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The pear ester ethyl (*E*,*Z*)-2,4-decadienoate as a potential tool for the control of *Cydia pomonella* larvae: preliminary investigation

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Abstract

Studies on the impact of the pear volatile ethyl (*E*,*Z*)-2,4-decadienoate (pear ester) on the behaviour of newly-hatched *Cydia pomonella* (L.) larvae were conducted. In general, on apple and pear fruits treated with the pear ester, the number of *C. pomonella* larvae that entered the fruit was lower than on untreated fruits, and so was the damage caused by the larvae. These effects are of potential importance for direct applications of the pear ester in *C. pomonella* control strategies, especially for improving the efficacy of larvicidal insecticides.

Key words: *Cydia pomonella*, kairomones, ethyl (*E*,*Z*)-2,4-decadienoate (DA 2313), host location, pear.

Introduction

Plant and fruit volatiles can affect the searching and host location behaviour of adults and larvae of *Cydia pomonella* (L.) (Lepidoptera Tortricidae), the most important and dangerous pest of apple, pear and walnut.

Several compounds affecting *C. pomonella* behaviour have been described: (*E*, *E*)-α-farnesene (Wearing and Hutchins, 1973; Hern and Dorn, 1999) that attracts adult and newly-hatched larvae over a short range; the esters hexyl and butyl hexanoate (Hern and Dorn, 2004), attractive to females in the olfactometer, and (*E*,*Z*)-2,4-decadienoate (pear ester), attractive to both males and females in the field (Light *et al*., 2001; De Cristofaro *et al*., 2002; Ioriatti *et al*., 2003; Coracini *et al*., 2003; Ansebo *et al*., 2004; De Cristofaro *et al*., 2004; Villa *et al*., 2004). Ioriatti *et al.* (2003) and Knight and Light (2005) suggested that the pear ester could be used for *C. pomonella* monitoring in combination with mating disruption control strategies. The attractant probably could also be applied directly on the crop, because it causes host location disruption in *C. pomonella* females (Pasqualini *et al*., 2004).

Many authors report that (*E*, *E*)-α-farnesene is attractive not only to *C. pomonella* adults, but also to newly-hatched codling moth larvae (Sutherland and Hutchins, 1972, 1973; Wearing and Hutchins, 1973; Sutherland *et al*., 1974; Susky and Sokolowsky, 1985; Bradley and Suckling, 1995; Hughes *et al*., 2003). In their olfactometer laboratory bioassays, Knight and Light (2001) demonstrated that also the pear ester was attractive to codling moth larvae. We therefore investigated the impact of the pear ester on the behaviour of *C. pomonella* larvae when in contact with pear ester-treated apple and pear fruits. In particular, we investigated the effects of the pear ester on newly-hatched larvae searching for the fruits.

Materials and methods

Laboratory and semi-field trials were conducted in 2003 and 2004. *C. pomonella* eggs and larvae used in the trials were mass-reared at the research station CRPV (Crop Production Research Centre), Cesena, Italy.

Laboratory experiments

No choice test on pear and apple fruits

The pear ester (microencapsulated formulation of ethyl (*E*,*Z*)-2,4-decadienoate; see also table 1) was applied at a rate of 12 ml/hl on 19 pear fruits and 20 apple fruits with a handheld sprayer ensuring thorough wetting of the fruits. An equal number of fruits were not treated, thus acting as control. Fruits were left to dry for one hour. Each fruit was then placed inside a transparent plastic glass (∅ 8 cm, 12 cm high), and 5 larvae were transferred on each fruit with an entomological brush.

