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21 **Studies on the codling moth (*Lepidoptera: Tortricidae*) response to different codlemone**

22 **release rates**

23

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33

34

35 **ABSTRACT**

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

51

52 **KEYWORDS:** *Cydia pomonella*, pheromone, mesoporous dispensers, release rate, mating
53 disruption

54

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

67 In the case of the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) control,
68 methods based on pheromones have become a cornerstone in orchard management programs
69 offering an alternative to conventional insecticides, together with the microbial control agents,
70 such as codling moth granulovirus (Miñarro and Dapena 2000, Arthurs et al. 2005) or
71 entomopathogenic nematodes (Lacey et al. 2006). From its discovery and synthesis, the main
72 component of the codling moth pheromone, 8E,10E-dodecadien-1-ol, codlemone (Roelofs et al.
73 1971), has been widely used for monitoring and implementing mating disruption as a
74 commercially viable pest management technique. In recent years, mating disruption is a
75 successful technique used to control codling moth on more than 160,000 ha worldwide (Witzgall
76 et al. 2008).

77 Although a few studies have reported on codling moth response to traps baited with increasing
78 pheromone loads (Kehat et al. 1994, Mitchell et al. 2008), emission rates were not assessed.

79 Thus, trap catches have not been correlated with emission values and optimal release rates for
80 attraction have not been proposed. Moreover, thresholds of pheromone concentration, and thus
81 emission rates, needed for communication disruption of codling moth are not yet established with
82 certainty. Many studies have reported mating disruption thresholds for codling moth based on the
83 experimental results of efficient treatments (Cardé et al. 1977, Charmillot 1990, Knight 1995,
84 Vickers et al. 1998). However, the minimum emission rate for effective mating disruption has not
85 yet been established.

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

92

93

Material and Methods

94 **Pheromone Dispensers and Traps.** New pheromone dispensers, with different loads and
95 sizes, were elaborated based on the technology of inorganic molecular sieves (Corma et al. 1999,
96 2000). The dispenser matrix is sepiolite, a natural clay mineral with a high adsorptivity for
97 organic molecules. Sepiolite is impregnated with the corresponding amount of pheromone in
98 dichloromethane solution and different additives to give consistency and protect the dispenser
99 against humidity. The impregnated material is then compressed in a cylindrical mold by means of
100 a hydraulic press. The technology of mesoporous dispensers has been employed as part of the
101 Adress System commercialized by Syngenta (Madrid, Spain) against *Ceratitidis capitata*
102 (Wiedemann) (Navarro- Llopis et al. 2007) or more recently, for mating disruption dispensers

103 against the California red scale (Vacas et al. 2009, 2010). The manufacturing process has been
104 licensed to Ecología y Protección Agrícola S.L. (Valencia, Spain) who has manufactured the
105 dispensers for these trials.

106 Two mesoporous cylindrical tablets were formulated for the preliminary Lleida-2011 trial: C5
107 with 5 mg of pheromone load, 10 mm in diameter and 4 mm high; C30 loaded with 30 mg (13
108 mm diameter, 11 mm high). The C5 formulation was also employed in the trials carried out later
109 in Asturias. A new mesoporous dispenser loaded with 50 mg of pheromone (C50) was included
110 in the Asturias-May 2012 trial to obtain higher emission levels; these were also cylindrical tablets
111 13 mm in diameter and 11 mm high. The Asturias-July 2012 trial included a new formulation C1
112 loaded with 1 mg of pheromone (10 mm diameter, 3 mm high). Codlemone was employed as sex
113 pheromone at 93% purity, provided by Bedoukian Research Inc. (Danbury, CA, USA).

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

117

118 **Field Trials**

119 *Preliminary Lleida-2011 Trial.* The first field experiment was carried out in a 10 year-old 7-ha
120 apple orchard located in the municipality of Bellpuig (province of Lleida – NE Spain; 41° 38' N,
121 1° 2'E) in July and August 2011. The orchard cultivars were Royal Gala and Golden Suprema.
122 Orchards received one ovicidal treatment (fenoxycarb) to control the first generation and four
123 organophosphate insecticide applications throughout the season, using pheromone traps as
124 indicators of the pest level. Orchards did not have mating disruption treatments. To evaluate the
125 capture efficiency of different pheromone emission levels, five traps were used in five fully
126 randomized blocks, baited with the following pheromone dispensers: (A5) 1x5-mg dispenser,

127 (A15) 3x5-mg dispensers, (A30) 1x30-mg dispenser, (A60) 2x30-mg dispensers, and (A90)
128 3x30-mg dispensers. Traps were hung in the canopy of apple trees at an approximate height of
129 2.5 m and were spaced at least 25 m apart, while blocks were placed 30 m away. Traps were
130 rotated once a week in the block, and trials finished after two complete trap position rotations.
131 Traps were placed in the field from 22 July 2011 to 29 August 2011.

