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PHEROMONE STUDIES AT THE PLANT PROTECTION INSTITUTE, BUDAPEST, DURING THE LAST QUARTER OF THE PAST CENTURY

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It goes without saying that the international boom in pheromone research which began in the late nineteen-sixties and early seventies would excite the interest of such a keen ethologist as TIBOR JERMY. Consequently, behavioural observations and trapping with female-baited traps were soon initiated on several pest Lepidoptera at both the Budapest and Keszthely laboratories of the Institute. Monitoring trials with already known synthetic pheromones (i.e., the codling moth *Cydia pomonella* L.; Lepidoptera, Tortricidae) were conducted in the early seventies in connection with a large sterile-male project supported in part by the International Atomic Energy Agency.

Having joined the Institute in 1974, the research topic of pheromones was suggested to me as a line of study in 1975 by TIBOR JERMY. During the first years, I was lucky to be able to conduct the first experiments under his personal supervision – in some cases in his own garden. TIBOR JERMY's acute observational prowess and the thorough organization of experimental design, omitting no detail however unimportant it might seem to be, impressed me deeply and had a highly determining impact on my later scientific career as well, for which influence I cannot be too grateful.

Observations on the natural behaviour of the target insect is of crucial importance in pheromone studies. An outstanding example of how direct observation of behavioural events can lead to novel understanding of pheromonal regulation of mating, even after several decades of intensive studies, is readily shown by the work on the Colorado potato beetle (*Leptinotarsa decemlineata* SAY; Coleoptera, Chrysomelidae) by JERMY and BUTT (1991).

Consequently the main objective of the studies at our laboratory during those early years was the description of behavioural processes connected with pheromone production and response in several lepidopteran (mainly noctuid) pests, in order to gain evidence for the presence and possible role of pheromones in the courtship process. The first publishable results were obtained with the cabbage armyworm, *Mamestra brassicae* L. (Lepidoptera, Noctuidae), where the presence of both a long-range female-produced pheromone, and a male-produced hairpencil pheromone could be demonstrated (SZENTESI *et al.* 1975).

In addition, considerable efforts were dedicated to the study of role of hair pencils in the courtship of the oriental fruit moth, *Grapholita molesta* BUSCK. (Lepidoptera, Tortricidae) (ISTVÁNOVICS & TÓTH unpubl. data), and quite extensive tests were run using the known pheromone inhibitor dodecyl acetate (ROTHSCHILD & MINKS 1977). In fact, successful suppression of mating and also of damage levels was achieved in preliminary air-permeation trials with this compound; however, the industrial sponsor (EGYT Pharmaceutical Co., Budapest, Hungary) never allowed these studies to be published.

In the following years, pheromone extracts were prepared from several moth species, and simple laboratory bioassays were developed for monitoring the activity of these extracts (TÓTH 1979, SZŐCS & TÓTH 1979). The extraction and bioassay of the sex pheromone of the winter moth (*Operophtera brumata* L.; Lepidoptera, Geometridae) is of special interest in this respect because it was among the first pheromone papers on this family (SZŐCS & TÓTH 1978). Interest in geometrids was enhanced by a contribution of my friend GÁBOR SZŐCS when he joined the laboratory in the late seventies. He had specialized in this group of Lepidoptera in his student years as moth collector. As we shall see, the study of geometrid sex pheromones became a significant research area in our laboratory.

