Effect of sex pheromone emission on the attraction of *Lobesia botrana*

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Abstract

Since the discovery of *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) sex pheromone, it has played an important role in the control and detection of this pest, for example, through the use of pheromone-baited traps and mating disruption techniques. Rubber septa are the most common pheromone dispensers used in monitoring traps, but often dispenser performance is not optimized. The key to improve methods based on pheromones as attractants (monitoring, mass trapping, or 'attract and kill') is to know the optimum emission interval, because release rates can strongly affect the attraction. In this work, five levels of pheromone load with different release rates were compared in traps using mesoporous pheromone dispensers to investigate the optimum release rate maximizing *L. botrana* catches. Residual pheromone loads of the dispensers were extracted and quantified by gas chromatography, in order to study release profiles and to estimate the various emission levels. The efficacy of pheromone emission was measured in field trials as number of moths caught. A quadratic model was fitted to relate the numbers caught vs. the daily emission rates. The resulting quadratic term was statistically significant, confirming the existence of a relative maximum for *L. botrana* catches. Taking into account that the trial was carried out only in one location, an optimum emission value of ca. 400 µg per day could be considered to enhance the attraction of *L. botrana* under West-Mediterranean weather conditions.

Introduction

The European grapevine moth, *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae), is a key pest of grapes in Central Europe and most Mediterranean countries (Anshelevich et al., 1994). Pest damage is mainly caused by larvae feeding on grapes, which leads to fungal colonization of wounds and fruit rot. Traditional chemical control was the main tool to fight *L. botrana*, but since the identification of its sex pheromone in the 1970s (Roelofs et al., 1973), it has been widely used for almost 2 decades against this pest in Germany, Switzerland, and Northern Italy. In other European regions, however, the introduction of pheromone-based methods has been slower (Witzgall et al., 2010). Roelofs et al. (1973) described the main compound of *L. botrana* sex pheromone as (*E*,*Z*)-7,9-dodecadienyl acetate. Two related compounds were identified later, (*E*,*Z*)-7,9-dodecadien-1-ol and (*Z*)-9-dodecenyl acetate, having a synergistic effect on male catches (Arn et al., 1988; El-Sayed et al., 1999). These findings were crucial for the application of pheromone-based control and monitoring techniques. In fact, mating disruption is nowadays the most successful and widespread technique for controlling the moth in Europe. In addition, sex pheromone-baited traps were developed for monitoring *L. botrana* populations, playing an important role in pest detection and treatment timing. Rubber septa are the most common pheromone dispensers used in monitoring traps, but in most cases their performance is not optimized. A dispenser with an appropriate pheromone release rate is necessary to reach a good efficiency and to expand the use of pheromones in pest control systems.

The ideal dispenser should have a constant release rate during the whole flight period of the pest, independent of weather conditions (Jutsum & Gordon, 1989; Leonhardt et al., 1989; Bradley et al., 1995). In order to improve control methods based on pheromones as attractants (monitoring, mass trapping, or 'attract and kill'), the key factor is to know the optimum emission interval, because release rates will strongly affect the attractiveness of the lure, and catches could decrease below and above this interval (Jacobson & Beroza, 1964; Anshelevich et al., 1994; Zhang & Amalin, 2005). There are some reports of responses of *L. botrana* to different pheromone loads of dispensers (Roehrich et al., 1983; Anshelevich et al., 1994). However, emission rates were not assessed, so trap catches were not correlated with emission values and optimal release rates were not proposed.

The goal of our study was to correlate field trap catches with different pheromone emission values in order to study the optimum emission rate that maximizes the efficiency of the attractant for the control of *L. botrana*. For this purpose, five levels of pheromone load with different release rates of (*E*,*Z*)-7,9-dodecadienyl acetate (major active compound) were compared in traps using mesoporous pheromone dispensers. The efficiency of each emission level was measured in field trials as number of moths caught.

