Farnesyl acetate
  - $((E)-3,7,11)$-trimethyl-2,6,10-dodecatrienyl acetate
  - i.e. *A. ustulatus*

Permutations and combinations of such compounds are present in the pheromones of several *Agriotes* spp.

Pheromone structures - geranyl/farnesyl esters

Structures for many spp. were reported, however, a great part of these compounds showed no behavioral activity.

<table>
<thead>
<tr>
<th></th>
<th>I = compound identified but no behavioral activity shown</th>
<th>P = compound identified, found attractive</th>
<th>A = synthetic compound attractive, but not known whether present in female</th>
</tr>
</thead>
</table>

Total: 24 spp.

Data from <www.pherobase.com> and my own files
One reason for this may be that most early identifications were based on direct gland extracts, which may not necessarily represent composition emitted into the air by the females.

(After Ivaschenko Zool. Zh. 59: 225 (1980).)
Gland extract vs. volatile collection (example No. 1)

In *A. sordidus*, in gland extracts large amounts of \((E,E)\)-farnesyl hexanoate were present, with only traces in volatile collections.

In gland extracts, the ratio of geranyl hexanoate to \((E,E)\)-farnesyl hexanoate was 1:1, whereas in volatile collections, the ratio was 1:<0.1.
Gland extract vs. volatile collection (example No. 1)

In field tests on *A. sordidus*, no influence of the addition of the farnesyl compound could be observed.

In field tests on *A. sordidus*, no influence of the addition of the farnesyl compound could be observed.
Consequently, by definition, only geranyl hexanoate can be termed a pheromone component in this species.
Gland extracts of *A. lineatus* were dominated by a single very large peak of geranyl octanoate, geranyl butyrate was hardly detectable (ratio of octanoate:butyrate = 100:<0.01)
In volatile collections the butyrate was well visible (ratio of octanoate:butyrate = 100:10)
In the field the presence of the butyrate was absolutely necessary for attraction.

Total caught in test: 279 beetles

<table>
<thead>
<tr>
<th>Geranyl octanoate</th>
<th>Geranyl butanoate</th>
<th>E,E-farnesyl acetate</th>
<th>Geraniol</th>
<th>Geranyl hexanoate</th>
<th>Neryl isovalerate</th>
<th>A. lineatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>273</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(Rümlang, May 23 - August 14, 1997)

It appears that volatile collections reflect better the true pheromone composition of a given species than gland extracts do.

<table>
<thead>
<tr>
<th></th>
<th>Geranyl octanoate</th>
<th>Geranyl butanoate</th>
<th>E,E-farnesyl acetate</th>
<th>Geraniol</th>
<th>Geranyl hexanoate</th>
<th>Neryl isovalerate</th>
<th>A. lineatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rümlang, May 23 - August 14, 1997</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>273</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Total caught in test: 279 beetles

Another reason for inactivity of identified structures may be that in some cases geometrical purity of synthetic compounds is crucial - as commonly found also in moth pheromones.

It is well known that *Agriotes ustulatus* responds only to synthetic \((E,E)\)-farnesyl acetate batches which have >95% \((E,E)\) geometric purity.

Presence of higher percentages of the other isomers inhibits catches.

(After Tóth et al., unpublished)
A recent report described the first neryl ester (nerol is the geometrical isomer of geraniol) as a pheromone component in *Agriotes acuminatus* also suggesting the importance of geometrical isomerism in click beetles.

“Good” isomer

![Neryl butyrate](image)

**neryl butyrate**

[(Z)-3,7)-dimethyl-2,6-octadienyl butyrate]

“Wrong” isomer

![Geranyl butyrate](image)

**geranyl butyrate**

[(E)-3,7)-dimethyl-2,6-octadienyl butyrate]

The full pheromone composition in *A. acuminatus* contained also another ester with an unusual structure, which was necessary for field activity.

**“Good” isomer**

neryl butyrate

[(Z)-3,7]-dimethyl-2,6-octadienyl butyrate]

**second component**

(Z,E)-2,6-dimethyl-2,6-octadien-1,8-diyl dihexanoate]

Other structures

Some *Melanotus* spp. from the far east appear to use dodecenyl compounds (structurally similar to moth pheromones)

![Chemical structures](image)

- dodecyl acetate
  - *Melanotus okinawensis*

- (E)-9,11-dodecadienyl acetate
  - *Melanotus sakishimensis, M. tamsuyensis*
Other structures

As new structure, a methyloctanol ester has been identified from the non-pest *Ectinus aterrimus* click beetle.

7-methyloctyl 9-methyldecanoate

*Ectinus aterrimus*

Tolasch Chemoecology 18:177 (2008)
Other structures

Four similar esters were recently shown out from *E. ferrugineus*, an endangered click beetle.

