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Effects of the kairomone ethyl (2*E*, 4*Z*)-2,4-decadienoate (DA 2313) on the oviposition behaviour of *Cydia pomonella*: preliminary investigations

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Abstract

In 2002, 2003, and 2004, semi-field trials on the impact of ethyl (2*E*, 4*Z*)-2,4-decadienoate (pear ester), a pear volatile, on the oviposition behaviour of *Cydia pomonella* L. (Lepidoptera Tortricidae) were conducted. The effects of pear ester applications on oviposition (No. eggs laid) and egg distribution (distance of the eggs from the fruit) were investigated on two crops (apple and pear) on early and late varieties in different phenological stages (fruitlet stage and ripe-fruit stage). The pear ester did not affect the number of eggs laid by *C. pomonella* females, but it significantly affected egg distribution, favouring oviposition at greater distance from the fruit on late varieties in the ripe fruit stage. In some cases, the pear ester disrupted the oviposition behaviour of *C. pomonella* females (host location disruption), which could result in higher larval mortality (direct effect) and improve the efficacy of larvicidal insecticides (indirect effect).

Key words: *Cydia pomonella*, pear ester, oviposition, host location disruption.

Introduction

The economic impact of *Cydia pomonella* L. (Lepidoptera Tortricidae) on pome fruits is continuously increasing. Because of several reasons, e.g. climatic changes favouring *C. pomonella* development, the rather scarce efficacy of various insecticides on numerically growing populations, and the appearance of resistant populations (e.g. Forti *et al.*, 2000; Charmillot *et al.*, 2005), maintaining *C. pomonella* populations at acceptable damage thresholds has become difficult. The interest in alternative non conventional chemical strategies is consequently increasing. Among these strategies, the use of semiochemicals is particularly promising.

Semiochemicals can be used for *C. pomonella* control. Sexual pheromones are well-known and widely used for direct control all over the world, while studies on the effect of host plant volatiles that act as kairomones on *C. pomonella* adults and larvae are mostly restricted to their potential as attractants. α-farnesene has been demonstrated to be attractive for adults (Dorn and Hern, 1999) and larvae of codling moth (Sutherland and Hutchins, 1972, 1973; Sutherland *et al.*, 1974; Landolt *et al.*, 2000; Hughes *et al.*, 2002). Coracini *et al.* (2004) captured male and female of *C. pomonella* in the field and in the wind tunnel by some apple volatiles.

Recently, a pear derived isolated from Bartlett pear (ethyl (2*E*, 4*Z*)-2,4-decadienoate = Et-*E*,*Z*-DD) and commonly referred to as "pear ester", was shown to attract both male and female *C. pomonella* (Light *et al.*, 2001; Knight and Light, 2005) and to be a potential tool to monitor codling moth. In extensive monitoring study, promising results were obtained with pear ester (Ioriatti *et al.*, 2003; Sauphanor *et al.*, 2005; Trimble and El Sayed, 2005). Some authors (Avilla *et al.*, 2003; De Cristofaro *et al.*, 2004) investigated the mode of perception and electrophysiological response of pear ester in *C. pomonella* females and males. Ethyl decadienoate is responsible of the typical Bartlett pear odour, and is one of the substances released by ripening pear fruits (Jennings *et al.,* 1964; Quamme, 1984; Sawanagul and Richardson, 1998).

Plant metabolites also play a role as cues for egg laying by the females. Besides the volatiles compounds, Lombarkia and Derridj (2002) showed that certain substances (non volatile primary metabolites) present on the surface of apple leaves and fruits, act as kairomones by stimulating oviposition in *C. pomonella* females. *C. pomonella* females usually lay their eggs close to fruits or directly on fruits, but also on leaves and bark (Putman, 1962; Geier, 1963; Balachowsky, 1966; Wearing *et al*., 1973; Jackson, 1978, Thiéry *et al*., 1995). Usually, one single egg is laid on each fruit or leaf (Geier, 1963; Wood, 1965; Jackson, 1979). On apple, 75% of the eggs are laid within 6 cm distance from the fruit, whereas 90% of the eggs can be found within 10 cm distance (Wildbolz, 1958; Geier, 1963; Wearing *et al*., 1973; Blomfield *et al*., 1997).