Materials and methods

Pheromone dispensers and traps

Three kinds of pheromone dispensers were employed for this trial. All of them were based on a mesoporous material (Corma et al., 1999, 2000), but they differed in size and pheromone load. Dispenser PD1 contained a pheromone load of 1 mg, and it was a cylindrical tablet, 9 mm in diameter and 3.5 mm high. The second (PD10) was loaded with 10 mg of pheromone, and the tablet was 13 mm in diameter and 7.5 mm high. A third dispenser (PD30) was loaded with 30 mg of pheromone, it was 13 mm in diameter and 20 mm high. (*E,Z*)-7,9-dodecadienyl acetate was used as the sex pheromone at 86% isomeric purity. The remaining 13% was the isomer (*E,E*)-7,9-dodecadienyl acetate, according to NMR analysis in our laboratory (data not shown). Previous work on *L. botrana* pheromone synthesis showed that the presence of the (*E,E*)-isomer in the blend did not interfere with the biological activity of the pheromone (Ideses et al., 1982). Pheromone was provided by Ecología y Protección Agrícola (Carlet, Spain) and dispensers were loaded with dichloromethane as solvent. For this trial, the mesoporous dispensers were manufactured by means of an industrial process that has around 15% of variability in the initial amount of pheromone (Ecología y Protección Agrícola).

Delta traps and sticky bases used in the field test were supplied by Biagro (Valencia, Spain). Each trap was baited with the corresponding pheromone dispensers, as described below.

Field trial

The field experiment was carried out from June to August 2009. The trial was designed as follows: four blocks of four traps were placed in a 4-ha Merlot vineyard, cultivated in trellis training. The orchard was in the centre of a 16-ha vineyard area located in Fontanars dels Alforins (Valencia, Spain); (Coordinates 38º 45’N, 0º 50’ E). Separation was 3 m between rows and 2 m between plants within each row. Distance between blocks was around 45 m and inter-trap distance was 50 m. Traps at each block were baited with a different pheromone dose and will be referred to hereafter as PD1 (one PD1 dispenser), 3PD1 (three PD1dispensers), PD10 (one PD10 dispenser), and 3PD10 (three PD10 dispensers). Thus, their initial pheromone load was 1, 3, 10, and 30 mg, respectively. All traps were hung at 1 m above the ground and their position inside each block was rotated weekly. None of these dispensers were replaced during the test period. The traps were placed on 2 June 2009 and the moths caught were counted weekly during 2 months. According to the results of the first weeks, it was decided to include a higher additional emission level, referred to as 3PD30, so four replicates of the trap baited with three PD30 dispensers (i.e., initial pheromone load 90 mg) were placed in the field 1 month later (24 June). Weather parameters were obtained from the nearest meteorological station located in Montesa (Valencia, Spain), at 20 km from the orchards.

Pheromone emission rates

During the trial, the three types of dispensers were aged in a vineyard located more than 2 km from the catch traps. Dispensers were placed on 2 June inside delta traps for 96 days. At different aging intervals a set of nine dispensers, three of each type, was taken to the laboratory to be analyzed.

In order to determine daily emission rates, initial pheromone loads, and the residual pheromone content of aged mesoporous dispensers were extracted in our laboratory by solvent extraction at 40 ºC for 2 h, using dichloromethane/methanol (2:3). The yield of all extractions was around 99%.

Extracts were centrifuged at 3 024 ***g*** for 8 min. The supernates were quantified by gas chromatography (GC) with flame ionization detector (GC/FID), using 1-dodecanol as internal standard. For these analyses, a Clarus 500 gas chromatograph from Perkin Elmer (Wellesley, MA, USA) was employed. All injections were made onto a ZB-5MS column (30 m × 0.25 mm × 0.25 µm) that was held at 150 ºC for 3 min and programmed at 20 ºC per min to 170 ºC, held at 170 ºC for 4 min, and then at 35 ºC per min to 260 ºC for 2 min. Helium was used as carrier gas at 1.2 ml per min with a split flow value of 30 ml per min.

Retention time of the pheromone component was confirmed by GC/FID analysis of commercial pheromone (86% isomeric purity; >99% chemical purity), provided by Shin-Etsu Chemical (Tokyo, Japan). The pheromone amount was calculated based on the ratio between the peak areas of the pheromone component and 1-dodecanol, by means of a simple regression model.

