Entomol. Gener.	15 (2): 121–125	Stuttgart 1990-06	ISSN: 0171-8177

EGT-Nr 672 VI

Courtship, Pheromone Titre and Determination of the Male Mating Success in the Oriental Fruit Moth, Grapholita molesta (Lepidoptera: Tortricidae)*

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Received: 1990-02-15/1990-03-20 Accepted: 1990-03-24

Löfstedt, C., Vickers, N. J., & Baker, T. C. [Dep. Ecol., Univ., S-223 62 Lund; Sweden]: **Courtship, Pheromone Titre and Determination of the Male Mating Success in the Oriental Fruit Moth, Grapholita molesta (Lepidoptera: Tortricidae).** — Entomol. Gener. 15 (2): 121–125; Stuttgart 1990. — [Article]

Sequestration, into the hairpencils, of the behaviourally most active courtship pheromone component, ethyl *trans*-cinnamate, in male *Grapholita molesta* (Busck 1916) results in an increased mating success over control ♂♂. ♀♀ attracted to ethyl cinnamate-treated ♂♂ moved significantly faster than ♀♀ attracted to control ♂♂. Since control ♂♂ that were successful at coupling on average took more attempts to do so than treated ♂♂, their successful attempt tended to be later and closer than for EC ♂♂.

Key words: Hairpencil — pheromone — *Grapholita molesta* — Lepidoptera: Tortricidae — Oriental fruit moth — ethyl *trans*-cinnamate — sequestration — sexual selection — mate choice.

Löfstedt, C., Vickers, N. J., & Baker, T. C. [Abt. Ökol., Zool. Inst., Univ.; S-22362 Lund]: **Werbeverhalten, Pheromon-Titer und Bestimmung des männlichen Kopulationserfolges bei der Orientalischen Fruchtmotte, Grapholita molesta (Lepidoptera: Tortricidae).** — Entomol. Gener. 15 (2): 121–125; Stuttgart 1990. — [Abhandlung]

Künstliche Einführung von Äthyl-Transcinnamat (EC), dem aktivsten Bestandteil des für Werbeverhalten verantwortlichen männlichen Sexualpheromons, in die Haarpinsel der ♂♂ von *Grapholita molesta* (Busck 1916) bewirkt einen erhöhten Paarungserfolg im Vergleich mit unbehandelten Kontroll-♂♂. ♀♀, die durch EC-behandelte ♂♂ angelockt werden, bewegen sich signifikant schneller als durch Kontroll-♂♂ angelockte ♀♀. Die KOntroll-♂♂ führen im ∅ mehr Paarungsversuche aus und gelangen weniger häufig und dann nur viel später als die behandelten ♂♂ zu einem Paarungserfolg.

1 Introduction

The choice of a conspecific ♂ that a ♀ makes in order to mate is one that may influence the fitness of her offspring and, if the selection factor is genetically based, will also affect the reproductive success of her progeny. Fisher [1958] explained the existence of female preference as the outcome of an evolutionary process in which both preference and preferred character evolve together, and O'Donald [1967]

* Based on a lecture in the symposium "Chemical Communication in Sexual and Social Selection" at the XVIII International Congress of Entomology, Vancouver/Canada, July 1988.

0171-8177/90/0015-0121 $ 1.25

and Lande [1981] constructed mathematical models that supported Fisher's explanation. Many case studies demonstrate sexual selection through *male competition* and this theory has become widely accepted. However, only a few experiments, such as Andersson's work [1982] on the long-tailed widowbird, have been performed where the theory of sexual selection by *female choice* has been experimentally supported [Majerus 1986: review].

