Studies on the codling moth (Lepidoptera: Tortricidae) response to different codlemone release rates

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ABSTRACT

The response of the codling moth \( Cydia pomonella \) L. (Lepidoptera: Tortricidae) to different emission values of its main pheromone component, \( 8E,10E \)-dodecadien-1-ol (codlemone), was investigated in three field trials conducted in plots without mating disruption treatments. Moth catches obtained in traps baited with pheromone dispensers were correlated with the corresponding codlemone release rates by multiple regression analysis. In a preliminary trial conducted in Lleida (NE Spain), a decreasing trend of captures was observed based on increasing pheromone levels. After this, the pheromone release profiles of the pheromone dispensers were studied, in parallel with the field trials, by residual codlemone extraction and gas-chromatography quantification. In the trials carried out in Asturias (NW Spain), a correlation between trap catches and emission levels (within the range from 11 to 1078 \( \mu \)g/day) was found and fitted a logarithmic model. Captures followed a decreasing linear trend in the range of emission rates from 11 to 134 \( \mu \)g/day. Given that release values comprised between 11 and 67 \( \mu \)g/day did not lead to significantly different catches in traps, this emission range could be considered to develop effective formulations for attraction purposes when mating disruption is not acting in the environment.

KEYWORDS: \( Cydia pomonella \), pheromone, mesoporous dispensers, release rate, mating disruption
The implementation of pheromone-delivery technologies in pest management programs requires practical decisions on pheromone loads, blends, release rates and densities of dispensers. All these aspects depend on each particular release device and potentially impact efficacy of the control method (Weatherstone et al. 1985). Knowledge about optimum emission levels is a key factor to improve the control methods based on the use of pheromones to attract insects to traps or other kind of devices (monitoring, mass trapping, or ‘attract-and-kill’) because release rates severely affect the attractiveness of the lure, and catches may decrease below and above this level (Jacobson and Beroza 1964, Anshelevich et al. 1994, Zhang and Amalin 2005). In the same way, a dispenser with an appropriate pheromone release rate is also necessary to achieve good mating disruption efficiency and to extend its implementation. The cost of pheromone applied per hectare is critical for mating disruption treatments; thus, pheromone emission from dispensers must be controlled and optimized.

In the case of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) control, methods based on pheromones have become a cornerstone in orchard management programs offering an alternative to conventional insecticides, together with the microbial control agents, such as codling moth granulovirus (Miñarro and Dapena 2000, Arthurs et al. 2005) or entomopathogenic nematodes (Lacey et al. 2006). From its discovery and synthesis, the main component of the codling moth pheromone, 8E,10E-dodecadien-1-ol, codlemone (Roelofs et al. 1971), has been widely used for monitoring and implementing mating disruption as a commercially viable pest management technique. In recent years, mating disruption is a successful technique used to control codling moth on more than 160,000 ha worldwide (Witzgall et al. 2008).

Although a few studies have reported on codling moth response to traps baited with increasing pheromone loads (Kehat et al. 1994, Mitchell et al. 2008), emission rates were not assessed.
Thus, trap catches have not been correlated with emission values and optimal release rates for attraction have not been proposed. Moreover, thresholds of pheromone concentration, and thus emission rates, needed for communication disruption of codling moth are not yet established with certainty. Many studies have reported mating disruption thresholds for codling moth based on the experimental results of efficient treatments (Cardé et al. 1977, Charmillot 1990, Knight 1995, Vickers et al. 1998). However, the minimum emission rate for effective mating disruption has not yet been established.

The aim of this study was to determine which maximum emission should be employed for monitoring purposes in orchards without a background level of pheromone. For this purpose, dose-response correlations were studied by comparing different codlemone release rates using traps baited with pheromone dispensers in three field trials conducted in two different provinces of Spain with different climates. Calculated emission rates were correlated by multiple regression analysis with their corresponding catches achieved.