Statistical analysis

Our main goal was to study the pheromone emission effect on moth attraction and to determine the optimum emission value. First, a multiple linear regression analysis was carried out to model the evolution of residual pheromone load vs. time for each type of dispenser. The first derivative of the resulting equation provides an estimation of the daily emission rate.

Catch data were collected six times for 3PD30 traps and nine times for the others, once every week, during the trial period. The √x-transformation of the numbers caught was used to normalize the data. Following the methodology applied in a previous study (Vacas et al., 2009), multiple linear regression (MLR) was used to relate catch data to the emission rate, and to determine the relative maximum. The average number caught was highly variable from week to week. Therefore, polynomial terms of time were introduced as independent variables. Indicator variables were also considered in order to take into account the effect of block. This approach resulted in a rather complicated regression model. In order to obtain a simpler polynomial equation, the effect of time was removed prior to applying MLR by subtracting from each catch datum the average number of moths caught recorded in all traps at a given day. Statistical analyses were performed using the Statgraphics plus 5.1 package (StatPoint Technologies, Warrenton, VA, USA).

Results

Pheromone emission rates

The release profiles of (*E,Z*)-7,9-dodecadienyl acetate for the three types of dispensers employed in this study are shown in Figure 1. The residual pheromone load [P (µg)] was fitted by polynomial regression in the case of PD1 and PD10 dispensers. The independent variable was the number of days since dispensers were installed in the orchard [t (time)]. For PD1 dispensers, a cubic equation was obtained (equation 1), resulting in a coefficient of determination $R^2 = 0.951$. No outliers were identified.

$$P_{PD1} = 946.8 - 24.284 \cdot t + 0.311 \cdot t^2 - 0.001488 \cdot t^3 \qquad (1).$$

A cubic equation was also obtained for PD10 dispensers. Data at t = 0 did not fit properly and they were disregarded, as well as three outliers, resulting in $R^2 = 0.983$ (equation 2).

$$P_{PD10} = 11605 - 281.25 \cdot t + 3.40 \cdot t^2 - 0.01511 \cdot t^3 \qquad (2).$$

In the case of PD30 dispensers, the residual pheromone load follows an asymptotic trend (Figure 1) and it was fitted by means of a non-linear exponential model (equation 3; $R^2 = 0.891$).

$$P_{PD30} = 19333 + 11148 \cdot \exp(-0.06367 \cdot t) \qquad (3).$$

The constant in equation 1 (946.8) coincides with the nominal load of PD1, which was close to 1 000 µg. Similarly, when t = 0 in equation 3, P becomes 30 481, which is consistent with the initial load of PD30 dispensers. In the case of PD10, Figure 1 shows that the initial pheromone content was 10.8 mg, which is also close to the nominal value. The observed small differences are due to variability of the industrial manufacturing process.

The slope of the lines based on equations 1-3 is not constant (Figure 1), which implies that the daily emission rate of these pheromone dispensers decreases over time. This rate was estimated at day t_i as the first derivative of the fitted equations, i.e., dP/dt ($t = t_i$). Equations 4, 5, and 6 correspond to the first derivative of equations 1, 2, and 3, respectively. For example, 3PD1 traps inspected on 17 June correspond to traps collecting moths in the period of days 8-15 (i.e., t = 8 to t = 15). This trap contains three PD1 dispensers. Thus, the pheromone emission rate was estimated by applying equation 4 at t = 11.5 (i.e., the midpoint of the 8-15 period), and the resulting value was multiplied by 3. The release rate was assumed to be constant along the time interval. All estimated emission values are indicated in the Appendix.

$$\frac{dP_{PD1}}{dt} = -24.284 + 0.622 \cdot t - 0.004464 \cdot t^2 \quad (4),$$

$$\frac{dP_{PD10}}{dt} = -281.25 + 6.8 \cdot t - 0.04533 \cdot t^2 \quad (5),$$

$$\frac{dP_{PD30}}{dt} = -709.8 \cdot \exp(-0.06367 \cdot t) \quad (6).$$

Field trial: Trap catches

The period under study was characterized by the following average weather conditions (from June to August 2009): daily mean T = 25.8 ºC, 59% r.h., and 0.8 m/s wind speed. All traps showed population fluctuations of the pest though at different levels (Figure 2). First flight began around day 8 (10 June 2009), and the largest catches were recorded on day 22 (24 June). Second and third flights appeared on day 43 (15 July) and day 64 (5 August), respectively. These days correspond to the three flights of the moth cycle.