In our studies we investigated the influence of the pear ester on the oviposition behaviour of *C. pomonella*. The effects of pear ester on oviposition (number of eggs laid) and egg distribution (distance of the eggs from the fruit) were studied under semi-field conditions on different crops (apple and pear) and varieties (early and late), and in different phenological stages (fruitlet stage and ripe-fruit stage). Trials were conducted in 2002 in commercial apple and pear orchards of the Emilia-Romagna Region, in 2003 and 2004 on pear in Emilia-Romagna Region and in 2004 on apple in Trentino Alto Adige Region.

As the response of *C. pomonella* to pear ester applications could depend on the amount of the attractant released by the crops themselves, in 2002 fruit volatiles were collected during the entire growing season in order to verify whether the attractant was released by apple and pear fruits in different phenological stages.

Materials and methods

Collection of fruit volatiles

In 2002, the volatiles released by the fruits of the four varieties used in the semi-field trials (pear: 'William' and 'Abate Fétel'; apple: 'Gala' and 'Golden delicious') were collected. Once a month, from June to September, that is, three times on the two early varieties 'William' and 'Gala', and four times on the late varieties 'Abate' and 'Golden', 500-550 g of apple / pear fruits were inserted into a glass jar (volume: 3,000 cc) with a glass lid. Two volatile collection techniques were used:

- SPME head space analysis
- Analysis of hexane extract

SPME (Solid Phase Microextraction) was used to collect volatile substances from the head space. Polydimethylsiloxane (100 µm) was the solid sorbent (Hern and Dorn, 2001). The syringe with the fibre was inserted through a lateral opening of the jar, so that only the fibre protruded into the head space. For each sample, volatile collection was performed for 22 ± 1 h at 25 °C. The fibre was then inserted immediately in the GC-MS. To collect the volatiles on cartridge filter, we used the methodology described in Benggtson *et al.* (2001). The volatiles were then extracted from the cartridge filter with 0.5 ml hexane (RS for HPCL, purity > 98%, Carlo Erba), and samples were stored in vials at – 15 °C. The presence of pear ester in the volatiles collections was analysed using GC-MS as reported in Tasin *et al.* (2005).

Effects on oviposition and egg distribution

In 2002, trials were conducted in four orchards near Lugo, province of Ravenna, Northern Italy, on early and late pear and apple varieties (pear varieties: 'William' and 'Abate Fétel'; apple varieties: 'Gala' and 'Golden delicious') in two phenological stages (fruit-let stage and ripe-fruit stage). On each variety, 8 branches of different length bearing one fruit, were selected on different plants. Distance among branches was at least 5 m, and branches were oriented vertically. Four of the selected branches were treated with a black gel formulation of pear ester (a.i. 5%; code Tre #9278, provided by Trecé Inc., Adair, OK, USA), by applying equally-sized pear ester drops along the entire branch (distance between drops: 1 cm). The other four untreated branches acted as control. Treated and untreated branches were then caged with white nylon bags (length 1.2 m; diameter 0.5 m; mesh size 1 mm^2), and 5 *C. pomonella* pairs 2-day-old were released in each cage. Prior to being transferred inside the cages, the *C. pomonella* males and females had been kept in mating cages for 2 days, to make sure that mating had occurred. Once transferred inside the cages including the branches, females were allowed to lay eggs for 4 days. After 4 days, the caged branches were cut off, and brought to the laboratory. Each branch was cut into 20 cm long sections, except for the section bearing the fruit, which was 10 cm long. The sections, leaves and side branches included, were examined under a binocular microscope, and the number of eggs on each section was counted.

Trials were repeated on the late pear variety 'Abate Fétel' in the fruit-let stage in 2003, and in the ripe-fruit stage in 2004. Branches (90 cm long in 2003; 50 cm long in 2004), each bearing one fruit, were selected and caged with nylon bags as described above. Half of them were previously treated with microencapsulated pear ester (5%) applied at a rate of 12 ml/hl water with a handheld sprayer, ensuring thorough wetting of the branches. The other branches were not treated, thus acting as control. Once the treatment dried 2 mated *C. pomonella* pairs were released in each cage in the fruit-let stage, while in the ripe-fruit stage 5 pairs/cage were released. For each treatment, 4 and 7 replicates were used, respectively in 2003 and 2004. In each year and trial, after 4 days, caged branches were brought to the laboratory, cut in sections (2003: 20 cm long sections except for the fruit-bearing section, which was 10 cm; 2004: 10 cm long sections), and the number of eggs present on each section was counted.