132 *Asturias-May 2012 Trial.* Based on the preliminary results, we decided to perform a second
133 field trial to test the existence of pheromone release thresholds that reduce trap catches by
134 including higher emission rates. Six cider-apple orchards located in Asturias (NW Spain; 43°
135 30'N, 5° 30'W) were selected. All the orchards were managed following organic guidelines
136 (Table 1). The distance between orchards varied between 150 m and 18.5 km. To evaluate the
137 catch efficiency of the different emission levels, five traps with different pheromone dose were
138 placed at each orchard. Pheromone dose in each one of the five traps per orchard was: C5 (1 x 5-
139 mg dispenser), C20 (4 x 5-mg dispensers), C50 (1 x 50-mg dispenser), C100 (2 x 50-mg
140 dispenser), and C200 (4 x 50-mg dispensers). The intertrap distance was at least 30 m. Traps
141 were hung at 1.5 m above the ground, and were revised and rotated weekly from 10 May 2012 to
142 6 June 2012. The characteristics of each plot are described in Table 1.

143 *Asturias-July 2012 Trial.* The experiment was carried out in July and August 2012 in the same
144 apple orchards and with the same methodology described above. The traps in each block were
145 baited with a different pheromone dose and are referred to hereafter as C1 (1 x 1-mg dispenser),
146 C3 (3 x 1-mg dispensers), C5 (1 x 5-mg dispenser), C10 (2 x 5-mg dispensers), and C20 (4 x 5-
147 mg dispensers). Traps were placed on 11 July 2012, and the moths caught were counted weekly
148 for five weeks.

149

150 **Pheromone Release Profiles.** Additional dispensers were simultaneously aged under field
151 conditions in nearby areas of the trial orchards in Asturias, to be periodically gathered and
152 analyzed to study their release profiles. The residual codlemone content was extracted at different
153 aging dates. Three dispensers per ageing date were extracted by solvent extraction at 40°C for 2
154 h, with magnetic agitation, in a particular volume of dichloromethane as follows: 2, 5 or 25 ml
155 for dispensers C1, C5 and C50 respectively. Extracts were then analyzed by gas chromatography
156 with a flame ionization detector (GC/FID), and pheromone content was quantified using *n*-
157 heptadecane as the internal standard. After 1 hour of extraction, 0.5 ml of the internal standard
158 solutions were added with the following concentrations: 1 mg/ml in extracts of dispensers C1 and
159 6 mg/ml in extracts of dispensers C5 and C50. All the analysis were performed using a
160 Clarus®500 gas chromatograph (PerkinElmer Inc., Wellesley, USA) equipped with a ZB-5 (30 m
161 × 0.25 mm × 0.25 mm) capillary column (Phenomenex Inc., Torrance, CA), maintained at 120°C
162 for 2 min and then raised by 20°C/min to 260°C, to be then maintained for 3 min. Temperature of
163 the injection port was 250°C, and FID detector was set at 300°C. The carrier gas was helium at
164 1.5 ml/min.

165
166 **Statistical Analysis.** The quantified residual pheromone loads [P (mg)] for each dispenser
167 were fit by polynomial regression with the independent variable *t* (number of ageing days). The
168 first derivative of the resulting equations provided an estimation of the daily emission rate.