**Material and Methods**

**Pheromone Dispensers and Traps.** New pheromone dispensers, with different loads and sizes, were elaborated based on the technology of inorganic molecular sieves (Corma et al. 1999, 2000). The dispenser matrix is sepiolite, a natural clay mineral with a high adsorptivity for organic molecules. Sepiolite is impregnated with the corresponding amount of pheromone in dichloromethane solution and different additives to give consistency and protect the dispenser against humidity. The impregnated material is then compressed in a cylindrical mold by means of a hydraulic press. The technology of mesoporous dispensers has been employed as part of the Adress System commercialized by Syngenta (Madrid, Spain) against *Ceratitis capitata* (Wiedemann) (Navarro- Llopis et al. 2007) or more recently, for mating disruption dispensers.
against the California red scale (Vacas et al. 2009, 2010). The manufacturing process has been licensed to Ecologia y Protección Agrícola S.L. (Valencia, Spain) who has manufactured the dispensers for these trials.

Two mesoporous cylindrical tablets were formulated for the preliminary Lleida-2011 trial: C5 with 5 mg of pheromone load, 10 mm in diameter and 4 mm high; C30 loaded with 30 mg (13 mm diameter, 11 mm high). The C5 formulation was also employed in the trials carried out later in Asturias. A new mesoporous dispenser loaded with 50 mg of pheromone (C50) was included in the Asturias-May 2012 trial to obtain higher emission levels; these were also cylindrical tablets 13 mm in diameter and 11 mm high. The Asturias-July 2012 trial included a new formulation C1 loaded with 1 mg of pheromone (10 mm diameter, 3 mm high). Codlemone was employed as sex pheromone at 93% purity, provided by Bedoukian Research Inc. (Danbury, CA, USA).

The delta traps and sticky bases used in the trials were supplied by Sanidad Agrícola Econex, SL (Murcia, Spain). Each trap was baited with the corresponding pheromone dispensers, as described in the next section.

Field Trials

**Preliminary Lleida-2011 Trial.** The first field experiment was carried out in a 10 year-old 7-ha apple orchard located in the municipality of Bellpuig (province of Lleida – NE Spain; 41° 38’ N, 1° 2’E) in July and August 2011. The orchard cultivars were Royal Gala and Golden Suprema. Orchards received one ovicidal treatment (fenoxycarb) to control the first generation and four organophosphate insecticide applications throughout the season, using pheromone traps as indicators of the pest level. Orchards did not have mating disruption treatments. To evaluate the capture efficiency of different pheromone emission levels, five traps were used in five fully randomized blocks, baited with the following pheromone dispensers: (A5) 1x5-mg dispenser,
3x30-mg dispensers. Traps were hung in the canopy of apple trees at an approximate height of 2.5 m and were spaced at least 25 m apart, while blocks were placed 30 m away. Traps were rotated once a week in the block, and trials finished after two complete trap position rotations.

Traps were placed in the field from 22 July 2011 to 29 August 2011.

**Asturias-May 2012 Trial.** Based on the preliminary results, we decided to perform a second field trial to test the existence of pheromone release thresholds that reduce trap catches by including higher emission rates. Six cider-apple orchards located in Asturias (NW Spain; 43º 30'N, 5º 30'W) were selected. All the orchards were managed following organic guidelines (Table 1). The distance between orchards varied between 150 m and 18.5 km. To evaluate the catch efficiency of the different emission levels, five traps with different pheromone dose were placed at each orchard. Pheromone dose in each one of the five traps per orchard was: C5 (1 x 5-mg dispenser), C20 (4 x 5-mg dispensers), C50 (1 x 50-mg dispenser), C100 (2 x 50-mg dispenser), and C200 (4 x 50-mg dispensers). The intertrap distance was at least 30 m. Traps were hung at 1.5 m above the ground, and were revised and rotated weekly from 10 May 2012 to 6 June 2012. The characteristics of each plot are described in Table 1.

