Lobesia botrana males mainly fly at dusk – video camera-assisted pheromone traps and implications for mating disruption

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Abstract

Pheromone-mediated mating disruption (MD) is currently considered an effective strategy to control the European grapevine moth (EGVM), *Lobesia botrana*, with a successful interference on natural female calling during the male searching flight. However, little is known on the impact of the hour of the day on EGVM male flight. While various models forecasting the day of maximum presence of males per flight have been developed, field research on the male flight activity over the hours of the day is scarce. Hence, we used video camera-assisted pheromone traps to allow a continuous monitoring of EGVM flights over daylight and night hours, quantifying captures of males. Experiments were carried out in three vineyards located in northern Spain over two years (2016 and 2017). Results showed that EGVM flight mainly occurred between 21:00 and 23:00 hours. Furthermore, male catches significantly differed over the study year, annual flight period and vineyard. Most of the dispensers used worldwide for *L. botrana* MD continuously release the main sex pheromone component [(7*E*,9*Z*)-7,9-dodecadien-1-yl acetate], except for some automatic devices releasing puffs of sex pheromones at selected time intervals. The findings presented here can be useful to optimize the MD technique, identifying selected time intervals when the release of EGVM synthetic pheromones can be concentrated, boosting MD efficacy against this important pest, minimizing the release of synthetic sex pheromone molecules in the environment and reducing application costs.

Keywords: chemical ecology; European grapevine moth; Integrated Pest Management; pheromone dispenser; Tortricidae

Key message

- Video camera-assisted pheromone traps allowed a continuous field monitoring of EGVM male catches over 24 hours.
- EGVM male activity mainly occurred between 21:00 and 23:00 h; all catches significantly differed over the annual flight period, study year and vineyard.
- This study can help to optimize mating disruption programs, identifying time intervals when the release of EGVM synthetic pheromones can be restricted.
- Automatic devices releasing pheromone puffs at selected time frames can be tuned, boosting mating disruption efficacy.

Introduction

Grapevine production is currently endangered by a number of important insect pests, among which the European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) plays a key role (Reineke and Thiéry 2016). Nowadays, pheromone-mediated mating disruption (MD) is considered a reliable and effective strategy to control *L. botrana* (Ioriatti et al. 2008, 2011; Witzgall et al. 2010; Cooper et al. 2014; Lucchi et al. 2018). Currently, more than 249,000 hectares of vineyards are managed using MD against EGVM in Europe, with about 76,000, 60,000, 47,000 and 36,000 hectares in Spain, Germany, France and Italy, respectively (Lucchi and Benelli 2018). No negative effects on human health and non-target organisms have been observed so far, allowing researchers to claim this method as fully compatible with modern Integrated Pest Management (IPM) criteria (Welter et al. 2005; Millar 2006; Miller et al. 2006; Ting and Eya 2010; Ioriatti et al. 2012).

In the latest decades, a rather wide array of pheromone dispensers and close-related technologies have been proposed to boost the efficacy of MD, as well as to promote the employment of biodegradable tools or to reduce the number of dispensers per hectare, thus labour cost (Lance et al. 2006; Anfora et al. 2008; Brockerhoff et al. 2012; Miller and Gut 2015; Ioriatti and Lucchi 2016; Hummel 2017; Lucchi et al. 2018). The large majority of dispensers currently used for *L. botrana* MD are "passive" reservoir devices, which continuously release plumes of the main sex pheromone component [i.e., (7*E*,9*Z*)-7,9-dodecadien-1-yl acetate], except for automatic "active" dispensers releasing puffs of sex pheromones at selected time intervals (Ioriatti and Lucchi 2016). However, the MD efficacy of synthetic sex pheromone plumes can be optimized concentrating the release of multiple synthetic plumes in the hours of the day when EGVM male flights are most abundant.

