NEW INSECT PHEROMONE, ERECTIN-LIKE SUBSTANCES

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Abstract

A new type of sex pheromone, which is not an attractant, was recently isolated by us from Callosobruchus chinensis L. From its function to release copulation behaviour by extrusion of the genital organ - termed "copulation release" (CR) - it was named erectin. It consists of two synergistically acting fractions, neither of which has any activity when used alone. Evidence for the presence of an erectin-like CR pheromone was obtained in the cases of Callosobruchus maculatus (F.) and Tenebrio molitor L. In the latter, 4-methyl-1-nonanol as a sex attractant potentiated the action of CR pheromone. When the volatile fraction which contained 4-methyl-1-nonanol was removed from the extract, the non-volatile fraction obtained showed only CR activity. Such a CR pheromone was found both in males and in females.

Synthetic erectin evoked the same activity as natural erectin. Confronted with a dummy consisting of an aluminium tube and erectin, the male of C. chinensis underwent copulation and ejaculation as it does with the live female. The possible presence of such a pheromone in stored-product insects led us to examine the use of erectin for pest control, and attempts were made to investigate the possibility of using erectin-like substances to lower the population density.

Introduction

The use of sex attractants with stored-product insects has been well documented by Burkholder and Ma, (1985). Recently a new type of sex pheromone, which is not an attractant, was found in Callosobruchus chinensis L. by Tanaka et al.,(1981) and from its function in releasing copulation behaviour, it was given the name erectin. There are other cases indicating the presence of erectin-like substances among stored product insects.

Sex pheromones of Callosobruchus chinensis

Mating behaviour. When in the proximity of a female a male raises his antennae and runs towards her in a zigzag pattern. As he contacts her, he lowers the antennae, bends his head, extends the tip of his abdomen towards her, extrudes his genital organ, and eventually establishes copulation. The behaviour which takes place from either the stationary, walking or flying position to the zigzag running is termed "sex attraction", and that from lowering the antennae to the extrusion of the genital organ as "copulation release" (CR) (Fig.1).
Sex Attractant. We first tried to isolate a sex attractant which might be involved. The volatile material from virgin females was trapped by Poropak Q and fractionated. A sex attractant was isolated and was estimated to be of a magnitude of 7pg/female, although it has not yet been identified. When the attractive fraction or the isolated attractant was placed at the tip of a glass rod, the male was attracted to it and walked around the sample in frustration, but did not extrude its genital organ. However, when offered the dead body, by freezing, of a female, or even, only the tip of the abdomen, the male was attracted, extruded its genital organ and copulated. Once extracted with ether, the female body showed neither attractancy nor CR activity. Thus, the presence of a mating factor other than sex attractant was suggested.

Copulation Release Pheromone. The ether extract of the filter paper on which the virgin females were reared showed both attractancy and CR activity, but after warming at 90°C under an air stream, it lost its attractancy while keeping its CR activity. The details of isolation and identification have been reported elsewhere (Tanaka et al., 1981) and will not be discussed here. This pheromone has no attractancy, but it induces all the other mating responses, and was thus named "erectin". Erectin is released from both male and female (more from the female), but the activity is shown only in the male.
Chemistry of Erectin. Erectin consists of two synergistically acting fractions, neither of which have any activity when used alone. One is a mixture of C_{26}-C_{35} hydrocarbons (ca. 12ug/female) and the other is a novel dicarboxylic acid (ca. 15ng/female), named "callosobruchus acid", (E)-3,7-dimethyl-2-octene-1,8-dioic acid (Fig. 2 & 3).

A mixture of:
hydrocarbons: 3-methylpentacosane, 11-methyloctacosane, 3-methylocta-
cosane, 11-methylnonacosane, 13-methylnonacosane, 11,15-dimethylnonacosane, 9,13-dimethylhentriacontane, and 11,15-dimethyltritriacontane, and
(E)-3,7-dimethyl-2-octene-1,8-dioic acid.

Copulation Release Pheromones of Callosobruchus maculatus

The mating behaviour of C. maculatus is very close to that of C. chinensis and we expected the presence of an erectin like substance in the former species. The extract of C. maculatus when placed on a glass rod caused a distinct CR activity in male C. chinensis, but almost no activity in male C. maculatus. The extract of female C. chinensis also did not affect male C. maculatus. This apparent contradiction was solved by improving the bioassay method. The size of the male organ of C. maculatus is far shorter than that of C. chinensis. This makes a

Copulation release activity of Callosobruchus acid.

