

Olfactory activity of ethyl (*E,Z*)-2,4-decadienoate on adult oriental fruit moths

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Abstract—We investigated whether or not pear ester (ethyl (*E,Z*)-2,4-decadienoate) attracted adult oriental fruit moths, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae). The electroantennographic responses of *C. molesta* to pear ester were recorded and dose–response curves calculated. In laboratory bioassays, the attractiveness of different dosages was assessed in a dual-choice olfactometric arena. The responses of virgin males and females to pear ester in the presence and absence of pear (*Pyrus communis* L.), peach (*Prunus persica* (L.) Batsch.), and apple (*Malus ×domestica* Borkh.) (Rosaceae) shoots were evaluated. Electroantennographic recordings demonstrated that both male and female *C. molesta* were able to detect the pear ester. In our bioassay, however, pear ester readily attracted males but attracted very few females. The response of males was dose-dependent and they preferred pear ester over apple- and pear-shoot volatiles, whereas no apparent preference between pear ester and peach-shoot volatiles was observed. Therefore, this kairomonal compound could be more effective in attracting *C. molesta* when applied in orchards of secondary host plants, like apple or pear, than in peach orchards.

Résumé—Des essais ont été effectués pour étudier l'effet attractif de l'ester de poire, éthyle (*E,Z*)-2,4-decadienoate, sur les adultes de *Cydia molesta* (Busck) (Lepidoptera: Tortricidae). Les réponses électroantennographiques de *C. molesta* à l'ester de poire ont été enregistrées et les courbes de dose–réponse ont été calculées. L'attraction aux différentes doses a été évaluée grâce à des essais au laboratoire sous forme de tests de choix dans un olfactomètre à deux choix. La réponse des mâles et des femelles vierges à l'ester de poire a été évaluée en présence et en absence des rameaux de poire (*Pyrus communis* L.), pêche (*Prunus persica* (L.) Batsch.) et pomme (*Malus ×domestica* Borkh.) (Rosaceae). Les enregistrements électroantennographiques ont démontré que les mâles et les femelles de *C. molesta* sont capables de détecter l'ester de poire. Dans notre test de choix l'ester de poire a attiré les mâles alors que les femelles étaient moins sensibles. La réponse des mâles est dépendante de la dose. Les mâles ont montré une préférence pour l'ester de poire, comparativement à la pomme et aux volatiles des rameaux de poire, alors qu'aucune préférence n'a été observée lorsque l'ester de poire était en concurrence avec les volatiles des rameaux de pêche. Par conséquent, ce composé kairomonal pourrait être plus efficace pour attirer la tordeuse orientale lorsqu'il est appliqué dans les vergers de plantes-hôtes secondaires, comme la pomme et la poire, plutôt que dans le verger de pêches.

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Introduction

The oriental fruit moth, *Cydia molesta* (Busck) (Lepidoptera: Tortricidae), is an important pest in areas of the world where stone fruits are grown, including Asia, Europe, North and South America, North Africa, New Zealand, and Australia (CAB International 2004). Stone and pome fruit trees (Rosaceae) are the host plants of *C. molesta*. Its primary host plant is peach, *Prunus persica* (L.) Batsch.; the moth infests the shoots, before attacking the fruits as the season progresses (Rothschild and Vickers 1991). Heavy infestations of apple (*Malus × domestica* Borkh.) orchards are also currently observed in several areas (Popovich 1982; Reis *et al.* 1988; Zhao *et al.* 1989; Hickel and Ducroquet 1998; Bradlwarter *et al.* 1999; Kovanci *et al.* 2004). Conventional chemical treatments as well as environmentally safe strategies based on alternative insecticides or sex pheromones (mating disruption) are used to control *C. molesta* populations (Cardé *et al.* 1977; Molinari and Cravedi 1990; Angeli *et al.* 2003; Il'ichev *et al.* 2004; Anfora *et al.* 2007; Ioriatti *et al.* 2009). Semiochemical-based approaches have been explored but, thus far, studies of the biological effect on *C. molesta* of volatiles from its host plants have been limited to laboratory bioassays (Natale *et al.* 2003, 2004a, 2004b; Piñero and Dorn 2007).