Most catch data recorded on 10 June and all data recorded on 18 August were null. Therefore, they were not further considered. Data of periods 43-52 and 52-57 were also rather low, 63% being zero. In order to overcome this lack of data variability, which is a problem if studying the effect of emission, both consecutive periods were merged as a single 43-57 interval (see the Appendix).

It was observed that the numbers caught in blocks B and D tend to be higher than in blocks A and C. Actually, by means of one-way ANOVA it was found that the square root of the numbers caught is significantly different between blocks A and C vs. B and D (F = 8.60; d.f. = 1,124; P = 0.004). This result could be explained by the clumped natural distribution of grapevine moth populations (Coscollá et al., 1997; Ifoulis & Savopoulou-Soultani, 2006).

In order to properly fit the square root of the numbers caught ($\sqrt{Nc}$) to time, block, and emission, it would be necessary to use indicator variables for blocks and polynomial terms of variable t, resulting in a rather complex equation. Instead, it seems preferable in this case to eliminate the effect of block and time prior to applying MLR. For data collected at blocks A and C, we calculated the difference between $\sqrt{Nc}$ and ASB_{AC} (average square root of all catch data recorded at blocks A and C). Similarly, for data collected at blocks B and D, $\sqrt{Nc} - ASB_{BD}$ was calculated (ASB_{BD} as average square root of all catch data recorded at blocks B and D). The resulting variable $\sqrt{Nc} - ASB$ accounts for the variability not explained by time or block that could be attributed to emission. Finally, a quadratic model was fitted to relate $\sqrt{Nc} - ASB$ to the estimated emission rates (values available in the supplementary material). Taking into account that emission values follow a positive skewed distribution, SRE (square root of emission) was regarded as the independent variable (equation 7).

$$\sqrt{Nc} - ASB = -0.784 + 0.129 \cdot SRE - 0.00322 \cdot SRE^2 \qquad (7).$$

The goodness-of-fit of equation 7 was low (R^2 = 0.142) but the regression coefficients were statistically significant (P≤0.0001). This result confirms the existence of a relative maximum of catches (Figure 3). Equation 7 was derived and equaled to zero, resulting in a square root of the optimum emission (SRE) of 19.9. Thus, the pheromone emission rate that maximizes attractant activity is: 19.9^2 = 396 µg per day.

By means of a normal probability plot, it was checked that residuals of equation 7 (i.e., observed minus predicted values) followed approximately a normal distribution and no outliers were identified. It was also found that two of the three highest data of emission act as influential points. Nonetheless, results are very similar if both data are discarded, and the quadratic term is still clearly significant (P = 0.0011). In order to study whether the effects of block and time were properly eliminated with the procedure applied prior to MLR, residuals of equation 7 were used as a dependent variable in a two-way ANOVA with factors block and time. The effect of both factors was not statistically significant (F = 0.05; d.f. = 1,117; P=0.83 for block; F = 0.20; d.f. = 6,117; P = 0.98 for time) .