Further progress towards the chemical identification of the pheromones which had been extracted was hampered by the unavailability of sensitive equipment and special expertise inside Hungary. A breakthrough became possible when international contacts with laboratories specializing in the structure elucidation of hitherto unknown pheromones was established. In this respect, the most significant partners and professors were G. H. L. ROTHSCHILD from CSIRO, Canberra, H. ARN from the Wädenswil Federal Station, W. FRANCKE from Hamburg University and C. LÖFSTEDT from Lund University, to whom the author is greatly indebted for the invaluable training and assistance they provided. Consequently, a row of joint pheromone identifications were completed in the early eighties on important pest noctuids (TÓTH *et al.* 1983, 1986) and tortricids (GUERIN *et al.* 1986*a, b*). Of special taxonomic and chemical interest is the identification of the ketone pheromone component from the grapevine pest geometrid *Peribatodes rhomboidaria* SCHIFF. (Lepidoptera, Geometridae) (BUSER *et al.* 1985, TÓTH *et al*. 1987). Through further structure elucidation of several other geometrid pheromones (HANSSON *et al.* 1990*a*, TÓTH *et al.* 1991, TÓTH *et al.* 1992*a*), it became clear that this group of insects uses a polyene-derived set of compounds (mostly epoxides) crucially different from the usual mono- or dienic acetates and alcohols of well-known tortricid or noctuid pheromonal structures. The importance of the chiral composition of these epoxides in maintaining species-selective pheromonal communication channels was amply demonstrated in geometrid species sharing the same habitat and general flight season (SZŐCS *et al.* 1993, TÓTH *et al.* 1994*b*, LANDOLT *et al.* 1996).

Epoxide structures seem to prevail also in the family Arctiidae. In the fall webworm (*Hyphantria cunea* DRURY; Lepidoptera, Arctiidae), the presence of linoleic and linolenic aldehydes, together with a dienic C21 epoxide, *was shown out?* from pheromone extracts by North American and French scientists. However, no field activity was observed by any combination of the above three compounds (HILL *et al.* 1982, EINHORN *et al.* 1982). Cooperating with Swiss, Japanese, Russian and Spanish scientists (TÓTH *et al.* 1989*b*), we identified two further trienic epoxide components from the pheromone of the fall webworm, and the first baits active in field trapping tests were formulated with the novel trienic epoxide (3*Z*,6*Z*)-1,3,6-(9S,10R)-9,10-epoxy-heneicosatriene. Later studies revealed that a ternary mixture of linolenic aldehyde, (3*Z*,6*Z*)-3,6-(9S,10R)-9,10-epoxyheneicosadiene and (3*Z*,6*Z*)-1,3,6-(9S,10R)-9,10-epoxyheneicosatriene is needed for optimal field activity (BINDA *et al.* 1990).

A third structural type of moth pheromone is presented by branched hydrocarbons. Dimethylalkanes were identified for the first time by us in the mountain-ash bentwing *Leucoptera scitella* L. and the closely related coffee leafminer *Perileucoptera coffeella* GUÉR. – MÉNEV. (Lepidoptera, Leucopteridae) (FRANCKE *et al.* 1987, 1988). Later studies revealed that only one of the four possible enantiomers was responsible for biological activity in *L. scitella*, although the presence of other enantiomers did not interfere with activity (TÓTH *et al.* 1989*a*).

A secondary alcohol, a novel structural type for Lepidoptera, was discovered by us when studying the pheromone composition of *Stigmella malella* STAINTON (Lepidoptera, Nepticulidae) (TÓTH *et al.* 1995). The pheromone of this species consists of (6*E*)- and (6*Z*)-6,8-nonadien-2-ol and the only biologically active form of the molecule is the pure (S) enantiomer. Similar, but monounsaturated secondary alcohols and ketone derivatives, have since been identified exclusively from some ancient monotrysian Lepidoptera (ZHU *et al.* 1995, KOZLOV *et al.* 1996) and from Trichoptera (LÖFSTEDT *et al.* 1994, BJOSTAD *et al.* 1996, JEWETT *et al.* 1996, LARSSON & HANSSON 1998), suggesting that the seemingly ancient pheromonal pattern of secondary alcohols may reflect the presence of an evolutionary link between the orders Trichoptera and Lepidoptera.

In some cases, in the course of our pheromone identification projects, geographical differences in pheromone composition have been discovered within the target species. In the currant borer, *Synanthedon tipuliformis* CLERCK (Lepidoptera, Sesiidae), for example, the main pheromone component is (2*E*,13*Z*)-2,13-octadecadienyl acetate (VOERMAN *et al.* 1984, SZŐCS *et al.* 1985). The minor component (3*E*,13*Z*)-3,13-octadecadienyl acetate significantly synergizes biological activity when added at 3–5% (SZŐCS *et al.* 1990). This striking synergism was observed in currant borer populations in several countries in Europe (SZŐCS *et al.* 1991), New Zealand (SZŐCS *et al.* 1990) and Canada (SZŐCS *et al.* 1998). However, in tests conducted in Tasmania, no biological activity of the 3,13 dienic acetate was observed (SZŐCS *et al.* 1990).

