Flight and Molecular Modeling Study on the Response of Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae) to (*E*,*E*)-8,10-Dodecadien-1-ol and Its Geometrical Isomers

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In a previous study we have reported that both (*E*,*Z*)-8,10-dodecadienol (E,Z) and (*Z*,*Z*)-8,10-dodecadienol (Z,Z) isomers inhibit the attraction of male codling moth, *Cydia pomonella* L. when added to (*E*,*E*)-8,10-dodecadienol (E,E) while the (*Z*,*E*)-8,10-dodecadienol (Z,E) isomer induces slight increase in the number of males attracted to the pheromone source. In the present study, we have tested the behavioral activity of the individual geometrical isomers E,Z; Z,E and Z,Z. A few number of codling moth males flew to the Z,E-isomer while the other two isomers (i.e. E,Z and Z,Z) did not elicit any upwind orientation. Analysis of the flight behavior to the E,E- and Z,E-isomer showed significant differences in most of the flight parameters evaluated. Based on the biological observations and molecular modeling, we suggest that the behavioral activity of the Z,E-isomer is due to presence of specific receptors for this isomer on male antennae and not to its structural resemblance to the E,E-isomer. These results underline the importance of the Z,E-isomer in sex attraction of male codling moth.

Introduction

The codling moth, *Cydia pomonella* L., is the most important pest of pome fruit world-wide. (*E*,*E*)-8,10-dodecadienol (E8,E10–12OH; codlemone) was identified as the main sex pheromone compound (Roelofs *et al.*, 1971). In both the laboratory and the field, the two geometrical isomers (*E*,*Z*)-8,10-dodecadienol (E8,Z10–12OH) and (*Z*,*Z*)-8,10-dodecadienol (Z8,Z10–12OH) were found to inhibit male attraction. In contrast, the (*Z*,*E*)-8,10-dodecadienol (Z8,E10–12OH) slightly increases male attraction (El-Sayed *et al.*, 1998). This leads us to the hypothesis that Z8,E10–12OH might mimic E8,E10–12OH. In the field, both the E8,Z10–12OH and Z8,E10–12OH isomers, as well as an equilibrium blend of the four isomers of 8,10–12OH were shown to disrupt male orientation to females more efficiently than pure codlemone (McDonough *et al.*, 1994; McDonough *et al.*, 1996).

Codlemone is not abundant in the female sex pheromone glands of tortricid species, and it is behaviorally active only in codling moth (Arn *et al.*, 1992). This will result in a low selection pressure on the chemical communication channel in codling moth, which might explain the strong attractivity of codlemone as main pheromone compound. In the closely related species, e.g. the chestnut moth, *Cydia splendana* (Hübner) E8,Z10–12OH was identified as pheromone compound (Witzgall *et al.*, 1996) and Z8,Z10–12OH was identified as a sex pheromone component for another three tortricids (Arn *et al.*, 1992). In contrast, the Z8,E10–12OH isomer shows no pheromonal activity among tortricid species. This might explain the inhibitory effect of the two geometrical isomers

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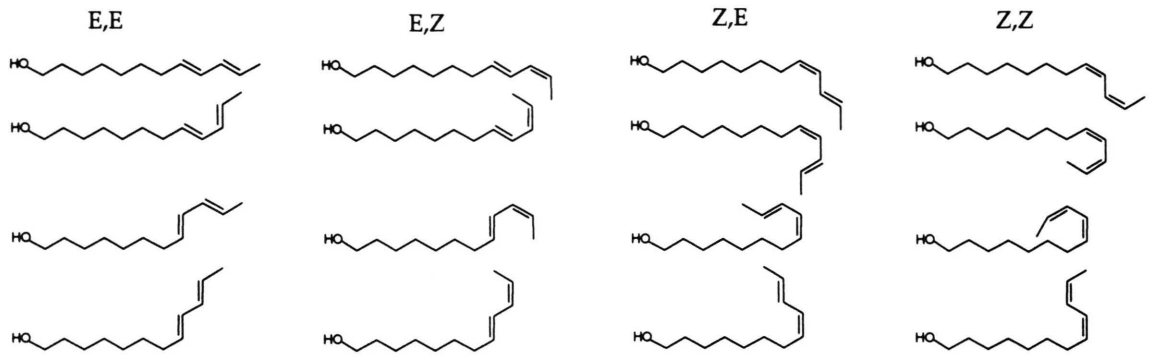