Choice test on pear

Nineteen pear fruits were virtually divided into two halves along two opposite longitudinal lines, running from the stem end to the calyx end. One half of each fruit was treated with the pear ester, while the other half was left untreated (control). Prior to applying the pear ester (rate: 12 ml/hl) with a handheld sprayer, each fruit was placed into a special device made of foam rubber. The device was oriented vertically and had the shape of half a pear fruit, thus enabling the exposure of only one half of each fruit to the treatment. Fruits were left to dry for one hour. Once the spray had dried, each fruit was

placed inside a plastic glass, and 5 larvae were transferred on each fruit along the two separation lines between the treated and the untreated half. The plastic glasses, each containing one fruit and 5 larvae, were closed with parafilm, and kept inside an incubator (temperature 23-24ºC; relative humidity approximately 80%; photoperiod L:D=16:8) for one week. After one week, on each fruit, the number of entries was counted, and the type of entry (sting or deep entry) was recorded. In both trials, ripe pear fruits (cv. 'Conference'; diameter 50-60 mm) that had been stored in a refrigerator after harvest were used.

Choice test on apple

Twenty apple fruits were virtually divided into two halves along two opposite longitudinal lines, running from the stem end to the calyx end. One half of each fruit was treated with the pear ester (rate: 12 ml/hl), while the other half was covered with parafilm, and thus left untreated (control). Once the spray had dried (after one hour), 5 larvae were transferred on each fruit next to the stem end. Each fruit with its larvae was then transferred into a transparent plastic glass closed with parafilm, and kept in an incubator (same as above). The parafilm was removed after two days. After two weeks, the number of larvae that had entered the fruit was counted, and the type of entry (sting or deep entry) was recorded. In this experiment, ripe apple fruits (cv. 'Golden delicious'; diameter 60-70 mm) that had been stored in a refrigerator after harvest were used.

Statistical analysis

For both pear and apple fruits, non-parametric Kruskal-Wallis-tests were used to compare the number of entries on pear ester-treated and untreated fruits in the "no choice tests", and on pear ester-treated and untreated halves in the "choice test".

Semi-field trial

In a pear orchard, cv. 'Abate Fètel', 60-cm long branches, each bearing one single fruit at its end, were selected on different plants (one branch per plant). Branches were caged with white nylon net bags (length 1.2 m; width 0.5 m; mesh size 1 mm^2). Two already mated *C. pomonella* pairs were released in each cage. Females were allowed to lay eggs for two days. When most of the eggs had reached the black head stage, branches (5 branches per treatment) were exposed to the different treatments (see table 1) using a handheld sprayer. Five branches were left untreated/treated with an equal volume of water, thus acting as control.

Ten days after treatment application, the branches were cut off, and brought to the laboratory. On each branch, the number of eggs laid on the leaves and on the fruit was counted, and eggs were scored as either hatched or unhatched. We then counted the number of living larvae on each fruit, and, for each branch, we calculated the percentage of living larvae on the fruit on the total number of hatched eggs on the fruit and leaves.

The percentages of living larvae were compared across treatments using the non-parametric Kruskal-Wallis test, followed by Dunn's multiple comparison procedure based on Kruskal-Wallis rank sums (Hollander and Wolfe, 1973). We furthermore counted the total number of entries on the fruits exposed to the different treatments, and we recorded the type of damage (stings or deep entries).

Results

Laboratory experiments

No choice test on pear and apple fruit

In the "no choice test", a lower number of entries was recorded on pear ester-treated fruits than on untreated ones, even though differences where not significant (table 2).

Choice test on pear and apple fruit

Similar results were obtained in the "choice test": the number of galleries was higher on untreated halves than on pear ester-treated ones, even though differences where not significant (table 3). Moreover on untreated halves, all entries were deep (>0.5 cm), while they were shallow (stings) on treated halves (<0.5 cm).

Table 1. Treatments applied to the branches.

