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Original article

Novel attractants of *Galleria mellonella* L (Lepidoptera Pyralidae Galleriinae)

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Summary — Various synthetic esters were tested for their attractiveness to females of *Galleria mellonella* in comparison with *n*-nonanal, a component of the sex pheromone of *Galleria mellonella*. It has been shown that methyl octanoate, ethyl octanoate and methyl decanoate attract females. Particularly, methyl octanoate is as effective as *n*-nonanal in bioassay and could be a potential pest control agent.

***Galleria mellonella* / sex pheromone / attractant / chemical control**

INTRODUCTION

The greater wax moth, *Galleria mellonella* L, is the most serious pest of honey bee colonies and stored combs. The rapidly growing larvae of *Galleria* feed upon all the hive products as they burrow through the comb and cause heavy damage. Therefore, the control of moths without harming the bees and bee products has great economic importance for bee keepers.

There are several physical, chemical and biological control methods applied against the greater wax moth invasion of honey bee colonies, but all of these have certain disadvantages that limit their use (Cantwell and Smith, 1970; Ali *et al*, 1973; Burges, 1977, 1978). Further investigations are needed to improve some of these techniques (Spangler, 1986, 1987) or to explore more effective and suitable new methods.

Sex pheromones of many Lepidopteran species are used as pest-management agents. The sex pheromone of the greater wax moth is secreted from the wing glands of males (Barth, 1937) in response to a low frequency sound produced by the wingbeat of females (Spangler, 1986, 1987) and has been identified as a 7:3 admixture of 2 aldehydes, *n*-nonanal and *n*-undecanal (Röller *et al*, 1968; Leyrer and Monroe, 1973). In laboratory and field bioassays live males were found to be significantly more attractive than a mixture of the above-mentioned aldehydes in pheromone baits, which did not attract beyond a few meters (Finn and Payne, 1977; Flint and Merkle, 1983).

Since the above-mentioned aldehydes are easily oxidized in air and lose their activity, their stimulating effect cannot reach females effectively unless they are continously supplied by living males. Hence, the aldehydes have a limited applicability in population control of *Galleria mellonella*. Esters are more stable under natural conditions.

In the present study, we tested the effectiveness of several synthetic ester molecules on the females of the greater wax moth.

MATERIALS AND METHODS

Experimental insects

Larvae of *Galleria mellonella* were cultured at 29° ± 1°C and at ≈ 70% relative humidity in an artificial diet prepared as previously described by Schmidt and Monroe (1976). The cultures were kept in complete darkness. The pupae were collected in individual vessels and newly-emerged female and male moths (6–24 h after adult emergence) were collected in separate groups.

Experimental chemicals

The synthesis of *n*-nonanal was carried out by the periodic acid oxidation of 9,10-dihydroxy stearic acid (Ernest, 1957). Methyl, ethyl, propyl, butyl, allyl esters of octanoic and decanoic acids were synthesized *via* the reaction of the corresponding acid chlorides and alcohols. Decyl and octyl acetates, methyl, ethyl, propyl, butyl and allyl esters of nonanoic acid were all synthesized *via* direct esterification reactions of the corresponding alcohols and acids. All the chemicals were purified repeatedly until spectroscopic purity was reached.

The test chamber

The test-apparatus for the bioassay was designed to observe the attractiveness or stimulatory potential of the synthesized chemicals. It consisted of 2 spherical glass chambers each with a volume of 1 l. The chambers were connected *via* a glass tube 3 cm in diameter and 30 cm in length.

To avoid saturation of the chamber where the insects were placed, 2 short plug holes in the chamber were left open. The apparatus was cleaned thoroughly, dried and reassembled before each test. The test compounds (table I) were introduced into chamber B in adsorbed form on a supended strip of filter paper (fig 1). Ten female moths were introduced into the other chamber, which was left open to air *via* the plug holes.

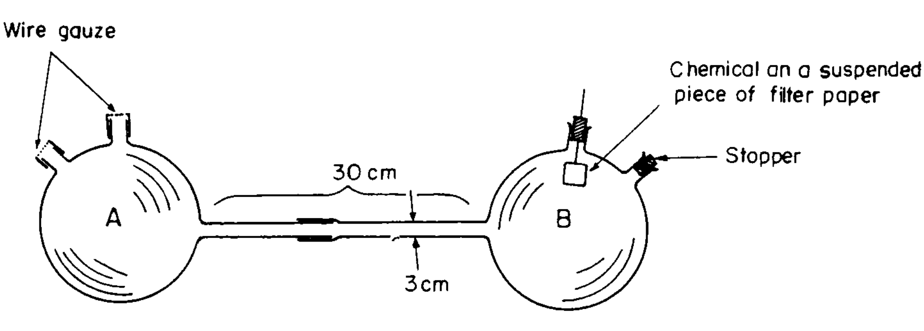

Fig 1. Apparatus used to test the behavioral effects of the chemicals.