Fig. 1. The four possible conformations of E8,E10–12OH molecule, and its geometrical isomers (E8,Z10–12OH, Z8,E10–12OH, Z8,Z10–12OH).

E8,Z10–12OH and Z8,Z10–12OH on the codling moth, thereby maintaining reproductive isolation among these species.

Male codling moth antennae contain receptors mainly tuned to E8,E10–12OH but still responding to the three geometrical isomers, E,Z, Z,Z and Z,E (Bäckman, 1999). In a cross-adaptation experiment where the receptors were exposed to the E,E isomer, Bäckman (1999) found that these receptors did not respond to E,E, E,Z and Z,Z-isomers but significantly responded to the Z,E-isomer. This suggests that there are neurones present on the male antennae that are specific only for Z,E-isomer and which are not habituated by the E,E-isomer. In addition, Preiss and Priesner (1988) have cited unpublished data that the antennae of male codling moth have receptors that are specific for the Z,E-isomer.

In a previous work, we have studied the synergistic and inhibitory effects of the three geometrical isomers (E,Z; Z,E and Z,Z) on the sex attraction of codlemone on male codling moth (El-Sayed *et al.,* 1998). In this article, we have studied the response of male codling moth to the individual geometrical isomers and applied molecular modeling to these molecules to determine the normal structural conformation. We have then compared the similarities of the structural configurations of the four geometrical isomers in order to see which isomer at best mimics the E,E-isomer.

Materials and Methods

Insect rearing

A culture of codling moth was maintained in the laboratory on a semi-artificial diet (Mani *et al.,* 1978). The larvae and pupae were reared at 22±°C, 18:6 (L:D) photoperiod and 50–70% R. H. Newly emerged moths were sexed daily and transferred to 33 × 33 × 33-cm PlexiglassTM boxes. Males were kept at 22±2 °C, 18:6 LD and 55–75% R. H. They were fed with saturated sucrose solution and were tested the day after emergence.

Chemicals

The E8,E10–12OH used in this study was purchased from S. Voerman, Institute for Pesticide Research, 6700 Wageningen, The Netherlands. The isomers of codlemone, E,Z-, Z,E- and Z8,Z10–12OH were synthesized as earlier described for the acetate analogs (Witzgall *et al.,* 1993) and were purified as described in El-Sayed *et al.* (1998). Analysis of chemical and isomeric purity was done on a Hewlett Packard 5890 GC with flame ionization detection on a DB-Wax column (30 m × 0.25 mm ID, J&W Scientific, Folsom, CA 96830) programmed from 60 °C (hold 2 min) at 10 °C/min to 100 °C, 1.5 °C/min to 150 °C and 20 °C/min to 230 °C. Chemical purity of the test compounds was >99.5%, their isomeric purity given in El-Sayed *et al.,* 1998. Pheromone test solutions were prepared in HPLC grade ethanol and were stored at −18 °C.

Flight tunnel

Tests were done in a flight tunnel with a flight section of 63 × 63 × 200 cm. Air was blown by a horizontal fan (Fischbach, GmbH, Neunkirchen, Germany) onto 4 × 4 activated charcoal filter elements (14.5 × 32.5 cm, Camfil, Trosa, Sweden), the