In the Oriental fruit moth (OFM), *Grapholita molesta* (Busck 0000) (Lepidoptera: Tortricidae), the ♂ is attracted to the ♀ by a sex pheromone released by her at dusk. After location of the ♀, usually by flight, the ♂ performs a series of courtship behaviours: the ♂ moves past the calling ♀ and, at a distance of a few cm, rhythmically extrudes and retracts 2 sets of hairpencils [Baker & Cardé 1979]. Volatile chemicals released from the hairpencils and the wind generated by wing vibration have been shown to be the main factors attracting the ♀ to the ♂ [Baker & Cardé 1979]. Without the ♀ signalling her acceptance of the ♂ by moving to and touching the end of his abdomen, coupling, and hence copulation, cannot occur. Thus, the ♀ can select a mate by moving only towards the display that she finds attractive.
Baker et al [1981] identified the behaviourally most active component of the courtship pheromone as ethyl *trans*-cinnamate. It has been suggested on a couple of occasions that the larval/adult diet may affect the compounds present in the courtship pheromone [Baker et al 1981, Nishida et al 1985] and that this may have repercussions upon the mating success of an individual raised on a particular diet. In the present study it is tried to determine whether such variation would indeed affect the ♂♂ ability to attract ♀♀ and succeed in mating. The following results are a part of a more extensive report to which it is referred for experimental details and a full discussion [Löfstedt et al 1989].

2 Materials and methods

A method was developed for providing adult moths with the opportunity to ingest and sequester ethyl *trans*-cinnamate (EC), following the evidence by Nishida et al [1985] that adults do take up this compound during breeding. At the time of peak responsiveness to sex pheromone by ♂♂ and pheromone gland extrusion by ♀♀, beginning about 2 h before lights off [Baker & Cardé 1979], a calling ♀ was transferred from one of the plastic cups to a glass vial and then onto the base of a $100 \times 30 \times 20$ cm^3 plexiglas box. A ♂ was then quickly introduced ca 10 cm downwind of the ♀, close enough to be able to detect and walk upwind in response to the ♀'s sex pheromone.
Successful ♂♂ were, within seconds after coupling teased apart from the ♀, the first few seconds of linkage being insecure [Baker 1982]. They were then stored in glass vials identical to those used for unsuccessful ♂♂.
After the mating experiments each individual ♂'s hairpencils were analysed by gas chromatography whether the ♂ was successful in coupling or not.

3 Results and discussion

The foremost result achieved was that of the measure of absolute mating success. The ethyl-*trans*-cinnamate-treated ♂♂ (EC ♂♂) experienced a success rate of 92% (34/37) whereas the control sugar water-treated ♂♂ (SW ♂♂) achieved coupling 68% (26/38) of the time (Fig 1). On their successful attempts, EC ♂♂ attracted ♂♂ from significantly further distance ($\bar{x} = 1.05$ cm $\pm$ 0.15 SE) than did SW ♂♂ ($\bar{x} = 0.73$ cm $\pm$ 0.08 SE) (t-test, $0.025 < p < 0.05$). The distance of an attempt was defined as the distance from the courting ♂'s abdominal tip

to the ♀'s head. ♀♀ also moved faster toward EC ♂♂ to touch their abdomens ($\bar{x}$ = 1.48 cm/sec ± 0.18, n = 12) than to SW ♂♂ ($\bar{x}$ = 0.99 cm/sec ± 0.07, n = 15) (t-test; 0.0005 < p < 0.005). When either an EC or SW ♂ is not successful at attracting a ♀ on one of his first displays then he tends to move in closer to the ♀ to display on subsequent attempts. Since SW ♂♂ that were successful at coupling on average took more attempts to do so ($\bar{x}$ = 2.54 ± 0.35 SE, n = 26) than EC ♂♂ ($\bar{x}$ = 2.09 ± 0.29 SE, n = 34), their successful attempt tended to be later and closer than for EC ♂♂ (t-test, p > 0.05). Typical gas chromatograms of extracts obtained from SW and EC ♂♂ respectively, are shown in Fig 1. No ethyl cinnamate peak was seen in any SW ♂ (n = 28).

On average, the hairpencils of EC ♂♂ contained 6.7 ng ± 0.47 SE (n = 26) of ethyl *trans*-cinnamate. Analyses of control ♀♀ fed ethyl cinnamate that had their abdomens snipped and extracted in a similar way to the ♂♂ also contained no trace of ethyl cinnamate. Mellein, another constituent of the extrusible organs, was present in all hairpencils analysed and measured about 50 ng.