Recently, ethyl (*E,Z*)-2,4-decadienoate, a volatile derived from pear, *Pyrus communis* L., and commonly referred to as pear ester, has been demonstrated to attract larvae and adult males and females of *Cydia pomonella* (L.) (Knight and Light 2001; Light *et al.* 2001). Other *Cydia* species, such as *C. fagiglandana* (Zeller), *C. splendana* (Hübner), and *C. pyrivora* (Danilevski), as well as another tortricid, *Hedya nubiferana* (Haworth), have also been caught in traps baited with pear ester and shown electrophysiological responses to this plant volatile (Schmidt *et al.* 2007; Tòth *et al.* 2009). In field trapping experiments, Knight and Light (2004) tested the attractiveness of pear ester to various lepidopteran species, including *C. molesta*. Although this tortricid was not attracted to pear ester in peach orchards, those authors suggested that further investigation was needed because it also attacks pears and may be sensitive to pear-fruit kairomone.

The objective of our research was to study the biological activity of pear ester on adult *C. molesta*. In laboratory bioassays we examined the electroantennographic (EAG) responses of *C. molesta* to pear ester and calculated EAG dose–response curves for males and females (Schmidt *et al.* 2007). In previous studies on the olfactory behaviour of *C. molesta*, the responses of males to pheromones have been investigated in a wind tunnel (Willis and Baker 1994; Valeur and Löfstedt 1996; Rumbo and Vickers 1997). However, the responses of females to host-plant volatiles were successfully investigated only by means of olfactometer assays (Natale *et al.* 2003, 2004a, 2004b; Piñero and Dorn 2007). For this reason, we used a dual-choice olfactometric arena, as described by Natale *et al.* (2004a, 2004b), to evaluate the attraction of virgin males and females to pear ester in the absence and presence of pear, peach, and apple shoots.

Materials and methods

Insects

Cydia molesta specimens were reared in the laboratory at the Università Cattolica del Sacro Cuore, Piacenza, Italy. Moths were obtained from an 8-year-old colony in peach orchards in Emilia Romagna (northern Italy) that had periodically been mixed with moths collected from other infested peach orchards. Larvae were reared on a semisynthetic diet consisting of corn semolina, wheat germ, and brewer's yeast. Pupae were sexed, placed in 1.5 L plastic bottles, and maintained at 23 ± 1 °C, a 16L:8D photoperiod, and $70 \pm 5\%$ RH. Adults 3–5 days old were used in the bioassays. Emergent males and females were kept separate and fed on a honey solution (10% in water). Moths were not exposed to any odour source before testing and each was used only once.

Electroantennography

We used a EAG technique similar to De Cristofaro *et al.* (2004) and Schmidt *et al.* (2007), using a standard EAG apparatus (Syntech, Hilversum, The Netherlands) and software for response analysis (EAG 2000, Syntech, Kirchzarten, Germany) EAG responses of *C. molesta* to pear ester (Sigma–Aldrich, Milano, Italy, >97% purity) were recorded. Nine hexane

solutions of the kairomone were prepared at concentrations ranging from 10^{-6} to 10^2 $\mu\text{g}/\mu\text{L}$. Aliquots of 10 μL of the test solutions, corresponding to dosages ranging from 1 mg to 10 μg , were adsorbed onto pieces of filter paper (1 cm^2) that were then inserted into individual Pasteur pipettes. The solutions were applied in ascending order of concentration at 60 s intervals. Before and after each series of stimuli, a reference stimulus (10 $\text{ng}/\mu\text{L}$ of pear ester) was applied in order to detect any decrease in the sensitivity of the antennal response. For each species, responses (mV) of males and females ($n = 10$) were recorded. Absolute EAG amplitudes were calculated as described by Den Otter *et al.* (1988, 1991).