In 2004, trials were conducted also in an experimental farm holding at the Istituto Agrario of San Michele all'Adige (TN, Northern Italy) on the late apple variety 'Golden delicious' in the fruitlet stage and in the ripe-fruit stage. 50 cm long branches, each bearing one fruit, were selected and caged as described above, and 2 mated *C. pomonella* pairs were released in each cage. Half of the branches were treated with microencapsulated pear ester (see above); the other untreated branches acted as control. Each treatment was replicated 5-7 times. After 6 days, the caged branches were retrieved from the field, brought to the laboratory, cut into 20 cm long sections (10 cm for the fruit-bearing section), and the number of eggs on each section was counted.

In each trial and for each replicate, we determined:

1. total oviposition: number of eggs laid on each branch including the fruit;
2. egg distribution: the mean distance from the eggs to the fruit without considering the eggs laid directly on the fruit;
3. oviposition close to the fruit (fruit + 10 cm branch; fruit + 30 cm branch): the number of eggs laid on the fruit and on the branch within 10 and 30 cm distance from the fruit;
4. percent oviposition close to the fruit: number of eggs laid on the fruit and on the branch within 10 and 30 cm distance from the fruit on the total number of eggs laid on that branch.

Statistical analysis

The recorded values (total oviposition, egg distribution, oviposition close to the fruit, and percent oviposition close to the fruit) were compared between treatments (pear ester-treated and untreated branches) using the parametric Student t-test. Levene's test was used to verify homogeneity of variances. To correct for variability among groups of females in the number of eggs laid, egg distribution (distance of the eggs from the fruit) and the percentages of eggs laid close to the fruit (fruit + 10 cm branch; fruit + 30 cm branch) were processed in base of weighted values. The weighting variable was the total number of eggs laid on the branch, and the value for the degrees of freedom was computed as the number of observations minus one. To improve homoschedasticity, percentages of eggs laid close to the fruit were arcsen-transformed. All analyses were performed with STATISTICA® 6.0.

Results and discussion

Fruit volatiles

In the different phenological stages of apple and pear fruits, ethyl decadienoate was never detected among the volatile substances collected in the head space and it was not found in hexane extract, either. Release of ethyl decadienoate by fruits is therefore a variable that must not be considered when analysing the response of *C. pomonella* females to pear ester treatments.

Total oviposition and egg distribution

In all trials and study years, all females inside the cages were still alive at the end of the study period (0% mortality). The results of the statistical analyses concerning the total oviposition and egg distribution are reported in table 1. For the total number of eggs laid per branch, in none of the trials, significant differences between pear ester-treated and untreated branches were recorded (table 1). However, previous laboratory experiments showed that the pear ester, a chemical from ripe pear odour, stimulated egg deposition by codling moth on artificial substrate (Knight and Light, 2004).

For the egg distribution only on the branch (fruit excluded), significant differences emerged in 2002 and 2003: on the late pear variety at ripe fruit stage, the mean distance of the eggs from the fruit was higher on the pear ester-treated than on untreated branches (table 1).

For the branch sections "fruit + 10 cm branch", differences between treated and untreated branches in the No. eggs never were significant, while significant differences were observed for percent oviposition (No. eggs on the fruit and within 10 cm distance from the fruit on the total number of eggs laid on that branch) in two cases (table 1): in 2004, on the late varieties at the ripe fruit stage of both apple and pear, the percentage of eggs close to the fruit was significantly lower on pear ester-treated than on untreated branches.

For the branch sections "fruit + 30 cm branch", differences between treatments were significant neither for the No. of eggs on that section nor for percent oviposition close to the fruit (table 1).

For both the entire branch (fruit included) and the branch sections close to the fruit (fruit + 10 cm, fruit + 30 cm branch), the total number of eggs laid by *C. pomonella* females on treated and untreated branches were comparable. The pear ester thus did not have any effect on the number of eggs laid by the females.