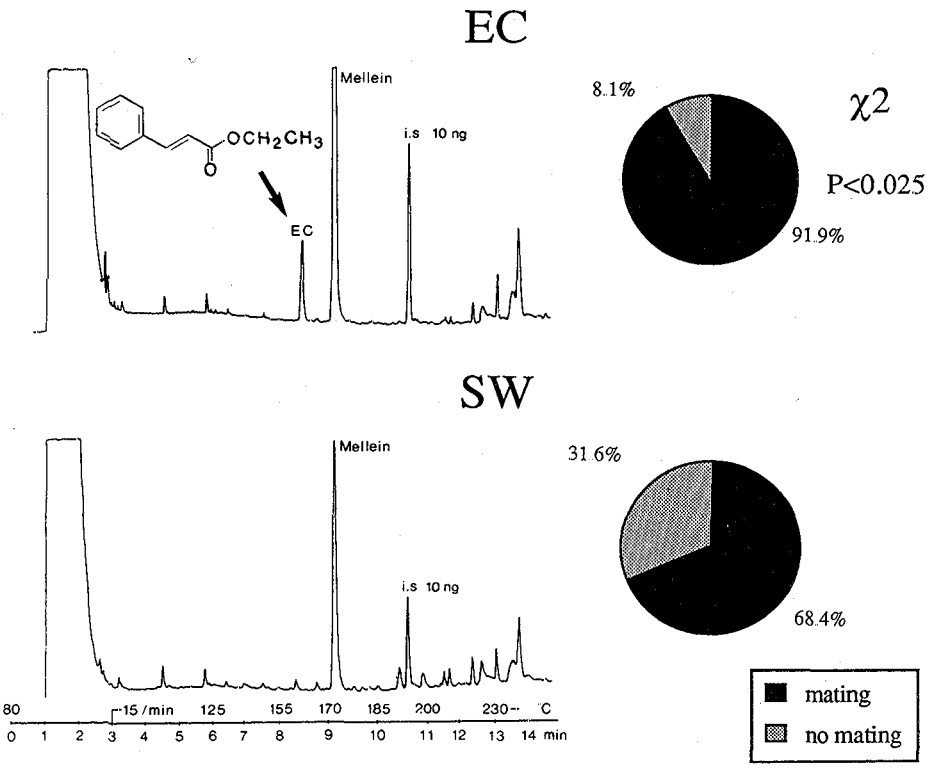

Fig 1: The proportion of ♂♂ achieving coupling estimated for control ♂♂ (SW, fed sugar-water only, n = 38) and ethyl *trans*-cinnamate treated ♂♂ (EC, fed sugar-water with ethyl *trans*-cinnamate, n = 37) and typical corresponding gas chromatograms from analysis of hairpencil extracts, showing the increased level of ethyl *trans*-cinnamate in an EC-treated ♂. The statistical significance of differences between SW and EC ♂♂ was tested by χ^2 2×2 analysis with Yates' correction.

This study demonstrates rigorously that the chemical message contained within the hairpencils of male *G. molesta* is a critical factor as to whether a ♀ accepts or rejects a particular ♂, how quickly she does so, and that OFM ♀♀ have a preference for increased development of this secondary sexual character. Adult ♂♂ on the ethyl *trans*-cinnamate diet had a significantly greater success rate in coupling. In a natural situation, several ♂♂ may arrive at a calling ♀ simultaneously or within a temporal distance of a few seconds [Baker & Cardé 1979]. The ability to attract a ♀ quickly and to get the ♀ to move speedily are critical in order to couple successfully before the arrival of another ♂. Baker [1982] has shown that competing ♂♂ may 'sneak' copulations or disrupt the first-arriving ♂'s courtship display. This fact makes the speed of attraction even more important.

Clearly, in view of the observed behavioural traits, the ability to sequester ethyl cinnamate from the diet and into the hairpencils is a very important feature. Indeed, ethyl *trans*-cinnamate has been found to be one of the components from rotting Japanese pears that actually attracted OFM in the orchard [Nishida et al 1985]. The question naturally arises as to where *G. molesta* ♂♂ obtain their ethyl cinnamate from in the wild. Male Oriental fruit moths are known to possess all hairpencil components without ingesting fruit juice as adults [Baker et al 1981; Nishida et al 1982, 1985]. Nishida et al [1985] reported that both ethyl cinnamate and jasmonic esters and/or their derivatives were plant constituents, and that these compounds might be sequestered from the host-plant by the insect for use at a later stage. It was apparent from an early stage of our study [Löfstedt et al 1989], in concordance with Nishida et al [1982, 1985], that ♂♂ reared on artificial diet as larvae and kept on sugar water as adults had a very low ethyl cinnamate titre.