out-coming air was aspired by another fan and cleaned by one set of charcoal filters. The flight tunnel was lit diffusely from the side at about 6 lux, the wind speed was 30 cm/s and the temperature ranged from 22 to 24 °C. Batches of 15 males were transferred to 2.5 × 15-cm glass tubes stoppered with gauze on both ends 15 min before the tests. Each test session comprised three to four batches of 15 males, and lasted 3 to 4 hr, starting one hr after lights off. Males were released individually in the flight tunnel and the following behavioral responses were recorded: walking and wingfanning during activation phase in the release tube (Activation); upwind flight in any direction (Take-off); initiating directed upwind flight (Lock-on); approaching the source (Close-in) touchdown at source (Touchdown). Males were allowed 2 min to respond. Each treatment was conducted on four days with 15 males/day. The presentation order of the treatments was reversed on alternate days. Timing was stopped when males landed on the tunnel walls or left the tunnel at the downwind end. Males were tested against two release rates of each compound i.e. 10 and 100 pg/min of E8,E10–12OH, and the individual geometrical isomers E8,Z10–12OH, Z8,E10–12OH and Z8,Z10–12OH and the monoenic alcohol E10–12OH. The pheromone solutions were released using the pheromone sprayer (El-Sayed *et al.,* 1999a). This apparatus allows the evaporation of known and constant amounts of pheromone chemicals at defined blend proportions and chemical purity.

Flight recording system

For recording the flight behavior, a flight tracker described by El-Sayed *et al.* (2000) was used. One side of the flight tunnel was illuminated using a light box covered with one sheet of white acrylic plastic, creating a light intensity of ca. 6 lux at the center of the tunnel. Two cameras were placed on the opposite side at angles of 45 and 135° to the tunnel axis, respectively. The sectors covered by the two cameras overlapped over a space with a maximum length of 60 cm along the tunnel axis. The live video images and the male's flight track were displayed on monitors for visual observation. The flight tracks were recorded at 0.04 Hz for off-line analysis. A software (trackevaluat8) was used to analyze the flight tracks off-line. Firstly, the camera co-ordinates were converted to 3D and then the flight parameters were calculated as follows: ground speed 3D (total distance travelled on 3D divided by time); angular velocity 3D (angular change in 3D divided by time); track angle 3D (the deviation of the observed flight path from the wind line, calculated for every frame along each flight path); course angle (the angle between the flight tunnel axis and a given track piece that was corrected for wind drift). Incomplete flight tracks, i.e. tracks with more than three image points missing were discarded.

Molecular modeling

Pheromone molecules are constantly changing their conformation at ambient temperature but the most stable conformation in the gas phase can be calculated by mathematical methods. At any time (e.g. when impinging on the antennae) the overwhelming majority of the pheromone molecules are in this or very closely related conformations. We have used molecular mechanics to obtain the most stable conformation of each of the four geometrical isomers of codlemone. The MM2 method is empirical and based on a large number of experimental data such as bond lengths, bond angels, conformational energies, energy barriers and heats of formation. The molecules are considered as an array of atoms held together by elastic or harmonic forces.

The software used for the molecular modeling was the MM2 for the Macintosh, version CS Chem3D 4.0, Cambridge Soft Corporation, Cambridge MA, USA. For the basic set of parameters, see Burkert and Allinger (1982). The minimum RMS gradient was set to 0.1. The computer used was a Macintosh iMac Special Edition, 400/128.

To avoid reaching a local energy minimum, the molecular modeling was started from four different conformations for each of the four geometrical isomers (Fig. 1). The transoid conformations of the double bonds were found to be the most stable ones. The next most critical dihedral angles were the dihedral angle between the carbon atoms C6-C7 and the double bond in C8-C9 position. To find the most stable conformation and not to overlook any conformation with lower energy, the "dihedral driver" tool in the program was used. It rotates the angle 10° and then recalculates the en-

ergy. Thereby the program in some cases crossed over a saddlepoint and reached conformations with lower energies than the ones originally obtained by the search for lowest energy conformation.

Statistical analysis

The percentage of males landed at the source for both release rates of E,E and Z,E-isomer were compared using Student's t-test, $P = 0.05$ (GraphPad software, 1993). The flight parameters were compared using one-way analysis of variance (ANOVA) and Duncan's new multiple-range test (Abacus Concepts, 1995). Significance level was set at 0.05 for difference between the treatments.

