Final report

Stimuli used in communication of beetles of hidden development – pests of forestry and agriculture

1. Results related to longhorn beetles (Coleoptera: Cerambycidae)

The chemical ecology of cerambycid beetles: what we know and what we would like to know (Imrei and Tóth 2012).

According to FAO data (http://www.fao.org/forestry) the extent of forests and other wooded land covers 45 % of Europe, which data is about 23 % for Hungary. The health status of woods are influenced by abiotic and biotic factors one of which are the xylophagous insects including longhorn beetles pests. Cerambycid beetles make damage to forests and to wood industry as they generally oviposit and colonize healthy, moribund or recently killed or decomposing woody plant material. There is a growing importance in other fields like in the case of cerambycid pests of nurseries and pests of the home and garden sector.

There are hardly if any measures available against longhorn beetle pests and the monitoring tools are generic and less effective. The application in practice of future, more extended knowledge on the chemical ecology of longhorn beetles could contribute to plant protection with effective detection and monitoring tools and in optimal, special cases with environmentaly sound, selective control methods.

An attractant for *Molorchus umbellatarum* Schreb. was found in the field screening studies with known pheromone components of longhorned beetles (Coleoptera: Cerambycidae) in Hungary (Imrei et al. 2013).

Five compounds known to be pheromone components of longhorned beetles (Coleoptera: Cerambycidae) in the subfamily Cerambycinae were field tested as attractants and possible pheromones for the cerambycid fauna of Hungary.

Nine cerambycid species were caught in the baited traps. Large numbers of both sexes of the cerambycine species *M. umbellatarum* Schreb. were caught in traps baited with (2R*,3S*)-octanediol, while the diastereomeric (2R*,3R*)-octanediol was to some extent attractive as well. This is the first report of an aggregation attractant and likely pheromone for a species in the cerambycine tribe Molorchini.

The results of our study support the hypothesis that the diol/hydroxyketone pheromone motif is characteristic of and highly conserved within the subfamily Cerambycinae. Intraspecific chemical

communication is summarized for the subfamily Cerambycinae, and possible links between taxonomy, insect behavior, and pheromone structures are described.

The pheromone compounds of *Plagionotus detritus* L. has been identified also finding it an attractive kairomone for a clerid predator in a beetle complex of a Hungarian oak forest (Coleoptera: Cerambycidae and Cleridae) (Imrei et al. 2014b).

Several cerambycid species of the tribe Clytini including *P. detritus* share habitat and overlap in flight period in the oak forests of the Mátra mountains of Hungary, where they gather on the sunlit parts of log piles. A predatory beetle species, *Clerus mutillarius* F. (Coleoptera: Cleridae) occurs on the same logs in large numbers, preying on bark beetles and longhorn beetles.

We identified 3-hydroxy-2-hexanone and (S)-2-hydroxy-3-octanone from air entrainment samples of male *P. detritus* beetles. The combination of the synthetic compounds attracted significantly more *P. detritus* than either of the two compounds alone or unbaited funnel traps. The blend of the compounds was significantly attractive to both males and females, suggesting that 3-hydroxy-2-hexanone and (S)-2-hydroxy-3-octanone are aggregation pheromone components of *P. detritus*.

Male and female specimens of the predatory *C. mutillarius* also were significantly attracted to the two components of the aggregation pheromone of *P. detritus*, indicating that C. mutillarius may use the aggregation pheromone of *P. detritus* as a kairomone to find prey items.

Development of a trap combining visual and chemical cues for the alfalfa longhorn beetle, *Plagionotus floralis* Pallas (Coleoptera, Cerambycidae, Clytini) (Imrei et al. 2014a).

The development of a trap comprised of both chemically and visually attractive stimuli was described for the alfalfa longhorn beetle, *P. floralis*, a pest causing increasingly serious damage each year in alfalfa fields in Central and Eastern Europe.