Dual-choice-arena bioassays

The olfactory responses of virgin male and female *C. molesta* to pear ester were determined in a dual-choice arena. Pear ester dosages of 0.1, 3, 10, and 40 mg were loaded into rubber-septum dispensers (Trécé Inc., Adair, Oklahoma, United States of America), which were held for three days in the open air at 23 ± 2 °C to allow release stabilization prior to assays. The dual-choice olfactometric arena was made up of a 10 L cylindrical glass bottle (test chamber) connected to a pair of 300 mL cylindrical flasks (odour chambers). Thirty insects were released into the test chamber simultaneously and allowed to move up to the odour chambers, where they were collected. The odour chambers were positioned in such a way as to preclude attraction by visual stimuli. The tests were performed for 16 h at 24 ± 1 °C, $60 \pm 5\%$ RH, starting 3 h before the onset of scotophase and ending 5 h after the onset of photophase (2500 lx). Response was recorded as the percentage of moths that chose one of the two odour sources. Percentages were calculated also by counting the individuals that remained in the test chamber (scored as “no choice”). The treatments were moved from one odour chamber to the other after each replicate. Two experiments were performed.

Test 1: pear ester dosage versus control

In the first trial the response behaviour of *C. molesta* to different dosages of pear ester

(0.1, 3, 10, and 40 mg) was compared with the behaviour of those in a blank control (empty flask). The dosages assayed were equal to or higher than those that elicited the strongest EAG responses (see Results). For each dosage, five replicates each with 30 different moths were performed. The control was performed using one empty chamber and a blank rubber-septum dispenser, to verify that the insects were not visually attracted to a particular chamber.

Test 2: pear ester versus host-plant odour

The second trial was carried out to assess the preference of the moths for pear ester (0.1 mg) over a natural host plant odour source. The 0.1 mg dosage was the most significantly active in test 1 (see Results). For test 2, a young shoot with six leaves, excised from a plant 10 min before the beginning of the test and with the cut end of the stem sealed with paraffin, was put in one of the odour chambers. The cultivars utilized were ‘Red Top’ peach, ‘Abate Fétel’ pear, and ‘Golden Delicious’ apple. The experiment was replicated three times for apple and pear and five times for peach. Thirty moths were released for each replicate. After each group of insects had been tested, the plant material was replaced.

Statistical analysis

Differences in EAG responses between males and females for each concentration were evaluated using an unpaired Student’s *t* test. Differences across concentrations in the EAG responses of both males and females were analyzed using a one-way analysis of variance followed by Fisher’s protected least significant difference test (Cosse and Baker 1999; Das *et al.* 2007).

The choices made by *C. molesta* in the dual-choice arena were analyzed using a one-sample χ^2 test (Yates-corrected) against an expected 50:50 ratio.

Results

Electroantennography

Male and female *C. molesta* showed significant increases in their EAG responses with the concentration of pear ester (males: $F_{[8, 81]} = 3.0$, $P < 0.05$; females: $F_{[8, 81]} = 3.4$, $P < 0.05$). Males responded significantly to 100, 10, and

Table 1. Electroantennographic (EAG) responses (mean \pm SD) of male and female *Cydia molesta* ($n = 10$ per sex) to nine concentrations (10^{-6} to 10^2 $\mu\text{g}/\mu\text{L}$) of ethyl (*E,Z*)-2,4-decadienoate (pear ester).

| Concn. (Log_{10} $\mu\text{g}/\mu\text{L}$) | EAG response (mV) | | Student's <i>t</i> test (df=18) |
|--|------------------------------|------------------------------|---------------------------------|
| | ♂ | ♀ | |
| -6 | 0.26 \pm 0.12a | 0.21 \pm 0.11a | $t = 0.2, P = 0.82$ |
| -5 | 0.25 \pm 0.12a | 0.20 \pm 0.10a | $t = 0.1, P = 0.89$ |
| -4 | 0.28 \pm 0.13a | 0.15 \pm 0.02a | $t = 0.3, P = 0.75$ |
| -3 | 0.29 \pm 0.12a | 0.16 \pm 0.10a | $t = 0.4, P = 0.70$ |
| -2 | 0.26 \pm 0.11a | 0.42 \pm 0.12b | $t = 0.4, P = 0.69$ |
| -1 | 0.25 \pm 0.12a | 0.41 \pm 0.10b | $t = 0.3, P = 0.80$ |
| 0 | 0.78 \pm 0.23b | 0.87 \pm 0.29c | $t = 0.3, P = 0.76$ |
| 1 | 0.77 \pm 0.24b | 0.94 \pm 0.24c | $t = 1.0, P = 0.32$ |
| 2 | 0.69 \pm 0.23b | 0.92 \pm 0.25c | $t = 0.6, P = 0.58$ |
| ANOVA | $F_{[8,81]} = 3.0, P < 0.05$ | $F_{[8,81]} = 3.4, P < 0.05$ | |