Discussion

Although it is demonstrated that the presence of minor compounds in *L. botrana* pheromone formulations increases biological activity (Arn et al., 1988; El-Sayed et al., 1999), this work employed (*E*,*Z*)-7,9-dodecadienyl acetate to determine the existence of an optimum sex pheromone release rate, as it is the major pheromone component and the main compound responsible for the attraction (Roelofs et al., 1973; Ideses et al., 1982; Witzgall et al., 2005). The key factor to improve control methods based on pheromones as attractants (monitoring, mass trapping, or 'attract and kill') is to know the optimum emission rate, because insect response to the attractant could decrease below and above this optimal value (Jacobson & Beroza, 1964; Roelofs et al., 1977; Howse, 1998; Zhang & Amalin, 2005). The inhibitory effect of high pheromone doses has been reported for a number of lepidopterans (Roelofs & Cardé, 1974; Wyman, 1979; Millar et al., 1996). However, most of these works discuss insect responses based on initial pheromone loads of the dispensers, which does not give a conclusive idea about the actual release of pheromone, given that daily emission rates, and therefore the amount of airborne pheromone, will depend on dispenser type and weather conditions. The effect of pheromone dispenser type has been studied on maize stalkborer catches: polyethylene vials loaded with 1 mg pheromone caught significantly more moths than rubber septa loaded with the same amount of ingredient (Critchley et al., 1997). Release kinetics and dispenser field performance are key factors to develop efficient formulations for dispensers, and must be known to establish the relationships between attractant power and pheromone emission.

Some studies compare catches and pheromone doses for lepidopteran pests, resulting in a variety of relationships. Leonhardt et al. (1990) tested cotton wick dispensers for gypsy moth [*Lymantria dispar* (L.)] and proposed an optimal reference release rate of 11.3 µg per day, but plastic laminate dispensers could remain highly attractive by emitting at least 0.72 µg per day. Kehat and coworkers (1994) found increasing catches of codling moth [*Cydia pomonella* (L.)] males with increasing pheromone doses, within the range of 0.1 to 100 µg, but rubber septa loaded with 5 000 µg were significantly less attractive than 100 or 1 000 µg dispensers. Similar behavior was observed for rice leaffolder moth, *Cnaphalocrocis medicinalis* (Guenée) (Kawazu et al., 2004). Vacas et al. (2009) found decreasing catches of *Chilo suppressalis* (Walker) below and above an optimal release rate of 34 µg per day. And Jactel and coworkers

(2006) found an asymptotic increase response of catches of pine processionary moth (*Thaumetopoea pytiocampa* Denis & Schiffermüller) according to increasing doses of its pheromone from 0.5 to 20 mg, with 95% of maximum catch obtained with the 10-mg dosage. This asymptotic pattern has also been observed in other Lepidoptera species (Evenden et al., 1995; Knutson et al., 1998; Rao & Subbaratnam, 1998).

Many papers have studied the effect of dispenser type and pheromone load for a variety of insect families (Mason et al., 1990; Cork et al., 2001; Franklin & Gregoir, 2001; Branco et al., 2004; Kovanci et al., 2006). However, only few studies determined the optimal release rate of attractants (de Groot & DeBarr, 1998; Cross et al., 2006; Vacas et al., 2009). As mentioned above, catches do not always increase with increasing pheromone doses. Usually, catches increase up to an optimal dose. For higher values, trap catches could remain constant or decrease due to a repellent effect. An optimum pheromone load for *L. botrana* monitoring dispensers has been suggested by Roehrich et al. (1983), who found that pheromone loads between 1 µg and 10 mg allowed the detection of moths. Anshelevich et al. (1994) reported that *L. botrana* males responded positively to sticky traps baited with rubber septa loaded with increasing doses from 0.1 µg to 0.1 mg pheromone, but loads of 1-10 mg caught significantly fewer moths. However, emission rates were only measured for 1-mg septa, so trap catches were not correlated with emission values and optimal release rates were not proposed. These studies only reported optimum pheromone loads, but the values cannot be adopted as a reference, because it has been demonstrated that similar initial loads in different dispenser types may result in different release rates (Leonhard et al., 1990; Dominguez-Ruiz et al., 2008). Instead, the most suitable reference value to optimize the dispenser performance would be the optimum daily release rate, as this is the actual variable responsible for the airborne pheromone acting in insect attraction. Determination of this value could be of interest to develop more effective dispensers, so that they are able to emit pheromone at the optimum level.