Fluorescent yellow funnel traps caught significantly more *P. floralis* beetles than traps with other colours, in some tests non-fluorescent yellow traps also attracted more beetles than non-yellow traps. Fluorescent yellow reflects at a high intensity at wavelengths of 500 to 550 nm, which may account for the far better response of *P. floralis*. This response to fluorescent yellow parallels those reported in the literature for the European cherry fruit fly (Rhagoletis cerasi L., Diptera, Tephritidae), the vine thrips (Drepanothrips reuteri Uzel, Thysanoptera, Thripidae) and two Oxythyrea scarabs (Coleoptera, Scarabaeidae).

A ternary synthetic chemical lure of (E)-anethol, 1-phenylethyl alcohol, and 3-methyl-eugenol generally increased the catches of the fluorescent yellow traps. Presented alone, 1-phenylethyl

alcohol or 3-methyl eugenol in fluorescent yellow traps caught significantly more P. floralis beetles than fluorescent yellow traps with no odor bait.

The establishment of a damage threshold for fluorescent yellow traps with the floral attractant to sample *P. floralis* would assist in decision making regarding the optimal application of agrotechnical measures. This protocol would improve plant protection practice with respect to both an economic and an environmental concern.

2. Results related to Scarabaeoidea with focus on chafer beetles (Coleoptera, Scarabaeidae)

Semiochemistry of the Scarabaeoidea (Vuts et al. 2014).

The superfamily Scarabaeoidea comprises a large and diverse monophyletic group. Members share ancestral characteristics, but often exhibit considerable differences in their ecology, physiology, or mating strategies. A large number of species are regarded as pests of crop or amenity plants, while others are beneficial to humans and even may be extremely rare as a result of anthropogenic activities. A significant number of chemical ecology-based studies have been conducted with the Scarabaeoidea in order to characterize semiochemicals influencing their behavior, such as pheromones and plant-derived allelochemicals. These may be used either to control or preserve populations of the beetles, depending upon pest or beneficial status. We have reviewed the role and identity of the semiochemicals of the Scarabaeoidea, with comments on possible future research and applied opportunities in the field of chemical ecology.

Evidence of a female produced pheromone in a cetoniin chafer, *Epicometis hirta* Poda (Coleoptera: Scarabaeidae: Cetoniinae) (Imrei et al. 2012).

Adults of the blossom chafer, *E. hirta*, are economically important horticultural pests in Central and Southern Europe, damaging the generative parts of plants. Although a funnel trap exploiting visual (light blue colour) and olfactory (synthetic floral blend) cues is available for detection and monitoring, application of the sex pheromone of *E. hirta* would presumably enhance trap efficacy or may open the possibility for new monitoring or control measures. Here, we report on the first successful trapping experiments with live *E. hirta*, which may lead to the identification of a pheromone.

Preliminary field observations on the behaviour of day-flying *E. hirta* suggested that males are attracted to females sitting on flowers, predominantly dandelions (*Taraxacum officinale* Weber,

Compositae). In order to quantify male attraction to females, *E. hirta* were field-collected in the early part of the flight period in large numbers using colour traps baited with floral volatiles.

Males were attracted to traps baited with females + apple, but not to the ones baited with males + apple, or apple alone. Females were not attracted to either sex or to apple pieces alone. However, simultaneously operated colour traps, baited with floral volatiles, caught significant numbers of females, indicating that they were present in the area. Based on the present results, the existence of a female produced sex pheromone is highly probable for *E. hirta*.

3. Results related to jewel beetles (Coleoptera, Buprestidae)

Trapping of European buprestid beetles in oak forests using visual and olfactory cues (Domingue et al. 2013).