**Asturias-July 2012 Trial.** The experiment was carried out in July and August 2012 in the same apple orchards and with the same methodology described above. The traps in each block were baited with a different pheromone dose and are referred to hereafter as C1 (1 x 1-mg dispenser), C3 (3 x 1-mg dispensers), C5 (1 x 5-mg dispenser), C10 (2 x 5-mg dispensers), and C20 (4 x 5-mg dispensers). Traps were placed on 11 July 2012, and the moths caught were counted weekly for five weeks.
**Pheromone Release Profiles.** Additional dispensers were simultaneously aged under field conditions in nearby areas of the trial orchards in Asturias, to be periodically gathered and analyzed to study their release profiles. The residual codlemone content was extracted at different aging dates. Three dispensers per ageing date were extracted by solvent extraction at 40°C for 2 h, with magnetic agitation, in a particular volume of dichloromethane as follows: 2, 5 or 25 ml for dispensers C1, C5 and C50 respectively. Extracts were then analyzed by gas chromatography with a flame ionization detector (GC/FID), and pheromone content was quantified using \( n \)-heptadecane as the internal standard. After 1 hour of extraction, 0.5 ml of the internal standard solutions were added with the following concentrations: 1 mg/ml in extracts of dispensers C1 and 6 mg/ml in extracts of dispensers C5 and C50. All the analysis were performed using a Clarus®500 gas chromatograph (PerkinElmer Inc., Wellesley, USA) equipped with a ZB-5 (30 m × 0.25 mm × 0.25 mm) capillary column (Phenomenex Inc., Torrance, CA), maintained at 120°C for 2 min and then raised by 20°C/min to 260°C, to be then maintained for 3 min. Temperature of the injection port was 250°C, and FID detector was set at 300°C. The carrier gas was helium at 1.5 ml/min.

**Statistical Analysis.** The quantified residual pheromone loads \( P \) (mg) for each dispenser were fit by polynomial regression with the independent variable \( t \) (number of ageing days). The first derivative of the resulting equations provided an estimation of the daily emission rate.

The captures recorded in each trap, as moths per trap and day, were transformed by \( \sqrt{x} \) to normalize variance prior to applying a multifactor ANOVA (Fisher’s LSD test at \( P \leq 0.05 \)) to study the differences between trap catches according to three factors: week, block and emission level. Following the methodology applied in a previous study (Vacas et al. 2009), multiple or simple regression was used to study the relationship between catch data and the pheromone...
emission rates tested. First, a two-way ANOVA was performed with catch data only with factors week and block. The residuals of this ANOVA did not account for variance due to the two factors week and block, and still provided evidence for variance due to the emission level factor. Thus, these residuals were employed in the regression analysis to obtain the correlation explaining the effect of the emission factor over trap catches. Statistical analyses were performed using the Statgraphics Centurion XVI package (StatPoint Technologies, Warrenton, VA, USA).

Results

Preliminary Lleida-2011 Trial. In our preliminary trial (Lleida-2011), population levels were very low throughout the study period; in fact, only 44 moths were captured in the 25 traps. Therefore, analysis of variance was performed with the total numbers of moths captured per trap and day throughout the trial for the different baited traps. No significant differences were found for emission factor (F = 1.14; df = 4,16; P = 0.371, in Fig. 1), whereas the block factor was significant (F = 7.81; df = 4,16; P = 0.001) due to the natural clumped distribution of the pest. Despite not being significant, the data suggest a trend of decreasing capture with increasing pheromone release rates. According to this result, we tested higher pheromone emission rates in the Asturias-May trial to confirm the decreasing trend in the number of captures.