Treatment	Active substance (formulation, quantity of a. i.; company)	Applied rate
Untreated control	-	-
Gusathion (toxic standard)	azinphos-methyl (WP, 25 %; Bayer)	250 g/hl
Virus	CpGv (L, 10^{13} vg/l; Calliope)	100 ml/hl
Pear ester	(*E*,*Z*)-2,4-decadienoate (MEC, 5%; Trecé)	6 ml/hl
Pear ester + virus	(*E*,*Z*)-2,4-decadienoate (MEC, 5%; Trecé) + CpGv (L, 10^{13} vg/l; Calliope)	6 ml/hl+ 100 ml/hl
Pear ester	(*E*,*Z*)-2,4-decadienoate (MEC, 5%; Trecé)	12 ml/hl
Pear ester + virus	(*E*,*Z*)-2,4-decadienoate(MEC, 5%; Trecé) + CpGv (L, 10^{13} vg/l; Calliope)	12 ml/hl+ 100 ml/hl

Table 2. Number of entries (m ± s. e.) on pear and apple fruits in the *No Choice test*. Kruskal-Wallis ANOVA: pear H=2.02, p=0.15; apple H=2.96, p=0.08.

Treatment	pear		apple	
	pear ester	control	pear ester	control
Number of entries (m ± s. e.)	0.89±0.38	1.73±0.38	1.1±0.24	1.57±0.21

Table 3. Number of entries (m ± s. e.) on fruits and halves in the *Choice test*. Kruskal-Wallis ANOVA: pear H=2.21, p=0.13; apple H=0.35, p=0.55.

Treatment	pear		apple	
	pear ester	control	pear ester	control
Number of entries (m ± s. e.)	0.52±0.19	1.00±0.25	0.55±0.17	0.7±0.18

Table 4. Mean number of eggs laid, mean number and percentage of hatched eggs, and total number of stings and deep entries observed in the different treatments.

Treatment	No. eggs laid (m±s.e.)	No. hatched eggs (m±s.e.)	Percentage (m±s.e.) of hatched eggs (%)	Total no. stings	Total no. deep entries
Untreated control	27.0±0.6	7.8±0.1	44.0±0.8	7	3
Azinphos methyl	19.2±0.4	11.4±0.3	64.5±0.4	0	0
Virus	25.0±0.8	5.4±1.0	23.3±1.4	1	1
Pear ester (6 ml/hl)	34.8±0.7	14.2±1.1	49.1±0.8	3	1
Virus + pear ester (6 ml/hl)	29.8±1.2	9.6±0.8	63.0±0.6	0	1
Pear ester (12 ml/hl)	32.0±0.6	11.8±0.6	49.4±0.7	1	0
Virus + pear ester (12 ml/hl)	20.4±0.8	10.2±0.8	53.1±0.4	0	0

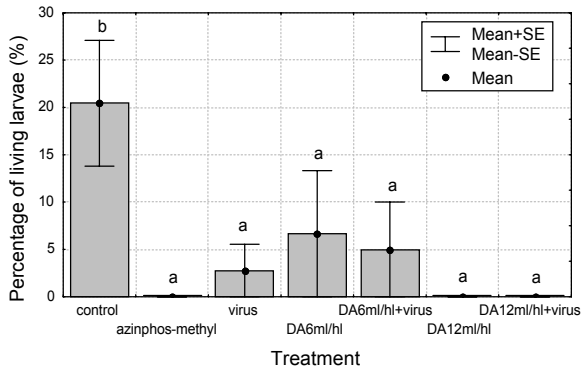

Figure 1. Percentage of living larvae on the total number of hatched eggs (%) in the different treatments (different letters indicate statistically significant differences: P<0.05).

Semi-field trial

The mean number of eggs laid, the mean number and percentage of hatched eggs on the branches exposed to the different treatments, and the total number of deep entries and stings observed on the fruits are reported in table 4.

In the untreated control, overall fruit damage consisted of 3 deep entries and 7 stings (table 4). On the branches treated with the toxic standard azinphos-methyl and on those treated with the pear ester at 12 ml/hl alone and in tank mixture with the virus, no living larvae were observed on the fruits, and thus also fruit damage was almost absent (1 sting for the treatment with pear ester at 12 ml/hl). In the other treatments (virus alone, pear ester at 6 ml/hl alone, and virus + pear ester at 6 ml/hl), fruit damage was highest when the pear ester had been applied alone (3 stings and 1 deep entry), intermediate with the virus alone (1 sting and 1 deep entry), and lowest when the two products had been applied together (1 deep entry).