Results and Discussions

Response to the individual geometrical isomers

The number of males completing successive steps of their behavioral sequence in response to the individual isomers in the flight tunnel is shown in Fig. 2. Males flew upwind and contacted the source only to the main pheromone compound, E,E-isomer and the geometrical Z,E-isomer (Fig. 2, A & C). In contrast, only few males initiated upwind orientation for approximately 5 to 20 cm in the pheromone plume but did not maintain their upwind progress towards the odor source of the two geometrical isomers E,Z and Z,Z and the monoenic alcohol E10–12OH (Fig. 2, B, D & E). Furthermore, increasing the release rate of the E,E-isomer from 10 to 100 pg/min caused significant increase in the number of males that arrived at the source ($\tau = 3.2$; df = 6; $P = 0.03$). However, a significant decrease in the number of males arriving at the source was observed ($\tau = 2.82$; df = 6; $P = 0.04$) when the release rate of the Z,E-isomer was increased to 100 pg/min. The observed increase in the response to a 100 pg/min of the E,E-isomer indicated that receptors were not habituated at this release rate while the reduction in male response at 100 pg/min of Z,E-isomer might indicate that the receptors were overdosed at this release rate. Female codling moth releases approximately 100 pg/min of E,E (Bäckman, 1997). This explains the increase in male response at 100 pg/min of the E,E-isomer since this is about the optimum release rate. In contrast, female gland extracts contain ca. 1% of the Z,E-isomer (Arn *et al.*, 1985), therefore, 100 pg/min of Z,E-isomer will be considerably higher than the amount released by a calling female. This might explain the reduction in male response observed at 100 pg of the

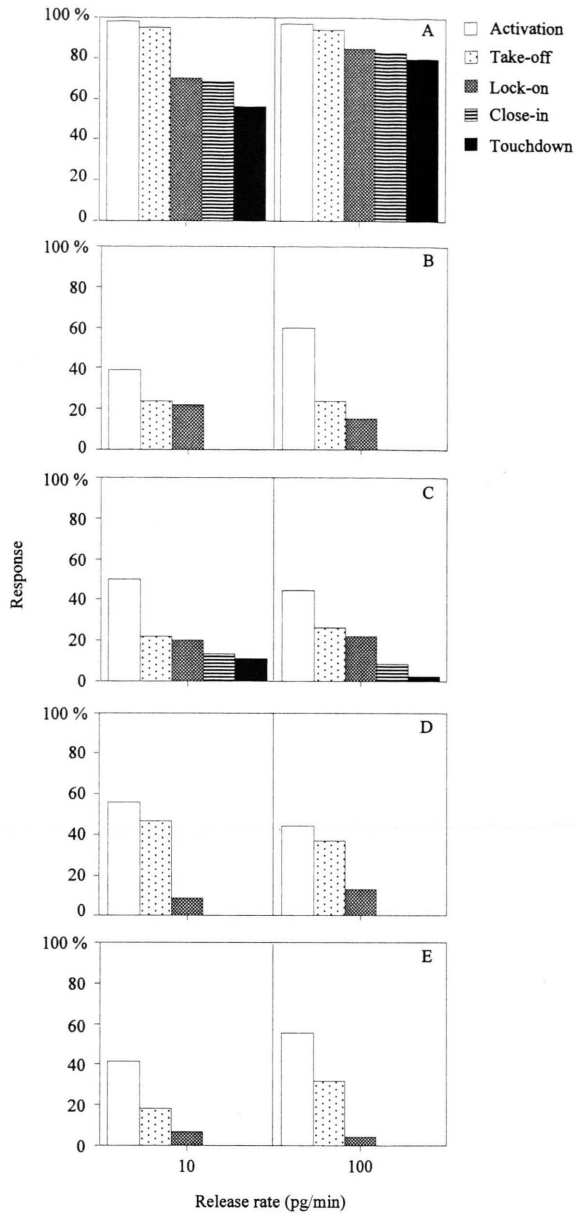

Fig. 2. Behavior response of male codling moth to 10 and 100 pg/min of, E8,E10–12OH (A), its geometrical isomers, E8,Z10–12OH (B), Z8,E10–12OH (C), Z8,Z10–12OH (D) and the monoenic alcohol E10–12OH (E) in the flight tunnel.