This trial employed different mesoporous dispensers, with pheromone loads ranging from 1 to 30 mg, to obtain the optimum daily emission rate. Release profiles of PD1 and PD10 were fitted to cubic equations, implying that their emission rates were not constant. However, their life span was at least 100 days (Figure 1) and their residual pheromone loads, at the end of the period under study, were 15% of the initial load for PD1 (equation 1, $t = 100$) and 22% for PD10 (equation 2, $t = 100$). On the other hand, the release profile of PD30 was fitted to a model (equation 3) with an asymptote at 19 333 µg, which means that about 63% of its initial load was not released, and more than half of the pheromone load was wasted. This is not a suitable feature for an ideal dispenser, as pheromone accounts for 95% of the cost of the dispensers and the use of pheromone must be optimized. Thus, PD30 would need changes in its formulation or design to gain efficiency. However, the life span of PD30 dispensers was enough for the purpose of this work, which was to monitor the main flights of *L. botrana* in the study area.

This study concludes that releasing (*E,Z*)-7,9-dodecadienyl acetate, the major pheromone component of the European grapevine moth, at a rate of about 400 µg per day would maximize moth attraction under West-Mediterranean weather conditions. Although significant, the scope of the statistical relationship found between catches and emission could be somewhat limited. It should be stressed that the field trial was carried out only in one location and the optimum release rate could be affected by environmental conditions, specially the wind, in so far as pheromone plume is modified (Murlis et al., 1992). Nevertheless, this value could be generalized to catches of *L. botrana* under the average climatic conditions required for its development in temperate Mediterranean areas. An optimum release value is, in any case, a key datum for dispenser manufacturers, as well as a tool to improve *L. botrana* management methods based on pheromones.

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Figure legends

Figure 1 Release profiles of (*E*,*Z*)-7,9-dodecadienyl acetate, the major *Lobesia botrana* pheromone component, from the three kinds of dispensers tested. Fitted curves describe the pheromone content of the dispenser [P (μg)] vs. time (t = number of days in orchard). For equation 2, points indicated as diamonds (◊) were not taken into account to obtain the regression equation.

Figure 2 Average number of moths caught per trap and week (MTW) for each of five types of baited trap, with t the day of inspection (day 0 corresponds to 2 June 2009 at which most traps were installed). Baited traps were delta traps and dispensers were not replaced.

Figure 3 Scatter plot and fitted regression model (equation 7) of $\sqrt{\mathrm{Nc}} - \mathrm{ASB}$ vs. SRE (square root of emission). The dependent variable is the square root of numbers caught minus the average square root of catches collected at blocks A and C, or B and D (ASB).

Appendix Pheromone emission rates and numbers caught of *Lobesia botrana* in traps baited with pheromone dispensers