Significant differences among treatments emerged for the percentage of living larvae on the fruit (Kruskal-Wallis test: $H_{(6, 34)}$=14.4427, p=0.0251): the percentage was significantly higher in the untreated control than in all the other treatments, while differences among the other treatments were not significant (figure 1).

Conclusions

The results of our preliminary laboratory studies indicate that (*E*,*Z*)-2,4-decadienoate (pear ester) may affect the behaviour of newly-hatched *C. pomonella* larvae, and that pear ester treatments may result in a quantitative and qualitative reduction of fruit damage. In fact, on pear ester-treated fruits, the number of entries was lower than on untreated fruits, and also the damage caused to the fruits was lower (more stings than deep entries). This lower number of entries may be due to *host location disruption*: in the presence of pear ester, the larvae are probably unable to locate with the same ability the fruits in which to develop, and are thus forced to "wander" without being able to reach the fruit. The lower number of deep entries may be due to a sort of lower aggressiveness, once the fruit has been reached. Those larvae that finally reach the fruit are un-

able to enter it. Their activity is limited to simple entry attempts, which result in shallow entries.

In our semi-field studies, the results obtained with applications of pear ester and the CpGv granulosis virus both alone and in tank mixture were comparable to those observed with the toxic standard azinphos-methyl. As under normal field conditions, the percentage of hatched eggs ranged from 21.6 to 59.4%, but the number of larvae that eventually reached the fruit was low also in the untreated control (Tremblay, 1986). We therefore think that these interesting and promising results should be corroborated in further experiments.

In conclusion, when exposed to pear ester treatments, *C. pomonella* larvae show reduced capacity to attack host fruits. Other studies showed that the pear ester may also directly and indirectly affect the oviposition behaviour of *C. pomonella* females (Pasqualini *et al*., in prep.). Therefore, since the pear ester can affect the behaviour of different developmental stages of *C. pomonella*, treatments with this pear ester could be useful to improve codling moth control, which has become increasingly difficult. (*E*,*Z*)-2, 4-decadienoate could be, as these first preliminary results show, a valuable tool in *C. pomonella* control strategies, to be used in combination with different insecticides and applied rate.

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References

ANSEBO L., CORACINI M. D. A., BENGTSSON M., LIBLIKAS I., RAMIREZ M., BORG-KARLSON A.-K., TASIN M., WITZGALL P., 2004.- Antennal and behavioural response of codling moth *Cydia pomonella* to plant volatiles.- *Journal of Applied Entomology*, 128 (7): 488-493.

BARDLEY S. J., SUCKLING D. M., 1995.- Factors influencing codling moth larval response to α-farnesene.- *Entomologia Experimentalis et Applicata*, 75: 221-227.

CORACINI M. D. A., BENGTSSON M., LIBLIKAS I., WITZGALL P., 2004.- Attraction of codling moth males to apple volatiles.- *Entomologia Experimentalis et Applicata*, 110: 1-10.

DE CRISTOFARO A., IORIATTI C., MOLINARI F., PASQUALINI E., ROTUNDO G., 2002.- Electrophysiological responses of codling moth populations from different host plants to (E,E)-8,10-dodecadien-1-ol and ethyl (2E,4Z)-2,4-decadienoate and interactions in perception of the two attractants, pp. 108-109. In: *Book of Abstracts of the OILB wprs Working Group Meeting "Pheromones and Other Semiochemicals in Integrated Production"*, Erice (TP), Italy, 22-27 September 2002.

DE CRISTOFARO A., ANFORA G., IORIATTI C., GERMINARA G. S., ROTUNDO G., 2004.- Occurrence of olfactory cells responding to pheromone components and plant volatile compounds in different species of Lepidoptera and Diptera: possible implications on semiochemical applications, pp. 83-84. In: *Book of Abstracts of the "OILB/srop 6th International Conference on Integrated Fruit Production – Use of pheromone and other semiochemicals in Integrated Control"*, Baselga di Piné (TN), Italy, 26-30 September 2004.