Note: A different letter within a column indicates a significant difference (Fisher's protected protected least significant difference test).

1 $\mu\text{g}/\mu\text{L}$, whereas females responded to these concentrations as well as to 0.1 and 0.01 $\mu\text{g}/\mu\text{L}$ (Table 1). Males and females exhibited their strongest responses at the three highest dosages, ranging from 1 to 0.01 mg. There was no significant difference in EAG responses between males and females ($P > 0.05$ in all cases) (Table 1).

Dual-choice-arena bioassays

Test 1: pear ester dosage versus control

Male *C. molesta* showed a significant preference for pear ester over the blank control at three of the dosages tested (Fig. 1). The number of males choosing pear ester was particularly high at the lowest dosages: 79 *versus* 10 in the 0.1 mg test ($\chi^2 = 29.7, P < 0.001$) and 44 *versus* 5 in the 3 mg test ($\chi^2 = 16.6, P < 0.001$). A higher percentage of males remained in the test chamber as the pear ester dosage increased. Similar numbers of insects were found in the control chamber at all dosages tested. In preliminary tests where the insects were given a choice between two empty chambers, almost all remained in the test chamber. There was no difference between the blank rubber-septum and empty-chamber controls, demonstrating the absence of visual stimuli (Fig. 1; $\chi^2 = 0.7, \text{n.s.}$). Females showed a low rate of response to pear ester at all dosages (Table 2). In this case, almost all insects opted for "no choice." For this reason, no differences in preference for pear ester

versus the control were recorded (10 mg pear ester: $\chi^2 = 2.9, \text{n.s.}$). In the control experiment, no influence of visual stimuli emerged (Table 2: $\chi^2 = 0.3, \text{n.s.}$).

Test 2: pear ester versus host-plant odour

The response rate was extremely low when females were tested with pear ester *versus* host-plant odour (Table 2). Males showed a significant preference for pear ester over pear and apple shoots ($\chi^2 = 4.3, P < 0.05$, and $\chi^2 = 28.3, P < 0.001$, respectively) (Fig. 2). The percentage of males attracted to pear ester decreased from 90% (0.1 mg pear ester *vs.* control) to about 65% (0.1 mg pear ester *vs.* pear or peach shoots: $\chi^2 = 9.5, P < 0.01$, and $\chi^2 = 6.4, P < 0.05$, respectively).

Discussion

EAG recordings demonstrated that male and female *C. molesta* were able to detect pear ester and that their responses were dose-dependent, even though their olfactory sensitivity was lower than that of other tortricid species (Light *et al.* 2001; Schmidt *et al.* 2007).

Only males were attracted to pear ester in test 1. Moreover, when pear ester and host-plant odour were presented simultaneously, males were also attracted to host-plant shoots. We could not demonstrate attractiveness of pear ester to females. However, Natale *et al.*

Fig. 1. Responses (%) of male *Cydia molesta* to various dosages of ethyl (*E,Z*)-2,4-decadienoate (pear ester) loaded into a rubber-septum dispenser, including one test to compare a blank septum (control) with an empty flask (test 1), in bioassays performed in a dual-choice olfactometric arena. The numbers denote numbers of moths collected in either of two odour chambers ($n = 5$ replicates with 30 moths each; χ^2 test: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P > 0.05$).