Trapping approaches developed for the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), were adapted for trapping several European oak buprestid species. These approaches included the use of natural leaf surfaces as well as green and purple plastic in sticky trap designs. Plastic surfaces were incorporated into novel 'branch-trap' designs that each presented two 5 × 9 cm^2 rectangular surfaces on a cardboard structure wrapped around the leaves of a branch. We used visual adult *Agrilus* decoys in an attempt to evoke male mating approaches toward the traps. Our first experiment compared the attractiveness of visual characteristics of the surfaces of branchtraps. The second looked at the effect on trap captures of adding semiochemical lures, including manuka oil, (Z)-3-hexen-1-ol, and (Z)-9-tricosene. In total, 1 962 buprestid specimens including 14 species from the genus Agrilus were caught on 178 traps in a 22-day time-span. Overall, the green plastic-covered branch-traps significantly out-performed the other trap designs. We further found that the presence of an EAB visual decoy placed on the trap surface often increased captures on these green traps, but this effect was stronger for certain *Agrilus* species than for others. The visual decoy was particularly important for themost serious pest detected, *Agrilus biguttatus* Fabricius, which was captured 13 times on traps with decoys, but only once without a decoy. There were some small but significant effects of odor treatment on the capture of buprestids of two common species, *Agrilus angustulus* Illiger and *Agrilus sulcicollis* Lacordaire. There were also 141 Elateridae specimens on these traps, which were not influenced by trap type or decoys. The results suggest that small branch-traps of this nature can provide a useful new tool for monitoring of buprestids, which have the potential to be further optimized with respect to visual and olfactory cues.

Bioreplicated visual features of nanofabricated buprestid beetle decoys evoke stereotypical male mating flights (Domingue et al. 2014).

Recent advances in nanoscale bioreplication processes present the potential for novel basic and applied research into organismal behavioral processes. Insect behavior potentially could be affected by physical features existing at the nanoscale level. We used nanobioreplicated visual decoys of female emerald ash borer beetles (Agrilus planipennis) to evoke stereotypical mate-finding behavior, whereby males fly to and alight on the decoys as they would on real females. Using an industrially scalable nanomolding process, we replicated and evaluated the importance of two features of the outer cuticular surface of the beetle's wings: structural interference coloration of the elytra by multilayering of the epicuticle and fine-scale surface features consisting of spicules and spines that scatter light into intense strands. Two types of decoys that lacked one or both of these elements were fabricated, one type nano-bioreplicated and the other 3D-printed with no bioreplicated surface nanostructural elements. Both types were colored with green paint. The light-scattering properties of the nano-bioreplicated surfaces were verified by shining a white laser on the decoys in a dark room and projecting the scattering pattern onto a white surface. Regardless of the coloration mechanism, the nano-bioreplicated decoys evoked the complete attraction and landing sequence of *Agrilus* males. In contrast, males made brief flying approaches toward the decoys without nanostructured features, but diverted away before alighting on them. The nano-bioreplicated decoys were also electroconductive, a feature used on traps such that beetles alighting onto them were stunned, killed, and collected.

Differences in spectral selectivity between stages of visually-guided mating approaches in a buprestid beetle (Domingue et al. 2016).

Spectral mating preferences were examined in male *A. angustulus* (Buprestidae: Coleoptera), a member of a taxon known for its high species diversity and striking metallic coloration. The spectral emission profile of a typical A. angustulus female displays low chroma, broadly overlapping that of the green oak leaves they feed and rest upon, while also including longer wavelengths. To pinpoint behaviorally significant spectral regions for *A. angustulus* males during mate selection, we observed their field approaches to females of five A. planipennis color morphs that have greater chroma than the normal conspecific female targets. *A. angustulus* males would initially fly equally frequently toward any of the three longest wavelength morphs (green, copper and red) whose spectral emission profiles all overlap that of typical *A. angustulus* females. However, they usually only completed approaches toward the two longest wavelength morphs, but not the green morphs. Thus, spectral preference influenced mate selection by *A. angustulus* males, and their discrimination of suitable targets became greater as these targets were approached. This increasing spectral discrimination when approaching targets may have evolved to allow female emissions to remain somewhat cryptic, while also being visible to conspecifics as distinct from the background vegetation and heterospecific competitors.

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