169 The captures recorded in each trap, as moths per trap and day, were transformed by sqrt (*x*) to
170 normalize variance prior to applying a multifactor ANOVA (Fisher's LSD test at $P \leq 0.05$) to
171 study the differences between trap catches according to three factors: week, block and emission
172 level. Following the methodology applied in a previous study (Vacas et al. 2009), multiple or
173 simple regression was used to study the relationship between catch data and the pheromone

174 emission rates tested. First, a two-way ANOVA was performed with catch data only with factors
175 week and block. The residuals of this ANOVA did not account for variance due to the two factors
176 week and block, and still provided evidence for variance due to the emission level factor. Thus,
177 these residuals were employed in the regression analysis to obtain the correlation explaining the
178 effect of the emission factor over trap catches. Statistical analyses were performed using the
179 Statgraphics Centurion XVI package (StatPoint Technologies, Warrenton, VA, USA).

180

181

Results

182 **Preliminary Lleida-2011 Trial.** In our preliminary trial (Lleida-2011), population levels were
183 very low throughout the study period; in fact, only 44 moths were captured in the 25 traps.

184 Therefore, analysis of variance was performed with the total numbers of moths captured per trap
185 and day throughout the trial for the different baited traps. No significant differences were found
186 for emission factor ($F = 1.14$; $df = 4,16$; $P = 0.371$, in Fig. 1), whereas the block factor was
187 significant ($F = 7.81$; $df = 4,16$; $P = 0.001$) due to the natural clumped distribution of the pest.

188 Despite not being significant, the data suggest a trend of decreasing capture with increasing
189 pheromone release rates. According to this result, we tested higher pheromone emission rates in
190 the Asturias-May trial to confirm the decreasing trend in the number of captures.

191

192 **Pheromone Release Profiles.** The release profile of mesoporous dispenser C1 is depicted in
193 Fig. 2A. Multiple linear regression performed with the mean residual pheromone values
194 demonstrated that the quadratic effect was not statistically significant for C1 (significance of the
195 quadratic coefficient: $P = 0.48$) and that the residual pheromone (mg) content fitted the linear
196 model ($P < 0.001$, $R^2 = 0.98$) given by Equation 1. Thus, the slope of the linear model gave the
197 emission rate of the dispenser, which was assumed constant and equal to $11.0 \mu\text{g/day}$ throughout

198 the study period. Likewise, the release profile of C5 (Fig. 2B) fitted the linear model in Equation
199 2 ($P = 0.002$, $R^2 = 0.94$; significance of quadratic coefficient: $P = 0.10$), corresponding to a mean
200 release value of $33.5 \mu\text{g/day}$, throughout the study period. Finally, multiple linear regression
201 showed that the quadratic effect was not statistically significant for formulation C50 (significance
202 of the quadratic coefficient: $P = 0.89$), and that the residual codlemone content once again fitted a
203 linear model (Equation 3; $P = 0.003$, $R^2 = 0.91$). Thus, the emission rate of dispenser C50 given
204 by the slope of the linear model (Fig. 2C) was constant and equalled $269.5 \mu\text{g/day}$.

$$205 \quad P_{C1} = 0.9485 - 0.0110 \cdot t \quad (\text{Eq. 1})$$

$$206 \quad P_{C5} = 5.2519 - 0.0335 \cdot t \quad (\text{Eq. 2})$$

$$207 \quad P_{C50} = 50.1351 - 0.2695 \cdot t \quad (\text{Eq. 3})$$

208

209 **Asturias 2012 Field Trials.** The sqrt-transformed catches were analyzed with multifactor-
210 ANOVA, considering the factors emission, week and block. None of the possible interactions
211 between factors resulted in statistically significant effects (week \times block: $F = 1.21$; $df = 15,59$; P
212 $= 0.29$, week \times emission: $F = 0.92$; $df = 12,59$; $P = 0.54$, block \times emission: $F = 1.18$; $df = 20,59$; P
213 $= 0.30$) and were disregarded from the final analysis. The emission factor was statistically
214 significant ($F = 10.55$; $df = 4,106$; $P < 0.001$), thus confirming the trend observed in Lleida-2011
215 trial. The higher the pheromone load, the fewer the catches obtained (Fig. 3A); the traps baited
216 with 5 mg dispensers trapped significantly more moths than those baited with 50 mg dispensers.
217 This suggests that the attractant power diminished with the emission level. Furthermore, the week
218 factor was statistically significant ($F = 7.71$; $df = 3,106$; $P < 0.001$), according to the pest
219 population dynamics, as well as the effect of the block factor ($F = 5.32$; $df = 5,106$; $P < 0.001$).