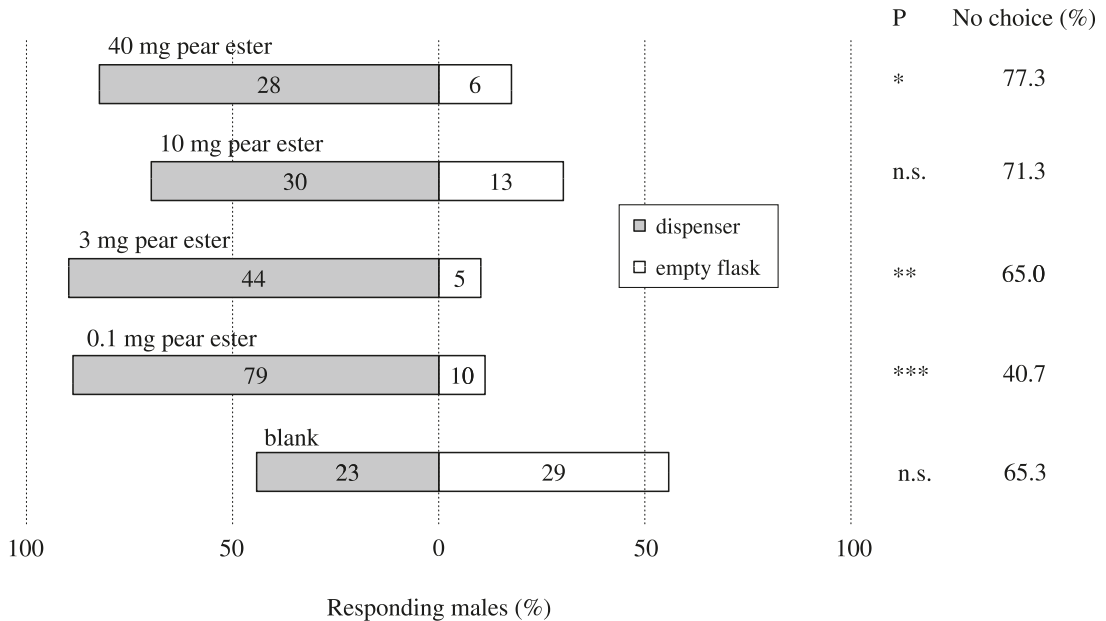


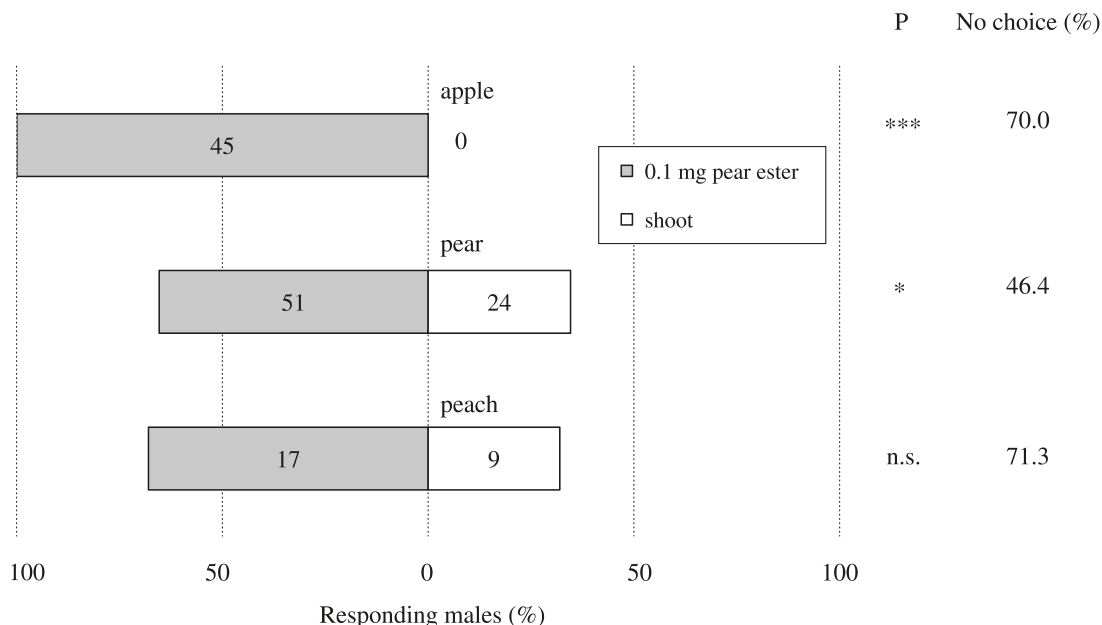
Table 2. Results of bioassays performed in a dual-choice olfactometric arena.