Pheromone Release Profiles. The release profile of mesoporous dispenser C1 is depicted in Fig. 2A. Multiple linear regression performed with the mean residual pheromone values demonstrated that the quadratic effect was not statistically significant for C1 (significance of the quadratic coefficient: P = 0.48) and that the residual pheromone (mg) content fitted the linear model (P < 0.001, R^2 = 0.98) given by Equation 1. Thus, the slope of the linear model gave the emission rate of the dispenser, which was assumed constant and equal to 11.0 μg/day throughout
the study period. Likewise, the release profile of C5 (Fig. 2B) fitted the linear model in Equation 2 (P = 0.002, R^2 = 0.94; significance of quadratic coefficient: P = 0.10), corresponding to a mean release value of 33.5 μg/day, throughout the study period. Finally, multiple linear regression showed that the quadratic effect was not statistically significant for formulation C50 (significance of the quadratic coefficient: P = 0.89), and that the residual codlemone content once again fitted a linear model (Equation 3; P = 0.003, R^2 = 0.91). Thus, the emission rate of dispenser C50 given by the slope of the linear model (Fig. 2C) was constant and equalled 269.5 μg/day.

\[ P_{C1} = 0.9485 - 0.0110 \cdot t \]  
(Eq. 1)

\[ P_{C5} = 5.2519 - 0.0335 \cdot t \]  
(Eq. 2)

\[ P_{C50} = 50.1351 - 0.2695 \cdot t \]  
(Eq. 3)

**Asturias 2012 Field Trials.** The sqrt-transformed catches were analyzed with multifactor-ANOVA, considering the factors emission, week and block. None of the possible interactions between factors resulted in statistically significant effects (week × block: F = 1.21; df = 15.59; P = 0.29, week × emission: F = 0.92; df = 12.59; P = 0.54, block × emission: F = 1.18; df = 20.59; P = 0.30) and were disregarded from the final analysis. The emission factor was statistically significant (F = 10.55; df = 4.106; P < 0.001), thus confirming the trend observed in Lleida-2011 trial. The higher the pheromone load, the fewer the catches obtained (Fig. 3A); the traps baited with 5 mg dispensers trapped significantly more moths than those baited with 50 mg dispensers. This suggests that the attractant power diminished with the emission level. Furthermore, the week factor was statistically significant (F = 7.71; df = 3.106; P < 0.001), according to the pest population dynamics, as well as the effect of the block factor (F = 5.32; df = 5.106; P < 0.001). According to the release studies described before, each baited trap had a different emission level. By considering release profiles of dispensers C5 and C50 and the calculated release rates
according to Eqs. 1 and 2, the emission factor could be considered a quantitative variable according to the following correspondence: C5 = 33.5 μg/day, C20 = 134 μg/day, C50 = 269.5 μg/day, C100 = 539 μg/day, and C200 = 1078 μg/day. A strong relationship was found by the regression analysis given the logarithmic model (P = 0.003, R² = 0.96) depicted in Fig. 4. Accordingly, catches dropped almost linearly with increasing emission rates from 33.5 to 269.5 μg/day; then, they continued to lower slightly up to the highest studied release level of 1,078 μg/day (Fig. 4). Then, captures were reduced by 86%, as compared with traps baited with C5 dispensers.

Smaller pheromone doses were tested in July, and the number of moths trapped in C1, C3, C5, and C10 traps were not significantly different (Fig. 3B); only when traps were baited with four C5 dispensers (C20) did mean captures start to decrease. The significance of the studied factors is given by the following statistics obtained by multifactor-ANOVA: week F = 13.88; df = 4,116; P < 0.001; block F = 6.21; df = 5,116; P < 0.001; and emission F = 5.25; df = 4,116; P = 0.003. Only the interaction between week and block was statistically significant and the other factors were consequently disregarded from the analysis (week × block: F = 2.78; df = 20,116; P < 0.001). This interaction resulted in a significant effect due to a reduction of captures in the block number 3 during the last week of trial, while captures increased in the other plots.