It appears likely that in the OFM a process of sexual selection, perhaps of a runaway type, may have been triggered by the avoidance of interspecific mating mistakes by those ♀♀ initially selecting ♂♂ that emitted specific host-plant derived volatiles, as suggested by Phelan & Baker [1986]. An alternative, not necessarily exclusive, explanation may be that as high EC titres are associated with adult ♂ feeding, a ♀ selecting for EC will mate with a well-fed ♂, able to provide a large spermatophore. This hypothesis remains to be tested.

4 Acknowledgements

This study was supported by grants from the National Science Foundation, USA, and from the Swedish Natural Sciences Research Council. We are grateful to the editors of *Oikos* for letting us publish material which has earlier appeared in this journal.

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Kikkawa, J., & Anderson, D. J. (Editors): **Community Ecology: Pattern and Process.** — [XI + 432 pages, numerous figures and tables, size 155 x 235 mm, soft cover]. — Publisher: Blackwell Scientific Publications, Melbourne-Oxford-London-Edinburgh-Boston-Palo Alto; ISBN: 0-86793-272-4; price: Brit £ 19.80. ——— EGR-Nr 1385].

Organized research, complex data analysis, community metabolism studies and new techniques of environmental measurement have all contributed to the considerable advances in methods of community ecology made over the last 25 years. In this stimulating text, the editors have assembled a truly international team of authors, each a leading authority in his specialist field, to take a fresh and critical look at major patterns and processes that must underlie all community organization. The volume is divided into 5 parts giving an historical perspective to the development of modern concepts necessary for the understanding of natural communities and covering such areas as limits of cummunities, community organization, ecological and evolutionary processes and other major approaches in community ecology. Clear and succint reviews of literature, combined with fresh ideas and interpretations, make this a stimulating text for advanced courses in ecology.

Danks, H. V.: **Insect Dormancy: An Ecological Perspective.** In: Biological Survey of Canada Monograph series No 1. — [IX + 439 pages, 40 figures and 45 tables, size 165 x 260 mm, balacron hard cover]. — Publisher: Biological Survey of Canada (Terrestrial Arthropods); ISBN: 0-9692727-0-7; price: US $ 50.—. ——— [EGR-Nr 1370].

Dormancy represents a remarkable aspect of insect life cycles, and comprehensive knowledge of dormancies can help to provide an approach to explain the single steps of life cycle in the diverse types of insect development. The well-written book is an detailed representation from an ecological rather than a physiological point of view to give a survey of the complexity of ecological aspects in dormancy. The fifteen chapters of the book cover a wide range of ecological perspectives in dormancy. Besides definitions, the attempts of classifications by several authors, the main characteristics of dormancy, internal and external mechanisms for control, cue reception, timers, hormonal control and structure of dormancy responses are reviewed. Furthermore, broader aspects of dormancy, such as evolutionary and genetic perspectives have been exactly documented and profoundly discussed. The text is supported by numerous tables. The discussions include a lot of information by viewing them from a broad ecological orientation as well as a qualitative outlook with new ideas in future work. In the author's opinion a "comprehensive view of insect dormancies must have a primarily ecological orientation" (p. 297). This makes it even less understandable why he hesitates to accept the possibility of an ecological classification of dormancies. Information is clearly organized and the chapters generally contain concise conclusions. An important part of the excellent work are the well documented references with 2750 items. Moreover, the book is completed by a summary of the results, authors, species, and subject indexes. This well presented book compiling the up-to-date data on dormancy is recommended to each ecologist studying insect life cycles and to each ecological library.

Rudolf Bährmann (Jena)

Walker, A. K., & Crosby, T. K.: **The Preparation and Curation of Insects.** — In: DSIR INFORMATION SERIES 163. — [91 pages, numerous figures, size: 150×210 mm, soft cover]. — Publisher: Wellington/New Zealand; ISBN: 0-477-02519-6; Price: $ 18.70. ——— [EGR-Nr 1520].
This handbook explains the methods and techniques used by workers of the New Zealand Arthropod Collection (NRAC) for preparing insects for its collection, and how the collection is curated and managed. Detailed information is given on the following topics: the preparation of specimens, including relaxing, pinning, card point mounting, double mounting, slide mounting, and labelling; organisation and storage of the collection; loans and the dispatching of specimens; restoration of specimens; hazardous properties of chemicals used; checklist of entomological supplies. Alternative techniques have been provided for many NZAC procedures, which may be more suitable for usage in other collections.