HERN A., DORN S., 1999.- Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to α-farnesene.- *Entomologia Experimentalis et Applicata*, 92: 63-72.

HERN A., DORN S., 2004.- A female-specific attractant for the codling moth, Cydia pomonella, from apple fruit volatiles.- *Naturwissenschaften*, 26:77-80.

HOLLANDER M., WOLFE D. A., 1973.- *Nonparametric statistical methods*.- John Wiley & Sons, New York.

HUGHES O. H. W., GAILEY D., KNAPP J., 2003.- Host location by adult and larval codling moth and the potential for its disruption by the application of kairomones.- *Entomologia Experimentalis et Applicata*, 106: 147-153.

IORIATTI C., DE CRISTOFARO A., MOLINARI F., PASQUALINI E., SCHMIDT S., ESPINHA I., 2003.- The plant volatile attractant (2E, 4Z)–2,4-ethyl-decadienoate for Codling Moth monitoring.- *Bollettino di Zoologia agraria e di Bachicoltura*, Ser. II, 35 (2): 127-135.

KNIGHT A. L., LIGHT D. M., 2001.- Attractants from 'Bartlett' pear for codling moth, *Cydia pomonella* (L.), larvae.- *Naturwissenschaften*, 88 (8): 339-342.

LIGHT D. M., KNIGHT A. L., HENRICK C. A., RAJAPASKA D., LINGREN B., DICKENS J. C., REYNOLDS K. M., BUTTERY R. G., MERRIL G., ROITMAN J., CAMPBELL B. C., 2001.- Ethyl (2*E*, 4*Z*)-2,4-decadienoate: a pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.).- *Naturwissenschaften*, 88 (8): 333-338.

PASQUALINI E., ESPINHA I., CIVOLANI S., MEDRZYCKI P., 2004.- Impact of the kairomone ethyl (2E, 4Z)-2,4-decadienoate (DA 2313) on the oviposition behaviour of *Cydia pomonella* on pear, p. 135. In: *Book of Abstracts of the "OILB/srop 6th International Conference on Integrated Fruit Production – Use of pheromone and other semiochemicals in Integrated Control"*, Baselga di Piné (TN), Italy, 26-30 September 2004.

SUTHERLAND O. R. W., HUTCHINS R. F. N., 1972.- α-farnesene, a natural attractant for codling moth larvae.- *Nature*, 239: 170.

SUTHERLAND O. R. W., HUTCHINS R. F. N., 1973.- Attraction of the newly hatched codling moth larvae (*Laspeyresia pomonella*) to synthetic stereo-isomers of farnesene.- *Journal of Insect Physiology*, 19: 723-727.

SUTHERLAND O. R. W., HUTCHINS R. F. N., WEARING C. H., 1974.- The role of the hydrocarbon α-farnesene in the behaviour of codling moth larvae and adults, pp. 249-263. In: *Experimental Analysis of Insect behaviour* (Burton Brown, ed.), Springer-verlag, Berlin.

SUSKI Z. W., SOKOLOWSKI R. J., 1985.- Some responses to α-farnesene of newly hatched larvae of the codling moth, *Laspeyresia pomonella* L.- *Ekologia Polska*, 33: 143-147.

TREMBLAY E., 1986.- *Entomologia applicata*, Vol. II (parte seconda), Ed. Liguori, Napoli.

VILLA M., MOLINARI F., PASQUALINI E., ESPINHA I., 2004.- Effects of the (E; Z)-2,4-ethyl decadienoate (pear ester) and synthetic pheromone blends to monitor *Cydia pomonella* adults, p. 143. In: *Book of Abstracts of the "OILB/srop 6th International Conference on Integrated Fruit Production – Use of pheromone and other semiochemicals in Integrated Control"*, Baselga di Piné (TN), Italy, 26-30 September 2004.

WEARING C. H., HUTCHINS R. F. N., 1973.- α-farnesene, a naturally occurring oviposition stimulant for the codling moth *Laspeyresia pomonella*.- *Journal of Insect Physiology*, 19: 697-710.

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