By considering the aforementioned release profiles for dispensers C1 and C5, the emission factor in this trial took the following values: C1 = 11 μg/day, C3 = 33 μg/day, C5 = 33.5 μg/day, and C20 = 134 μg/day. The linearity of the decreasing attraction of *C. pomonella* to codlemone-baited traps was confirmed by the multiple regression results depicted in Fig. 5 (P < 0.001, R² = 0.95). Thus, *C. pomonella* attraction could be promoted with codlemone emission rates up to 67 μg/day, while release rates above ca. 134 μg/day achieved significantly lower captures.
Discussion

The present work has employed different mesoporous dispensers, with pheromone loads ranging from 1 to 50 mg, as tools to study the codling moth response to different codlemone emission rates. Our preliminary trial suggested a decreasing trend of captures in accordance with increasing pheromone loads within the range 5-90 mg. This response has been previously reported in the literature: Kehat et al. (1994) found increasing catches of codling moth males with increasing pheromone doses, within the 0.1–100 μg range, but a 5,000 μg load was significantly less attractive than 100 or 1,000 μg loaded on a rubber septum. Similarly, Mitchell et al. (2008) showed that by increasing the load from 1 to 10 mg, the mean number of male moths captured decreased, while no differences were observed within the 0.01-0.1mg range. The same response was observed in the wind tunnel assays performed to develop an attract-and-kill strategy (Lösel et al. 2000). Maximal captures were achieved at a concentration of 0.065% codlemone in a 100 μl droplet, and a reduction of more than 50% in the average number of moths trapped was obtained with pheromone concentrations that were 10 times higher (0.65%). However, all these works address insect responses based on the initial pheromone loads of the dispensers, which do not provide a conclusive idea about actual pheromone release as it is highly affected by dispenser type. For example, Critchley et al. (1997) demonstrated that 1 mg-loaded polyethylene vials caught significantly more moths than rubber septa with the same amount of ingredient. In fact, rubber dispensers have non-linear kinetics, which means that emission can greatly vary between the beginning and the end of their lifespan and even on the same day due to temperature (Domínguez-Ruíz et al. 2008).

In the present work, field trap catches and pheromone release profiles of the dispensers employed were studied simultaneously and correlated to verify the existence of an optimum release value for attraction or whether the decreasing trend observed becomes asymptotic at
higher release rates. Although field trials were conducted in representative plots, the statistical
analysis performed takes the block factor as a fixed factor, and therefore results obtained are
valid only in the areas where trials were conducted. For this reason, field trials were conducted in
the two main apple growing areas of Spain; nevertheless, these results should be validated in
regions with different conditions and population levels.

In our experiments, it was found that emission rates within the range 11-67 μg/day did not lead
to significantly different catches in monitoring traps. At higher values, however, moth catches
decreased significantly. Nevertheless, the effect of very low emission rates (< 11 μg/day) remains
uncertain; probably, a positive relationship could be observed with increasing release rates in a
much lower range. With the data obtained, the multiple regression highlights a pronounced drop
in captures with codlemone emissions up to 269 μg/day, which continue slightly decreasing up to
the highest release level studied, that of 1,078 μg/day (only 2 moths were captured in the 6 traps
with this codlemone emission during the 4-week trial). This result was possibly due to sensory
adaptation or sensory overload effect in the vicinity of the lure, a mechanism that has been
proposed for mating disruption (Cardé and Minks 1995).

Although the minimum rate for effective mating disruption has not been established with
certainty, estimates vary widely and range from 2 to 40 mg/ha/h (Cardé et al. 1977, Charmillot
1990, Vickers et al. 1998), and may vary in any case depending on population density, tree size
and other environmental factors (Howell et al. 1990; McDonough et al. 1992). The
aforementioned mating disruption pheromone doses correspond to the individual dispenser
release rates within the 29-240 μg/day range, applied at 1,000-2,000 dispensers/ha. The
dispensers described by Angeli et al. (2007) fall within this emission range (mean ca. 56 μg/day);
however, these pheromone emission rates are 10-50 times lower than those of several other
commonly used dispensers for the conventional mating disruption of C. pomonella, with reported
mean release values from 0.6 to 3 mg/day (Brown et al. 1992, Knight 1995, Tomaszewska et al. 2005, Femenia 2011). These efficient mating disruption dispensers agree with the results presented herein as captures were virtually zero in the traps baited with codlemone dispensers releasing at a rate ca. 1 mg/day.