220 According to the release studies described before, each baited trap had a different emission
221 level. By considering release profiles of dispensers C5 and C50 and the calculated release rates

222 according to Eqs. 1 and 2, the emission factor could be considered a quantitative variable
223 according to the following correspondence: C5 = 33.5 µg/day, C20 = 134 µg/day, C50 = 269.5
224 µg/day, C100 = 539 µg/day, and C200 = 1078 µg/day. A strong relationship was found by the
225 regression analysis given the logarithmic model ($P = 0.003$, $R^2 = 0.96$) depicted in Fig. 4.
226 Accordingly, catches dropped almost linearly with increasing emission rates from 33.5 to 269.5
227 µg/day; then, they continued to lower slightly up to the highest studied release level of 1,078
228 µg/day (Fig. 4). Then, captures were reduced by 86%, as compared with traps baited with C5
229 dispensers.

230 Smaller pheromone doses were tested in July, and the number of moths trapped in C1, C3, C5
231 and C10 traps were not significantly different (Fig. 3B); only when traps were baited with four
232 C5 dispensers (C20) did mean captures start to decrease. The significance of the studied factors is
233 given by the following statistics obtained by multifactor-ANOVA: week $F = 13.88$; $df = 4,116$; P
234 < 0.001 ; block $F = 6.21$; $df = 5,116$; $P < 0.001$; and emission $F = 5.25$; $df = 4,116$; $P = 0.003$.
235 Only the interaction between week and block was statistically significant and the other factors
236 were consequently disregarded from the analysis (week \times block: $F = 2.78$; $df = 20,116$; $P <$
237 0.001). This interaction resulted in a significant effect due to a reduction of captures in the block
238 number 3 during the last week of trial, while captures increased in the other plots.

239 By considering the aforementioned release profiles for dispensers C1 and C5, the emission
240 factor in this trial took the following values: C1 = 11 µg/day, C3 = 33 µg/day, C5 = 33.5 µg/day,
241 and C20 = 134 µg/day. The linearity of the decreasing attraction of *C. pomonella* to codlemone-
242 baited traps was confirmed by the multiple regression results depicted in Fig. 5 ($P < 0.001$, $R^2 =$
243 0.95). Thus, *C. pomonella* attraction could be promoted with codlemone emission rates up to 67
244 µg/day, while release rates above ca. 134 µg/day achieved significantly lower captures.

245

Discussion

246
247 The present work has employed different mesoporous dispensers, with pheromone loads
248 ranging from 1 to 50 mg, as tools to study the codling moth response to different codlemone
249 emission rates. Our preliminary trial suggested a decreasing trend of captures in accordance with
250 increasing pheromone loads within the range 5-90 mg. This response has been previously
251 reported in the literature: Kehat et al. (1994) found increasing catches of codling moth males with
252 increasing pheromone doses, within the 0.1–100 µg range, but a 5,000 µg load was significantly
253 less attractive than 100 or 1,000 µg loaded on a rubber septum. Similarly, Mitchell et al. (2008)
254 showed that by increasing the load from 1 to 10 mg, the mean number of male moths captured
255 decreased, while no differences were observed within the 0.01-0.1mg range. The same response
256 was observed in the wind tunnel assays performed to develop an attract-and-kill strategy (Lösel et
257 al. 2000). Maximal captures were achieved at a concentration of 0.065% codlemone in a 100 µl
258 droplet, and a reduction of more than 50% in the average number of moths trapped was obtained
259 with pheromone concentrations that were 10 times higher (0.65%). However, all these works
260 address insect responses based on the initial pheromone loads of the dispensers, which do not
261 provide a conclusive idea about actual pheromone release as it is highly affected by dispenser
262 type. For example, Critchley et al. (1997) demonstrated that 1 mg-loaded polyethylene vials
263 caught significantly more moths than rubber septa with the same amount of ingredient. In fact,
264 rubber dispensers have non-linear kinetics, which means that emission can greatly vary between
265 the beginning and the end of their lifespan and even on the same day due to temperature
266 (Domínguez-Ruíz et al. 2008).