However, in some cases the pear ester disrupted the host location of the females (higher mean distance of the eggs from the fruit and lower percentage of eggs laid closest to the fruit on pear ester-treated than untreated branches). Significant effects were always observed on late varieties in the ripe fruit stage. *C. pomonella* mated females are able to detect ripe fruits because of their volatile substances. The pear ester probably covered these volatile substances. The females probably were not able to identify the natural source, which resulted in host location disruption.

Even though for the branch section closest to the fruit (fruit + 10 cm), the preferential oviposition site of codling moth females in nature on apple and pear (Wildbolz, 1958; Geier, 1963; Wearing *et al*., 1973; Blomfield *et al*., 1997), significant effects on percent oviposition were observed only in two cases, in 11 out of 12 trials the percentage of eggs laid was lower on treated than untreated branches. In an applied perspective, a higher concentration of eggs at greater distance from the fruit could determine an increase in larval mortality because of the greater distance that must be covered by the young larva to reach the fruit (direct effect). Preliminary studies indicate that pear ester may disrupt host location also in *C. pomonella* larvae (Pasqualini *et al*., 2005). Furthermore, the efficacy of larvicidal insecticides could be improved because of the potentially longer exposure time (indirect effect).

No significant differences in percent ovipostion were recorded for the branch section "fruit + 30 cm". We posit the following possible explanation: the pear ester is active exclusively next to the fruit (i.e. fruit + 10 cm), the preferential oviposition site of *C. pomonella.* In fact, except for the preferential oviposition site, on all the other branch sections eggs were laid randomly all over the branch.

Finally, in three out of four cases, significant effects were observed on pear. Given the results of our laboratory analyses on the volatiles released by apple and pear fruits from the fruitlet stage to the ripe-fruit stage, it can be excluded that naturally released ethyl (2*E*, 4*Z*)- 2,4-decadienoate affected the pear ester treatments. However, the effect of pear ester could also have been modified according to the other background odours released by the host plants.

Conclusion

Due to the high inherent variability and the limited number of replicates, our results do not enable us to draw straightforward conclusions and further studies are warranted. Nevertheless, in the different years and phenological stages and on the different crops and varieties, the mean distance of the egg from the fruit was generally higher, and the percentage of eggs laid closest to

Table 1. Mean values of total oviposition, egg distribution, and number and percentage of oviposition close to the fruit (fruit + 10 cm branch; fruit + 30 cm branch) recorded on pear ester-treated and untreated branches, and results of the statistical analyses (E = early variety; L = late variety; FL = fruit-let stage; RF = ripe-fruit stage; DA = treated by DA2313; c = control).