Day period[1]	Date[2]	Trap code[3]	Catches at each block[5] A	C	B	D	ASB[6] A-C	B-D	Emission (μg day^{-1})	proced.[7]
0-8	10 June	PD1	2	0	-	1	0.18	0.29		
		3PD1	0	0	1	0	0.18	0.29		
		PD10	0	0	0	0	0.18	0.29		
		3PD10	0	0	0	0	0.18	0.29		
8-15	17 June	PD1	1	0	5	4	2.54	3.90	18	$(4)_{t=11.5}$
		3PD1	4	5	10	13	2.54	3.90	53	$3\cdot(4)_{t=11.5}$
		PD10	15	8	16	16	2.54	3.90	209	$(5)_{t=11.5}$
		3PD10	15	20	37	37	2.54	3.90	627	$3\cdot(5)_{t=11.5}$
15-22	24 June	PD1	1	4	12	13	2.87	5.20	14	$(4)_{t=18.5}$
		3PD1	8	5	25	27	2.87	5.20	43	$3\cdot(4)_{t=18.5}$
		PD10	23	6	40	18	2.87	5.20	171	$(5)_{t=18.5}$
		3PD10	10	20	50	45	2.87	5.20	513	$3\cdot(5)_{t=18.5}$
22-29	1 July	PD1	8	4	19	12	2.44	4.18	11	$(4)_{t=25.5}$
		3PD1	3	4	21	25	2.44	4.18	34	$3\cdot(4)_{t=25.5}$
		PD10	7	6	33	17	2.44	4.18	137	$(5)_{t=25.5}$
		3PD10	13	3	20	27	2.44	4.18	412	$3\cdot(5)_{t=25.5}$
		3PD30[4]	-	9	10	3	2.44	4.18	1 704	$3\cdot(6)_{t=3.5}$
29-36	8 July	PD1	1	2	0	4	0.67	1.28	9	$(4)_{t=32.5}$
		3PD1	0	2	6	2	0.67	1.28	26	$3\cdot(4)_{t=32.5}$
		PD10	0	2	0	1	0.67	1.28	108	$(5)_{t=32.5}$
		3PD10	2	0	5	1	0.67	1.28	324	$3\cdot(5)_{t=32.5}$
		3PD30	0	0	-	2	0.67	1.28	1 091	$3\cdot(6)_{t=10.5}$
36-43	15 July	PD1	0	5	1	6	2.37	1.58	7	$(4)_{t=39.5}$
		3PD1	6	11	7	1	2.37	1.58	20	$3\cdot(4)_{t=39.5}$
		PD10	1	16	3	3	2.37	1.58	83	$(5)_{t=39.5}$
		3PD10	7	21	-	7	2.37	1.58	250	$3\cdot(5)_{t=39.5}$
		3PD30	6	1	1	0	2.37	1.58	699	$3\cdot(6)_{t=17.5}$
43-57	29 July	PD1	0	0	2	0	0.48	1.00	4	$(4)_{t=50}$
		3PD1	0	0	6	1	0.48	1.00	13	$3\cdot(4)_{t=50}$
		PD10	2	1	0	1	0.48	1.00	55	$(5)_{t=50}$
		3PD10	0	1	0	3	0.48	1.00	164	$3\cdot(5)_{t=50}$
		3PD30	0	2	2	1	0.48	1.00	358	$3\cdot(6)_{t=28}$
57-64	5 Aug	PD1	1	5	5	0	1.78	2.03	3	$(4)_{t=60.5}$
		3PD1	-	-	13	6	1.78	2.03	9	$3\cdot(4)_{t=60.5}$
		PD10	4	1	5	2	1.78	2.03	36	$(5)_{t=60.5}$

		3PD10	5	4	5	6	1.78	2.03	107	$3\cdot(5)_{t=60.5}$
		3PD30	-	4	5	2	1.78	2.03	183	$3\cdot(6)_{t=38.5}$
64-77	18 Aug	(all traps)	0	0	0	0	0	0		

[1]Day 0 corresponds to 2 June 2009, when all traps (except 3PD30) were installed.

[2]Date at which traps were inspected for counting.

[3]Initial pheromone load: 1 mg (PD1), 3 mg (3PD1), 10 mg (PD10), 30 mg (3PD10), and 90 mg (3PD30).

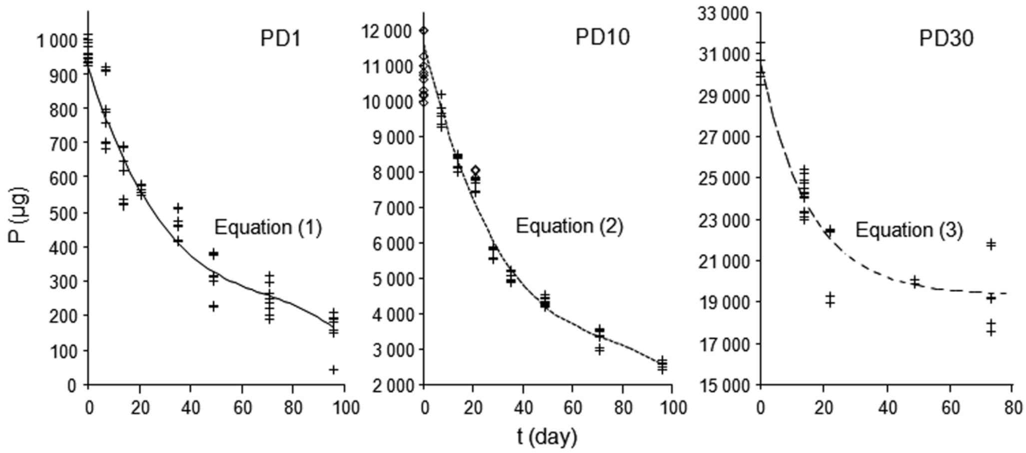
[4]Traps 3PD30 were set up on 24 June.