Table I. Volumes and corresponding weights of the chemicals for the behavioral threshold, latency periods, and the type of responses exhibited by the females. See text for the explanations of column headings.

Compound	*Behavioral threshold*			*Response type*
	Volume of chemical (µl)	*Weight of chemical (g)*	*Latency period (min)*	
Octyl acetate	200	0.1769	120–130	Agitative
Methyl octanoate	150	0.1316	90–100	Active
Ethyl octanoate	180	0.1564	100–110	Active
Allyl octanoate	400	0.3436	120–130	Agitative
Propyl octanoate	400	0.3455	120	Inactive
Butyl octanoate	400	0.3451	120	Inactive
Nonanal	120	0.0992	80–90	Active
Methyl nonanoate	400	0.3519	120	Inactive
Ethyl nonanoate	400	0.3462	120	Inactive
Allyl nonanoate	400	0.3522	120	Inactive
Propyl nonanoate	400	0.3516	120	Inactive
Butyl nonanoate	400	0.3460	120	Inactive
Decyl acecate	220	0.1907	110–120	Agitative
Methyl decanoate	200	0.1746	110–120	Active
Ethyl decanoate	200	0.1729	110–120	Agitative
Allyl decanoate	400	0.3455	120	Inactive
Propyl decanoate	400	0.3448	120	Inactive
Butyl decanoate	400	0.3444	120	Inactive
Nononoic acid	400	0.3620	120	Inactive

Bioassay for stimulatory activity of synthetic substances

Prior to experimentation with the test chemicals, a set of control experiments was performed on test groups of moths. The females were placed in the chamber, and the plug hole was opened to air for 2 h. Within that period of time no moths passed into the other chamber and they remained still. The control experiment was repeated 5 times.

All the experiments were carried out at atmospheric pressure in daylight and between 9 am and 3 pm.

Three classes of female responses were observed during the experiments. Upon exposure to some of the esters, the test insects were sessile, just as the insects in the control experiments were at all tested volumes of the compounds. The behavior of these insects has been described as "inactive response". If a high degree of mobility within the flask in which they were placed was initially observed, this behavior was called "agitative activity". If, on the other hand, the observed behavior among the test insects was very similar to that observed during exposure to *n*-nonanal, it was termed "active response". This last type of activity can be described as follows: first, movement ot the antennae, then rapid fanning of wings and dancing of each individual around itself were observed. These patterns of activity were accompanied by the transport of some or all the stimulated insects into the second flask where the test chemicals were placed. All the insects which moved into the second flask exhibited ovipositioning and fanning.

In the control groups, the effectiveness of solvents used for cleaning purposes such as acetone, ethyl alcohol and water were tested on female behavior and found to be ineffective. Afterwards, the synthesized compounds were tested for a 2-h period. Firstly, 1 μl of the compound was introduced into chamber B (fig 1). If no response was observed within *ca* 2 h, then the volume was increased up to 50 μl, followed by increments of 10 μl up to 400 μl. For each volume of the compounds, fresh groups of *Galleria mellonella* individuals were used. Ten females were used in each test (5 replicates).

The behavioral thresholds (the volume of chemical required to initiate the response in the females, the equivalent weights) and the latency periods (the time elapsed until the response was observed) were recorded (table I). Similar tests were carried out on *Galleria mellonella* males.

Statistical methods

Analysis of variance (ANOVA) was used to compare the means of the percent age of attracted females. To satisfy the assumptions of the method, the percentages were subjected to improved angular transformation (Sokal and Rohlf, 1981). Homogeneity of group variances were tested by F_{max} (Sokal and Rohlf, 1981). When the ANOVA *F*-statistic was significant at least at the 5% level, *T*-test was then used to partition means into significant ranges.

RESULTS AND DISCUSSION

The pattern of the "active response" type of behavior was very similar to the sexual responses of females to the sex pheromone *n*-undecanal as described by Röller *et al* (1968). Furthermore, all the tested compounds were found to be ineffective on males.

Types of female responses to the tested compounds are given in table I, along with the volumes and weights of the chemicals for the behavioral thresholds and latency periods.

Expression of the "active response" type of behavior by females but not males to methyl and ethyl octanoates and methyl decanoate as well as *n*-nonanal suggests that the first 3 esters constitute novel sex attractants in the greater wax moth. The percentage of females attracted by these compounds in each of the replicates (sets) has been presented in table II.

Pairwise comparision of means of the transformed percentages by a subsequent *T*-test (Sokal and Rohlf, 1981) revealed

Table II. Statistical analysis of percent *Galleria mellonella* expressing an "active response".