| Test 1.* | | | | |
|------------|----------------|-----------------------|-----------------|---------------|
| | Pear ester (%) | Blank dispenser (%) | Empty flask (%) | No choice (%) |
| Pear ester | | | | |
| 0.1 mg | 3.3 | | 3.3 | 93.3 |
| 3 mg | 2.7 | | 1.3 | 96.0 |
| 10 mg | 12.0 | | 3.3 | 84.7 |
| 40 mg | 0.7 | | 3.3 | 96.0 |
| Control | | 5.3 | 4.0 | 90.7 |
| Test 2.† | | | | |
| | Shoot (%) | 0.1 mg pear ester (%) | No choice (%) | |
| Apple | 0 | 0 | 100.0 | |
| Pear | 0.7 | 2.0 | 97.3 | |
| Peach | 1.7 | 0 | 98.3 | |

*Responses of female *Cydia molesta* to different dosages of ethyl (*E,Z*)-2,4-decadienoate (pear ester) loaded into a rubber-septum dispenser, including one test to compare a blank septum (control) with an empty flask.

†Responses of female *Cydia molesta* to young shoots (apple, pear, or peach) versus 0.1 mg pear ester.

Fig. 2. Responses (%) of male *Cydia molesta* to young shoots of apple, pear, or peach and to 0.1 mg ethyl (*E,Z*)-2,4-decadienoate (pear ester) (test 2) in bioassays performed in a dual-choice olfactometric arena. The numbers denote numbers of moths collected in either of two odour chambers ($n = 5$ replicates with 30 moths each for apple and pear and $n = 3$ replicates with 30 moths each for peach; χ^2 test: ***, $P < 0.001$; *, $P < 0.05$; n.s., $P > 0.05$).



(2004a) recorded a “no choice” response by about 55% of mated females and 75% of virgin females exposed to peach-shoot odour, and a significant preference by females for host-plant odour over a blank. In all treatments, our “no choice” responses were particularly high, ranging from 85% to 100%. This suggests that the presence of pear ester affects females’ behaviour, reducing their mobility or attraction to the host plant.

Males were attracted to pear ester in both the absence and the presence of host-plant shoots. However, they showed a preference for pear ester over pear or apple shoots; no preference was recorded when pear ester and peach shoots were present. The percentages of “no choice” males were similar in tests involving apple and peach shoots and in which high levels of pear ester were used. When pear shoots and pear ester at 0.1 mg were used, significantly more insects responded. Nonetheless, an effect of higher dosages of pear ester was noted in males during our dual-choice-arena

bioassay in that their “no choice” responses gradually increased.

Knight and Light (2004) did not capture males or females when testing attraction of *C. molesta* to traps baited with 10 mg pear ester in peach orchards. We obtained the same result in peach orchards, using lures ranging from 0.1 to 40 mg pear ester (data not shown). However, our laboratory bioassays showed that pear ester elicits an attractant effect in males and that the response rate depends on the dosage tested and the host-plant odour with which the pear ester is in competition. It has been previously shown that background odours and unbalanced dosages of attractant plant compounds can strongly affect insect behavioural responses (Knudsen *et al.* 2008; Anfora *et al.* 2009). In conclusion, as indicated by Knight and Light (2004), pear ester may be more effective in attracting *C. molesta* when applied in orchards of secondary host plants, such as apple or pear, than when it is used in peach orchards. The results reported here

encourage the undertaking of further studies to explore the feasibility of using pear ester in monitoring and controlling *C. molesta* in apple and pear orchards.

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