267 In the present work, field trap catches and pheromone release profiles of the dispensers
268 employed were studied simultaneously and correlated to verify the existence of an optimum
269 release value for attraction or whether the decreasing trend observed becomes asymptotic at

270 higher release rates. Although field trials were conducted in representative plots, the statistical
271 analysis performed takes the block factor as a fixed factor, and therefore results obtained are
272 valid only in the areas where trials were conducted. For this reason, field trials were conducted in
273 the two main apple growing areas of Spain; nevertheless, these results should be validated in
274 regions with different conditions and population levels.

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

285 Although the minimum rate for effective mating disruption has not been established with
286 certainty, estimates vary widely and range from 2 to 40 mg/ha/h (Cardé et al. 1977, Charmillot
287 1990, Vickers et al. 1998), and may vary in any case depending on population density, tree size
288 and other environmental factors (Howell et al. 1990; McDonough et al. 1992). The
289 aforementioned mating disruption pheromone doses correspond to the individual dispenser
290 release rates within the 29-240 $\mu\text{g}/\text{day}$ range, applied at 1,000-2,000 dispensers/ha. The
291 dispensers described by Angeli et al. (2007) fall within this emission range (mean ca. 56 $\mu\text{g}/\text{day}$);
292 however, these pheromone emission rates are 10-50 times lower than those of several other
293 commonly used dispensers for the conventional mating disruption of *C. pomonella*, with reported

294 mean release values from 0.6 to 3 mg/day (Brown et al. 1992, Knight 1995, Tomaszewska et al.
295 2005, Femenia 2011). These efficient mating disruption dispensers agree with the results
296 presented herein as captures were virtually zero in the traps baited with codlemone dispensers
297 releasing at a rate ca. 1 mg/day.

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

314 Attract-and-kill or attract-and-remove strategies are being studied as alternatives to mating
315 disruption treatments (Charmillot et al. 2000, Lösel et al. 2000, Krupke et al. 2002, Reinke et al.
316 2012). As mentioned before, knowledge about optimum release rates is essential for control
317 methods based on pheromones as attractants. When there is no pheromone background, the

318 emission range reported in this work (11-67 $\mu\text{g}/\text{day}$) could be considered to develop effective
319 formulations for attraction purposes because commercial dispensers can be designed in
320 accordance with this value for better pheromone use.

321

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325

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431

Figure legends

432
433 **Fig. 1.** Mean \pm SE number of moths caught per trap and day (MTD) for each of the five types of
434 baited trap (A5, A15, A30, A60 and A90) tested in preliminary trial Lleida-2011. Bars labelled
435 with the same letter are not significantly different (LSD test at $P > 0.05$).

436
437 **Fig. 2.** Release profiles of 8E,10E-dodecadien-1-ol (codlemone) from the C1 (A), C5 (B) and
438 C50 (C) mesoporous dispenser employed in field trials carried out in Asturias (2012). Fitted
439 linear models (Eqs. 1-3) describe the mean pheromone content of the dispenser [codlemone (mg)]
440 vs. time (days of ageing). Three replicates were extracted per ageing time.

441
442 **Fig. 3.** Mean \pm SE number of moths caught per trap and day (MTD) for each of the five types of
443 baited trap tested in trials Asturias-May (A) and Asturias-July (B). Bars labelled with the same
444 letter are not significantly different (LSD test at $P > 0.05$).

445
446 **Fig. 4.** Fitted regression (logarithmic) model, for trial Asturias-May data, of residuals vs.
447 emission rates. The dependent variable is the residuals from the ANOVA applied to capture data
448 (MTD) according to factors week and block.

449
450 **Fig. 5.** Fitted regression (linear) model, for trial Asturias-July data, of residuals vs. emission
451 rates. The dependent variable is the residuals from the ANOVA applied to capture data (MTD)
452 according to factors week and block.

453

454

Tables

455

456

Table 1. Description for Asturias trial orchards

| Orchard | Municipality | Size (ha) | Age (years) | Insecticide treatments |
|---------|--------------|-----------|-------------|------------------------|
| 1 | Villaviciosa | 1.1 | 11 | Granulovirus + Neem* |
| 2 | Villaviciosa | 0.5 | 5 | Granulovirus + Neem* |
| 3 | Villaviciosa | 0.9 | 15 | Granulovirus + Neem* |
| 4 | Nava | 1.1 | 15 | None |
| 5 | Villaviciosa | 2.0 | 8 | Granulovirus + Neem* |
| 6 | Sariego | 0.7 | 14 | None |

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

460