Set [a]	*Compounds* [c]			
	n-*Nonanal* (0.0992 g)	*Methyl octanoate* (0.1316 g)	*Ethyl octanoate* (0.1564 g)	*Methyl decanoate* (0.1746 g)
I	70	80	60	0
II	80	70	60	10
III	70	80	50	0
IV	90	90	50	10
V	90	90	60	6
Mean [b] of the transformed percentages	<u>62.38</u>	<u>60.75</u>	48.22	11.96

[a] The number of females in each set was 10; [b] 2 means underscored by the same line are not significantly different (*P* < 0.05). The minimum significant difference was 9.26; [c] doses of the chemicals used are given in parentheses.

that the percent attraction by *n*-nonanal and methyl octanoate were not significantly different at the 5% level and that these 2 compounds were significantly more attractive to the insects than ethyl octanoate or methyl decanoate (table II).

The amounts of test chemicals used in the present study should not be directly compared with the amount of pheromone produced by the insects under natural conditions because the signal is conveyed by the amount of chemical transferred into the vapor phase which should be considerably much less (all have high boiling points) than the amount used in the liquid phase of these compounds. If ventilation is not adequate, material transferred from one chamber into the other is mainly due to passive diffusion. In this case, it is expected that these doses would be beyond the physiological range. If the primary purpose is not the detection of potent compounds but that of their dosage, then one has to use a sophisticated olfactometer such as that utilized by Zagatti *et al* (1987).

Among the tested esters in the present study, methyl and ethyl octanoates and methyl decanoate acted as sex attractants on *Galleria*. Methyl octanoate was the most effective compound, and exhibited the same level of attractivity as nonanal, the prominent component of the sex-pheromone of *Galleria mellonella*. In the future, methyl octanoate should be utilized to lure females to insecticide-treated baits. It is expected to be a superior population controlling agent to *n*-nonanal which is easily oxidized to nonanoic acid under atmospheric conditions and neither the resulting acid itself nor its esters are found to be effective.

Résumé — Nouvelles substances attractives pour *Galleria mellonella* L (*Lepidoptera, Pyralidae*). La grande teigne de la ruche, *Galleria mellonella*, est un parasite important des colonies d'abeilles domestiques et des réserves entreposées dans les rayons. Aussi, une méthode de lutte non dangereuse pour les abeilles est-elle d'une grande importance économique pour les apiculteurs. Il existe divers procédés de lutte contre la teigne de la ruche, physiques, chimiques et biologiques, mais ils présentent tous des inconvénients. La phéromone sexuelle du mâle de la teigne est un mélange de *n*-nonanal et de *n*-undécanal dans le rapport de concentration de 7:3. Parce que ces aldéhydes peuvent facilement s'oxyder à l'air et qu'ils perdent alors leur activité, leur application est limitée. C'est pourquoi des composés apparentés ont été produits par synthèse, et leur pouvoir attractif testé dans un appareil (fig 1). Les tableaux I et II présentent les résultats statistiques. Finalement, ce sont les 3 composés, octanoate de méthyle, octanoate d'éthyle et décanoate de méthyle, qui se sont montrés efficaces en remplacement de la véritable phéromone sexuelle (*n*-nonanal) (tableau II). Les meilleurs résultats ont été obtenus avec l'octanoate de méthyle.

***Galleria mellonella* / phéromone sexuelle / attractivité / lutte chimique**

Zusammenfassung — Neuartige Lockstoffe für *Galleria mellonella* (*Lepidoptera, Pyralidae*). Die Große Wachsmotte, *Galleria mellonella*, ist ein wichtiger Schädling für Bienenvölker und gelagerte Wabenvorräte. Aus diesem Grunde ist eine für die Bienen ungefährliche Kontrolle der Motte für den Bienenzüchter von großer wirtschaftlicher Bedeutung.

Es gibt verschiedene physikalische, chemische und biologische Kontrollverfahren gegen die Große Wachsmotte, die jedoch alle auch mit Nachteilen verbunden sind. Das Sexualpheromon (Geschlechts-Lockstoff) der männlichen Wachsmotte ist

eine Mischung von n-Nononal und n-Undekanal im Verhältnis von 7:3. Da diese Aldehyde jedoch in der Luft leicht oxydiert werden und sie dabei ihre Wirkung verlieren, ist ihre Anwendbarkeit nur beschränkt.

Es wurden deshalb verwandte Verbindungen synthetisch hergestellt und in einer eigens entwickelten 2-teiligen Versuchskammer (Abb 1) getestet. Die Ergebnisse wurden statistisch überprüft (Tabelle I, II). Am Ende konnten die drei Verbindungen Methyloktanoat, Ethyloktanoat und Methyldekanoat an Stelle des echten Geschlechtspheromons (n-Nononal) als wirksam festgestellt werden (Tabelle II). Die besten Resultate wurden mit Methyloktanoat erzielt.

***Galleria mellonella* / Lockstoff / Sexpheromon / chemische Bekämpfung**

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