Sample | Attempt to copulate 0 | 100 (\%)
--- | --- | ---
Natural C-acid | 15ng + HC | 12ug
Natural C-acid | 15ng | 12ug
HC | 12ug | 12ug
Compound I | 100ng + HC | 12ug
Compound I | 15ng + HC | 12ug
Compound I' | 100ng + HC | 12ug
Compound I | 100ng + octadecane | 12ug

a) Callosobruchus acid.
b) Hydrocarbon mixture from the female of azuki bean weevil.
difference in the style of copulation of the two bean weevils. The male of *C. maculatus* holds the female with his fore and mid legs, while *C. chinensis* only touches the female with his forelegs to make copulation. The size of glass rod which was successful in the case of *C. chinensis* was too large to be held by the male *C. maculatus*, and therefore it did not respond. However, male *C. maculatus* responded to the extract of the female of *C. maculatus* or *C. chinensis*, when provided with a thinner glass rod having a rougher surface, or when the extract was placed on the body of a female which was exhaustively extracted with ether. An active fraction was obtained which seems different from that obtained from *C. chinensis*.

**Sex Pheromone of Tenebrio molitor L.**

The male is attracted to a female, mounts on the female with his genital organ extruded and copulates. Often the male attempts copulation with another male. Both male and female extracts show CR activity towards the male; the volatile fraction from the female, but not from the male, attracts the male, and shows CR activity towards the male. Such behavioural characteristics have caused confusion on the nature of sex pheromone(s) involved, but are now understood by the presence of at least two pheromones interacting with each other in the mating behaviour (Tanaka et al., 1986).

**Figure 4. Synergism between 4-methyl-1-nonanol and erectin like substance**
The volatile fraction was trapped with Poropak Q by passing air over virgin females, and from this 4-methyl-1-nonanol was isolated as a sex attractant. It is secreted only by the female and attracts only the male. The ether extract of the virgin female body showed a strong CR activity as well as attractancy and contained 4-methyl-1-nonanol. When the volatile fraction which contained 4-methyl-1-nonanol was removed from the extract under vacuum and warm conditions, the nonvolatile fraction obtained showed only CR activity. Although not isolated and identified yet, such CR pheromone was found both in males and females, and its activity is potentiated by 4-methyl-1-nonanol (Fig. 4). 4-methyl-1-nonanol itself showed also a distinct CR activity at higher doses. As the live male always bears CR pheromone, its activity is potentiated with 4-methyl-1-nonanol.

Possible Use of Erectin-Like Substances

The presence of erectin-like substances is also suspected from the mating behaviour of other insects such as Costelytra zealandica, Trogoderma glabrum Herbst, Limonius canus LeConte and Tribolium confusum DuV. Attempts were made to investigate the possible use of erectin-like substances in lowering population density. As a model, erectin of C. chinensis was used.

The first requisite is the availability of erectin in sufficient quantity. This was achieved by the synthesis of callosobruchisic acid and substitution of a complex hydrocarbon mixture with a single octadecane (Tanaka et al., 1982). Only (E) form was active. Synthesis of the two optical forms was also achieved, but both forms showed the same activity as the natural one, thus preventing us from assigning their absolute configuration at this moment (Mori et al., 1983) (Fig. 5).

Figure 5. Synthetic optical antipodes showing the same copulation release activity as the natural C-acid of C. chinensis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Attempt to copulate</th>
<th>100(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural C-acid (^a)</td>
<td>15ng + HC (^b)</td>
<td>12μg</td>
</tr>
<tr>
<td>Racemic C-acid</td>
<td>15ng + HC</td>
<td>12μg</td>
</tr>
<tr>
<td>(R) C-acid</td>
<td>15ng + HC</td>
<td>12μg</td>
</tr>
<tr>
<td>(S) C-acid</td>
<td>15ng + HC</td>
<td>12μg</td>
</tr>
</tbody>
</table>

Copulation release activity of Callosobruchisic acid.
\(^a\) Callosobruchisic acid.
\(^b\) Hydrocarbon mixture from the female of azuki bean weevil.
However, from the practical standpoint, this is as good as the use of the racemic mixture. An idea was evolved to make use of erectin-bearing dummies which can mimic the female. When an amount of erectin equivalent to that of one female was applied to a glass rod, the male attempted to copulate, but did not ejaculate.

Figure 6. Attempted copulation of male *C. chinensis* with a glass rod bearing erectin.

Males presented with a wire gauze (250 mesh) bearing erectin, succeeded in achieving erection and insertion only, whereas when presented with an aluminium foil tube bearing erectin the male was able to effect erection, insertion and ejaculation, (Fig. 7). The size was important and with a gap of 30-70um, 80% of the males made insertion and 30% made ejaculation as when presented with a live female. However, problems remain because the male copulates with an average of 15 different females. This indicates that the approach of using dummies for control is not an effective method. Although not yet fully conceived, the possibility of utilizing erectin-like substances of many insect species in the future cannot fail to stimulate the imagination.
Figure 7. Male response to various substrates treated with a female extract (3-5 female equivalent).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Attempt to copulate</th>
<th>Copulation</th>
<th>Sperm transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum foil dummy</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Metal wire gauge dummy</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Freezed female</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Glass rod</td>
<td>0</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Cigarette filter</td>
<td>0</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Plastic spongy</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sand paper</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Filter paper</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
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References