Borkovec, A. B., & Gelman, D. B. (eds): **Insect Neurochemistry and Neurophysiology.** — [XI + 484 pages, a number of figures and tables, size 160×240 mm, balacron hard cover, jacket]. — Publisher: Humana Press, Clifton-New Jersey, marketed and distributed by John Wiley & Sons Ltd, Chichester, ISBN: 0-89603-119-5, price: Brit £ 68.15.——— [EGR-Nr 1405].
This encyclopedic work, of interest to anyone concerned with the broad field of insect neuroscience is the result of the second International Conference on Insect Neurochemistry and Neurophysiology (ICINN-86) held at the University of Maryland in August 1986. The authors represent a veritable 'who's who' in this rapidly advancing field. Seven major reviews cover 'insect neuropeptides' (M. O'Shea), 'Prothoracic hormones and neurohormones in *Bombyx mori* (A. Suzuki), 'Endocrine timing signals that direct ecdysial physiology and behavior' (S. E. Reynolds), 'Hormonal control of diuresis in insects' (S. H. P. Madrell), 'Chemistry of synapses and synaptic transmission in the nervous system of insects (H. Breer), 'Biogenic amine receptors and their mode of action in insects' (P.D. Evans), and 'Embryonic formation of a simple neurosecretory nerve in the moth Manduca sexta' (P. H. Taghert et al.). These are followed by research summaries organized under the three headings 'neurochemistry', 'neurophysiology', and 'neuroanatomy'. The division is arbitrary. The control of insect visceral muscle by octopamimergic neurons treated in the first section represents neurophysiological research, the differential synthesis and allatotropic stimulation of JH-III and JH III diol in locust corpora allata presented under 'neurophysiology' relates more to neurochemistry, and all of the 'neuroanatomy' papers deal with neuropeptide immunoreactivity. As it is always the case, the camera-ready typewritten papers suggest a very transient nature of the information presented. As an up-to-date survey of the 'state of the art', the book can be recommended. References and an 18-page subject index make it very useful. Ernst Florey (Konstanz)

Law, J. H. (ed): **Molecular Entomology.** — In: UCLA Symposia on Molecular and Cellular Biology, Vol. 49. — [XVII + 512 pages, size: 235×160 mm, numerous figures and photographs, hardcover, jacket]. — Verlag: Alan R. Liss, Inc., New York; ISBN: 0-8451-2648-2; Price: $ 90.—. ——— [EGR-Nr 1542].
Again a symposium volume, replete with photo-ready typewritten manuscripts. In the age of desk-top-publishing, an anachronism that is hard to defend. Fortunately, the papers presented are not anachronistic. Some of the old hands in the field of insect science (commonly known as entomology), and many of the newer ones, provide a compact presentation of their views and findings, ranging from behavioral and molecular aspects of olfaction to genetic engineering for insect resistance factors.
The editor's preface, too, sounds anachronistic when it states that the conference (from which this volume resulted) "was predicated on the emergence of a new field — a blend of insect science, molecular biology, and biochemistry." Had this been written thirty years ago, it would have sounded exciting. As it is, the book represents a useful cross section through modern entomology at its best, up to the state of the art of early 1986. Reference lists at the end of the papers give only the first page number of the articles or chapters quoted, and they follow the unpleasant custom of running the initials of the author's first names together to yield YAI and MO, GA and SO. Fortunately, the editing is not consistent; the reader is grateful for R. G. H., E. C., and P. S. in one paper that presents even the last page of the articles cited. One is put off again by book citations such as "Compre Insect Physiol Biochem Pharmacol". Altogether, the book contains 42 papers. They are grouped into 6 sections: molecular aspects of the nervous system, molecular endocrinology and development, hemolymph proteins, insect-specific processes, sex-specific proteins, and applications of molecular entomology. The 22 page index is well prepared and most useful — it may be perused as a guide through present day terminology and research topics.
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