Z,E-isomer/min. This indicates that the male antennae might have two types of receptors; one for the E,E-isomer and another for the Z,E-isomer with a different adaptation threshold.

Flight behavior

Flight tracks analysis indicated that males reduced their ground speed as the release rate of the E,E- or the Z,E-isomer was increased from 10 to 100 pg/min. However, males flew with significantly higher ground speed and lower angular velocity to both release rates of the E,E-isomer than compared to the Z,E-isomer (Table I). Generally, the number of neurones specific for the main pheromone compounds on males antennae will be higher than the number of neurones specific for any other minor compound. Due to their small number, the receptors for the minor compounds will be quickly overdosed in contrast to the receptors for the main pheromone compound when exposed to equal number of pheromone molecules. This can explain the lower ground speed that was observed for the Z,E-isomer compared to ground speed observed for the E,E-isomer. Further analysis of the flight behavior showed that male reduces their track and course angle with increasing the release rate of E,E from 10 to 100 pg/min (Table 1). In contrast, increasing the release rate of Z,E from 10 to 100 pg/min did not alter the flight course but lead to significant increase in the track angle (Table 1). Together these observations indicate that perception of the two compounds take place via two different olfactory inputs that have

Table I. Mean ± SD of the flight parameters of male, *Cydia pomonella* tracks to E8,E10–12OH and Z8,E10–12OH, released at two rates, 10 and 100 pg/min.

Light parameter	10 pg/min		100 pg/min	
	E,E	Z,E	E,E	Z,E
Ground speed 3D (cm/s)	71.2 ± 09.7 a	38.3 ± 04.1 b	51.9 ± 12.8 a	19.6 ± 04.5 b
Angular velocity 3D (°/s)	579 ± 67.9 a	656 ± 17.4 b	795 ± 17.7 a	1021 ± 82.6 b
Tack angle 3D (°)	54.6 ± 11.5 a	50.1 ± 29.3 a	50.6 ± 15.5 a	65.7 ± 14.2 b
Course angle 3D (°)	38.7 ± 08.4 a	25.1 ± 12.2 b	30.9 ± 09.4 a	24.2 ± 07.2 b

Within each release rate (i.e. 10 pg/min or 100 pg/min), means in each raw followed by the same letter are not significantly different ($P > 0.05$).