trial		pear 2002 E FL		pear 2002 E RF		pear 2002 L FL		pear 2002 L RF		apple 2002 E FL		apple 2002 E RF		apple 2002 L FL		apple 2002 L RF		pear 2003 L RF		pear 2004 L RF		apple 2004 L FL		apple 2004 L RF	
variable analysed		**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**	**DA**	**c**
total oviposition	No. eggs	51.0	102.0	41.0	48.8	21.3	23.0	30.3	64.8	134.3	76.3	33.0	21.0	76.8	130.5	131.5	60.5	16.8	23.3	109.0	90.1	81.3	45.4	70.0	98.7
	σ	36.2	38.0	23.7	26.0	15.4	17.5	19.2	59.8	37.5	33.0	26.2	12.8	78.7	65.1	57.3	62.5	12.9	10.5	66.7	36.9	55.2	14.3	27.5	47.8
	t value	1.941		-0.441		-0.15		-1.098		2.32		0.824		-1.052		1.675		-0.779		0.655		1.405		-1.198	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.100		0.675		0.885		0.314		0.059		0.442		0.333		0.145		0.466		0.525		0.194		0.258	
egg distribution (only eggs on branch)	dist. (cm)	13.42	18.50	60.62	52.44	43.99	26.68	92.60	75.94	38.09	55.74	63.30	61.13	39.07	18.77	84.18	95.87	34.33	23.04	24.72	22.56	15.94	14.31	26.85	18.10
	σ	3.64	6.13	28.06	24.17	17.19	6.92	6.01	5.40	18.05	26.19	12.79	16.24	13.88	7.36	10.38	2.32	4.73	5.95	4.02	7.68	7.66	6.78	8.36	5.67
	t value	1.082		0.384		1.65		3.405		-0.974		0.182		2.342		-1.532		2.507		0.626		0.309		1.955	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.321		0.714		0.150		**0.014**		0.367		0.861		0.058		0.176		**0.046**		0.543		0.764		0.079	
oviposition close to the fruit (fruit + 10 cm branch)	No. eggs	30.5	38.0	8.0	17.5	6.0	7.0	0.5	12.5	18.5	10.8	1.8	3.0	23.3	62.0	0.0	0.0	1.0	3.5	28.3	42.1	36.2	26.4	13.4	42.9
	σ	16.1	24.0	11.6	20.6	5.0	4.9	0.6	22.4	20.4	21.5	3.5	2.9	27.8	42.6	0.0	0.0	0.8	3.7	17.5	13.0	48.1	12.2	11.6	29.4
	t value	0.519		-0.804		-0.287		-1.073		0.523		-0.547		-1.524		--		-1.321		-1.68		0.439		-2.103	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.622		0.452		0.784		0.325		0.620		0.604		0.178		--		0.235		0.119		0.671		0.062	
	%	59.80	37.25	19.51	35.90	28.24	30.43	1.65	19.31	13.78	14.10	5.30	14.29	30.29	47.51	0.00	0.00	5.97	15.05	25.95	46.75	44.47	58.15	19.14	43.42
	σ	21.55	13.17	28.04	23.36	7.33	19.78	2.89	15.70	9.94	23.88	5.57	22.15	15.21	21.22	0.00	0.00	7.10	13.56	7.55	21.58	31.08	26.25	15.27	15.81
	t value	1.592		-0.931		-0.217		-1.483		0.522		-0.578		-1.107		--		-1.119		-2.201		-0.694		-2.416	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.162		0.388		0.835		0.189		0.620		0.584		0.311		--		0.306		**0.048**		0.505		**0.036**	
oviposition close to the fruit (fruit + 30 cm branch)	No. eggs	48.8	89.5	11.8	21.8	9.5	13.8	0.8	14.0	44.8	19.8	3.0	5.5	33.8	108.5	2.0	0.0	3.5	12.0	71.3	58.1	71.3	38.6	36.6	83.3
	σ	32.8	53.3	18.4	25.6	7.9	8.4	0.5	21.5	38.9	39.5	5.4	3.4	38.5	65.4	4.0	0.0	2.1	7.4	57.9	13.1	53.6	11.9	21.8	48.3
	t value	1.302		-0.634		-0.738		-1.231		0.901		-0.787		-1.969		1		-2.201		0.586		1.326		-1.999	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.241		0.550		0.488		0.264		0.402		0.461		0.096		0.356		0.070		0.569		0.218		0.073	
	%	95.59	87.75	28.66	44.62	44.71	59.78	2.48	21.62	33.33	25.90	9.09	26.19	43.97	83.14	1.52	0.00	20.90	51.61	65.40	64.50	87.70	85.02	52.29	84.37
	σ	4.78	19.64	41.48	29.13	14.72	21.81	2.53	14.41	17.97	43.88	9.08	18.30	27.48	12.98	1.82	0.00	10.57	26.19	17.78	19.87	17.82	14.55	30.96	21.24
	t value	0.298		-0.653		-1.004		-1.433		0.387		-1.639		-2.114		1.152		-1.769		0.081		0.267		-1.925	
	d.f.	6		6		6		6		6		6		6		6		6		12		9		10	
	p	0.776		0.538		0.354		0.202		0.712		0.152		0.079		0.293		0.127		0.937		0.795		0.083	

Parameters evinced in grey are processed in according to weighted variables. The weighting variable was the total number of eggs on the branch.
Bold numbers indicate statistically significant differences (Student t-test: $p<0.05$)

the fruit was generally lower on pear ester-treated than on untreated branches. Thus, in presence of pear ester, *C. pomonella* females seem to be disoriented, and their ability of locating the normal oviposition sites (fruits or fruit proximity) seems to be impaired (host location disruption), especially on the branch section closest to the fruit, their preferential oviposition site, and in late varieties at the ripe fruit stage. On apple and pear varieties, pear ester applications could thus help to consistently reduce the risk of fruit damage because it may cause host location disruption in both larvae (Pasqualini *et al*., 2005) and adults.

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