Three components, (5*Z*)-5-decenyl acetate, (7*Z*)-7-dodecenyl acetate and (9Z)-9-tetradecenyl acetate, have been identified from the pheromone of the turnip moth (*Agrotis segetum* SCHIFF.; Lepidoptera, Noctuidae) by several authors (BESTMANN *et al.* 1978, ARN *et al.* 1980, TÓTH *et al.* 1980, LÖFSTEDT *et al.* 1982). Usually the presence of all three components is needed for maximal biological activity in Europe, although it appeared that the relative importance of the components may shift according to geographical region (ARN *et al*. 1983, LÖFSTEDT *et al.* 1986) – the decenyl compound being more important towards the west and the tetradecenyl compound towards the east (HANSSON *et al.* 1990*b*). In a field trapping study of several Eurasian and African sites, we found that the ternary mixture was working well at all sites in Eurasia and North Africa, while at the two sites south of the Equator in Africa, only the decenyl compound showed some field activity and the addition of the two others did not influence attractive activity (TÓTH *et al.* 1992*b*). This suggested the evolution of pheromonally distinct strains of the turnip moth in Africa south of the Equator. This idea was supported in a direct comparative study of Swedish and Zimbabwean turnip moth populations by the Lund (Sweden) pheromone group (WU *et al.* 1999).

The third example of geographical differences in pheromone composition came from a phycitid, the lima-bean pod borer *Etiella zinckenella* TR. (Lepidoptera, Phycitidae). From Hungarian and Egyptian populations, several tetradecenyl pheromone components have been identified (TÓTH *et al.* 1989*c*), among which the mixture of (*Z*)-9-tetradecenyl and (*Z*)-11-tetradecenyl acetates showed maximal biological activity (TÓTH *et al.* 1996*a*). This blend attracted male lima-bean pod borers in trapping tests at sites in several European countries, Egypt, Northern India, but no activity was observed in Taiwan, Japan, Australia or North America, suggesting a crucially different pheromone composition for pod borer populations in these latter regions (TÓTH *et al.* 1996*a, b*).

Apart from the scientific importance, such comparative studies of populations of widespread pests in several geographical regions can be very significant from the practical point of view. Naturally, the best results for agricultural monitoring or forecasting can be expected when the optimal pheromone composition for the given region is used.

More recently, the scientific interest in our laboratory shifted somewhat from Lepidoptera to the Coleoptera, which offer new and exciting challenges for the pheromone scientist. In the early nineties, we started to study the pheromones of click beetles (Coleoptera, Elateridae), discovering new pheromone components for several central and western European click beetle pest species (FURLAN *et al.* 1996, TÓTH & FURLAN unpubl. data), and optimising the activity of known pheromone components in the region for species where some information is already known about the pheromone composition (TÓTH *et al.* 1999).

Also in the Coleoptera, we have discovered attractants for several scarab beetle pests (i.e., *Epicometis hirta* PODA, *Anomala* spp., etc.) (TÓTH *et al.* unpubl. data) in the second half of the nineteen-nineties. Most recently, promising results were obtained in a preliminary mass-trapping test on *Anomala vitis* FABR. and *A. dubia* SCOP. (Coleoptera, Scarabaeidae, Melolonthinae) in peach and sour cherry orchards (VOIGT & TÓTH 2002) using the highly potent sex attractant discovered previously (TÓTH *et al.* 1994*a*).

Having discussed the main lines and results of pheromone studies of the past quarter of a century in our Institute, it just remains for me to offer these results as a tribute to the initiator of these studies, to TIBOR JERMY, as a humble contribution from this laboratory on his 85th birthday.

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