The use of pheromone dispensers for monitoring purposes allows following population dynamics, detecting the presence of adults, assessing mating disruption efficacy, and even establishing timings and thresholds for control measures. Yet the pheromone release rates should be standardized for many of these purposes. The application of synthetic pheromone in a mating disruption program may change the relative attraction of pheromone lures; consequently, monitoring dispensers loaded with 1 mg of pheromone can prove unreliable indicators of efficacy (Thomson et al. 2001), giving false negatives when used in a mating disruption environment. In this case, the sensitivity of pheromone traps can be improved by baiting traps with stronger lures (i.e. 10 mg of pheromone lures) to establish a high emission point source within a pheromone treated area (Charmillot 1990, Calkins et al. 2003). This applies not only to mating disruption efficacy assessments, but also in general to establish when control measures should be adopted. Insect response to the attractant can decrease below and above a particular emission interval (Jacobson and Beroza 1964, Roelofs et al. 1977, Howse 1998, Zhang and Amalin 2005); therefore, establishing treatment thresholds, according to trap catches, without including the actual release rates of the dispensers or employing suboptimal emission rates may result in underestimated population levels.

Attract-and-kill or attract-and-remove strategies are being studied as alternatives to mating disruption treatments (Charmillot et al. 2000, Lösel et al. 2000, Krupke et al. 2002, Reinke et al. 2012). As mentioned before, knowledge about optimum release rates is essential for control methods based on pheromones as attractants. When there is no pheromone background, the
emission range reported in this work (11-67 μg/day) could be considered to develop effective formulations for attraction purposes because commercial dispensers can be designed in accordance with this value for better pheromone use.

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Figure legends

Fig. 1. Mean ± SE number of moths caught per trap and day (MTD) for each of the five types of baited trap (A5, A15, A30, A60 and A90) tested in preliminary trial Lleida-2011. Bars labelled with the same letter are not significantly different (LSD test at P > 0.05).

Fig. 2. Release profiles of 8E,10E-dodecadien-1-ol (codlemone) from the C1 (A), C5 (B) and C50 (C) mesoporous dispenser employed in field trials carried out in Asturias (2012). Fitted linear models (Eqs. 1-3) describe the mean pheromone content of the dispenser [codlemone (mg)] vs. time (days of ageing). Three replicates were extracted per ageing time.

Fig. 3. Mean ± SE number of moths caught per trap and day (MTD) for each of the five types of baited trap tested in trials Asturias-May (A) and Asturias-July (B). Bars labelled with the same letter are not significantly different (LSD test at P > 0.05).

Fig. 4. Fitted regression (logarithmic) model, for trial Asturias-May data, of residuals vs. emission rates. The dependent variable is the residuals from the ANOVA applied to capture data (MTD) according to factors week and block.

Fig. 5. Fitted regression (linear) model, for trial Asturias-July data, of residuals vs. emission rates. The dependent variable is the residuals from the ANOVA applied to capture data (MTD) according to factors week and block.
Table 1. Description for Asturias trial orchards

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Municipality</th>
<th>Size (ha)</th>
<th>Age (years)</th>
<th>Insecticide treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Villaviciosa</td>
<td>1.1</td>
<td>11</td>
<td>Granulovirus + Neem*</td>
</tr>
<tr>
<td>2</td>
<td>Villaviciosa</td>
<td>0.5</td>
<td>5</td>
<td>Granulovirus + Neem*</td>
</tr>
<tr>
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<td>15</td>
<td>Granulovirus + Neem*</td>
</tr>
<tr>
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<td>1.1</td>
<td>15</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Villaviciosa</td>
<td>2.0</td>
<td>8</td>
<td>Granulovirus + Neem*</td>
</tr>
<tr>
<td>6</td>
<td>Sariego</td>
<td>0.7</td>
<td>14</td>
<td>None</td>
</tr>
</tbody>
</table>

*Granulovirus (Madex, Andermatt Biocontrol) was sprayed against the codling moth, and neem (NeemAzal-T/S, Trifolio GmbH) against the rosy apple aphid, *Dysaphis plantaginea* Pass.