[5]No. moths caught.. Missing data are marked as '-'.

[6]Average of the square root of catches recorded at blocks A and C, or B and D.

[7]Procedure used to calculate emission values (see text for a detailed explanation). The equation used is indicated within parentheses, and t is the median number of days in orchard.

Fig1


PD1
PD10
PD30
P (µg)
Equation (1)
Equation (2)
Equation (3)
t (day)

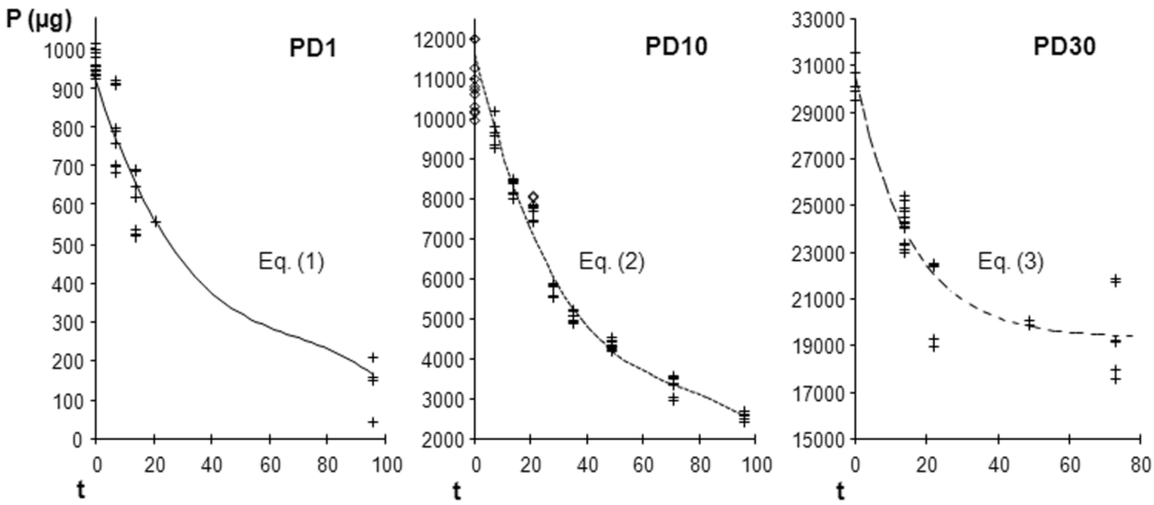

P (µg)
PD1
PD10
PD30
Eq. (1)
Eq. (2)
Eq. (3)
t
t
t

Fig2

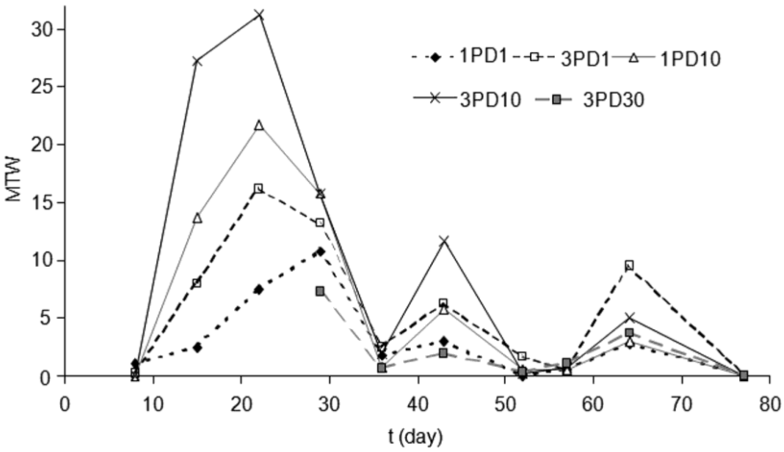

1PD1
3PD1
1PD10
3PD10
3PD30
MTW
t (day)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80

Fig3