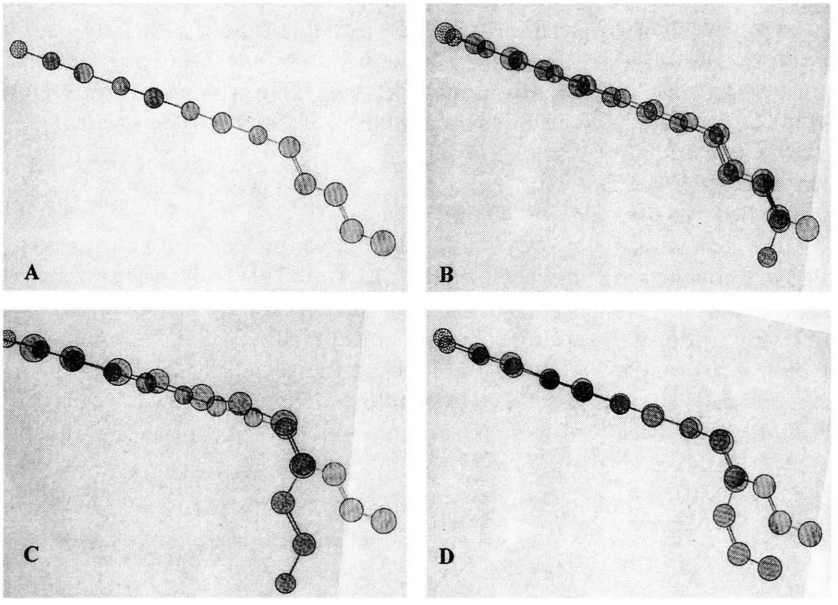

Fig. 3. Molecular structure of E8,E10–12OH (A), superimposition of E,E on E8,Z10–12OH molecules (B), E,E on Z8,E10–12OH (C), and E,E on Z8,Z10–12OH molecules (D).

different excitation characteristics which leads to different behavioral outputs.

Molecular modeling

The results from the MM2 experiments are shown in Fig. 3. Inspection of the energy-minimized structures (i.e. the "normal" conformation of the molecule) revealed that the E,Z-isomer is in fact the most structurally related to codlemone (the E;E-isomer). If the biological activity of the Z,E-isomer would be a result of its structural resemblance to codlemone then also the E,Z-isomer would be biologically active since it is more structurally related. In accordance with a suggested model of a pheromone receptor (Bestmann and Wostrowsky, 1982) we believe that the OH-group is coordinating with an electropositive group in the receptor, that the diene system is attached to a dienophilic protein moity and that the rest of the molecule is bound by van der Waals forces in a lipophilic half-pipe or tunnel. It seems natural that a pheromone mimic would be better if the anomality is as far away from the functional group as possible, thereby allowing the mimicing compound to bind optimal over as long part of the molecule as possible. From this rational, the E,Z-isomer, would bind better than the Z,E-isomer since in the most stable conformation the E,Z-isomer only differs from the most stable conformation of the E,E-isomer at the terminal position (the methyl group). One can conclude that the action of the Z,E-isomer has to be on a different receptor.

To exclude the possibility that the *trans* orientation of double bond at the C10 and OH group at C1 might be responsible for the biological activity of the Z,E-isomer we have also tested E10–12OH. However, non-of the males tested flew to this compound (Fig. 2, E). Based on the behavioral data and the molecular modeling, our conclusion is that male codling moths possess a receptor for the Z,E-isomer that causes upwind orientation.

Male codling moth antennae contain broadly tuned receptors which response mainly to E,E but still respond with larger extent to the other three geometrical isomers, E,Z and Z,Z and the Z,E-isomer, respectively. In a cross adaptation experiment, these receptors were habituated using E,E and were then stimulated with the E,E-; the E,Z-; the Z,E- and the Z,Z-isomer. After the habituation, the Z,E-isomer was the only isomer that elicited response from these receptors (Bäckman, 1999). This might indicate that there are neurones specific for the Z,E-isomer. Again this will support our results regarding the biological activity of the Z,E-isomer. Interestingly, Preiss and Priesner (1988) have cited unpublished data that male codling moth antennae contain receptors for the Z,E-isomer. Both the E,Z and Z,Z-isomers were found to inhibit male orientation to the pheromone source where the Z,E-isomer did not (El-Sayed *et al.,* 1998). It could be that the two isomers cause blockage to the E,E receptors where there are no apparent input channels for these two isomers E,Z and Z,Z which lead to the inhibition observed. In contrast, with Z,E isomer, even if it might cause excitation to E,E receptor, there are still other neurones responding to this isomer which leads to the augmentation observed in the previous study and upwind orientation in this study.

Recently, evidence for a multicomponent blend in codling moth was demonstrated by El-Sayed *et al.* (1999b). Our finding in this study that the Z,E-isomer can elicit upwind orientation in male moth highlights the importance of this compound in sex attraction of codling moth. However, additional electrophysiological and behavioral studies to determine whether the Z,E-isomer is a part of the pheromone system of codling moth are needed. This undoubtedly will have a positive impact on the environmentally safe methods to control this important pest.

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