7-methyloctyl 5-methylhexanoate

7-methyloctyl octanoate

7-methyloctyl 7-methyloctanoate

7-methyloctyl (Z)-4-decenoate

Kairomonal effect: 
*E. ferrugineus* also is attracted to the pheromone of its prey, *Osmoderma eremita* (Scarabaeidae).

(R)-gamma-decalactone

Pheromone information in click beetle genera

At present we have some information on chemical structures in 34 spp. Information on the genus *Agriotetes* predominates.

Data from <www.pherobase.com> and my own files
Catches of male and female *A. sordidus* in traps baited with different doses of the pheromone (Italy, Veneto, May 1 - 26, 2001, Furlan et al., unpubl.)
Females responding to the pheromone

However, L. Furlan observed that in traps of *A. sordidus* baited with synthetic pheromone, sizeable catches of females (besides large catches of males) were recorded.

Catches of male and female *A. sordidus* in traps baited with different doses of the pheromone (Italy, Veneto, May 1 - 26, 2001, Furlan et al., unpubl.)
Females responding to the pheromone

Female catches also showed a dose response, suggesting that the pheromone showed true attraction in female *A. sordidus*.

Catches of male and female *A. sordidus* in traps baited with different doses of the pheromone (Italy, Veneto, May 1 - 26, 2001, Furlan et al., unpubl.)
Females responding to the pheromone

Attraction of females to the respective synthetic pheromone has been confirmed in several species, so the case of *A. sordidus* is not unique.

**A. brevis**
pheromone components: 
\((E,E)\)-farnesyl butyrate + geranyl butyrate
Eraclea, Italy, 2000
Total caught: 194 beetles

**A. ustulatus**
pheromone component: 
\((E,E)\)-farnesyl acetate
Eraclea, Italy, 2000
Total caught: 20 beetles

**A. sordidus**
pheromone component: 
geranyl hexanoate
Berton-Greggio, Italy, 2001
Total caught: 42 beetles

Attraction of females to the respective synthetic pheromone has been confirmed in several species, so the case of *A. sordidus* is not unique.
What is more, female antennae responded well to the pheromone and gave similar response spectra in EAG to that of male antennae.

Agriotes sordidus

(after Vuts J. et al., unpublished)
Females responding to the pheromone

This again was true to a number of species, and strongly suggested that females are capable to perceive their respective pheromone components. (after Vuts J. et al., unpublished)
This brings up the possibility that the pheromones of click beetles are wrongly classified into the “classical” sex pheromone category.

Females responding to the pheromone

Agriotes brevis

(mean normalized responses (+SE))

(after Vuts J. et al., unpublished)
Sex pheromone vs. aggregation pheromone?

In *A. ustulatus*, a floral lure has been discovered, which attracts females. (White colour as attractive visual cue showed negligible influence)

Debrecen, 2008

Eraclea, 2008

(After Tóth M. et al., in preparation)
Sex pheromone vs. aggregation pheromone?

The addition of the pheromone to the floral lure in the same trap increases catches of females!

Debrecen, 2008

Eraclea, 2008

(totals caught: 4814 beetles)

(totals caught: 2532 beetles)

(After Tóth M. et al., in preparation)
Sex pheromone vs. aggregation pheromone?

Most female specimens are caught in traps with both floral AND pheromonal baits

Debrecen, 2008
(total caught: 34325 beetles)

Eraclea, 2008
(total caught: 6123 beetles)

(After Tóth M. et al., in preparation)
Sex pheromone vs. aggregation pheromone?

A similar phenomenon (that the presence of the pheromone increases the effect of floral bait on females) is unusual with sex pheromones.

Debrecen, 2008
(total caught: 34325 beetles)

Eraclea, 2008
(total caught: 6123 beetles)

(After Tóth M. et al., in preparation)
Sex pheromone vs. aggregation pheromone?

However, it is frequently reported with aggregation pheromones.

Debrecen, 2008  
(total caught: 6123 beetles)

Eraclea, 2008  
(total caught: 34325 beetles)

(After Tóth M. et al., in preparation)
This again suggests that the pheromone of *Agriotes* click beetles is not a "classical" sex pheromone.

(After Tóth M. et al., in preparation)
Acknowledgements

Scientists cooperating in unpublished results shown:

Bálintné Csonka, É. (Hungary)
Francke, W. (Germany)
Furlan, L. (Italy)
Subchev, M. (Bulgaria)
Szarukán, I. (Hungary)
Tolasch, T. (Germany)
Toshova, T. (Bulgaria)
Vuts, J. (Hungary)
Yatsynin, V.G. (Russia)