Unfortunately, little is known on the impact of the hour of the day on *L. botrana* flight. Earlier research focused on EGVM male flight responses to calling females, pheromone gland extracts and synthetic sex pheromones in wind tunnel assays (Witzgall and Arn 1990; El-Sayed et al. 1999), as well as on their flight trajectories, which have been deeply investigated in laboratory using computer-based devices (El-Sayed et al. 2000). Besides, remarkable attempts have been done to develop models forecasting the day of maximum flight of males per EGVM generation (also known as "peak flight") (Götz 1939; Gabel and Stockel 1988; Gallardo et al. 2009; Amo-Salas et al. 2011).

Several attempts have been made in the past to use sophisticated mechanical trapping devices (Metcalf et al. 1962). Some of them were driven by clockworks; by opening and closing slits at predetermined times, it was determined at which time flying insects would enter in the trap (Götz 1939, 1943). However, such devices were too expensive to produce in large numbers, to install and to monitor daily, thus they have been never used on a wide scale. In addition, field research on the occurrence of male flight over 24 hours of the day has never been conducted. However, in laboratory, Hurtrel and Thiéry (1999) pointed out that the circadian flight activity of females showed a peak before the onset of scotophase and sustained activity occurred during 6 hours around this peak. Notably, validating this information on male moths in the field can be crucial to understand selected time intervals on which the release of EGVM synthetic pheromones in vineyards can be concentrated, boosting mating disruption efficacy against this important pest, minimizing the release of *L. botrana* synthetic sex pheromone molecules in the environment, and reducing the MD application cost.

Therefore, in this study, we used video camera-assisted pheromone traps to allow a continuous monitoring of the EGVM male activity for 24 hours a day, providing a detailed quantification of male catches in the field for the three flights. Experiments were carried out

105 in three different vineyards located in northern Spain, over two years (growing seasons 2016
106 and 2017).
107
108 **Materials and methods**
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110 Field experimental sites and study period
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112 The field trials were carried out in Zaragoza prefecture (Aragon region, Spain) over
113 two consecutive years (2016 and 2017). Three vineyards located nearby Lucena de Jalón town
114 were selected for monitoring *L. botrana* male flight from the end of April till the end of
115 August, covering the three annual flights normally recorded in this area in previous years. The
116 selected vineyards (Testigo, La Noría, El Navarro, respectively, 41°31'24.06"N -
117 1°17'17.78"O, 41°35'16.69"N - 1°16'29.82"O, 41°32'13.18"N - 1°15'14.43"O) were 1 ha min
118 surface, managed with a conventional IPM strategy, not based on MD. They were isolated
119 (i.e., not surrounded by other vineyards) to avoid possible influences from vineyards relying
120 to MD for EGVM population management.
121 The pest daily flight was monitored with three camera traps deployed in the centre of
122 each vineyard. The traps were baited with standard pheromone lure produced by Trécé
123 Incorporated for *L. botrana*, code 3104-25 EGVM. The lure was changed every 4 weeks, as
124 for producer instructions, during all the trial period.
125 In 2016, the three pheromone traps were deployed in the vineyards on April 20th and
126 removed on September 8th; in 2017, the traps were installed on April 8th and removed on
127 August 31th. In both years, the three flight periods were determined by the first and the last
128 catch registered in at least one of the traps. Dates and duration of each flight period of *L.*
129 *botrana* in 2016 and 2017 are reported in Table 1.

Camera video traps

Camera video traps used in the research were provided by Trapview (Hruševje, Slovenia) and customized for the trials. They were fitted with higher capacity batteries and larger solar panels, so they were able to take up to 48 photos per day (one every 30 minutes). Each automated trap placed in the field was equipped with a high-resolution camera to take pictures of the trap sticky plate in which insects were being caught, and with the temperature and relative humidity sensor. The traps used cellular network to send the data and images to the cloud for processing, where image recognition algorithms were deployed to identify and mark targeted pests. Based on the automated image processing, some basic frequency statistics were calculated by the Trapview system. Even though the insect images were processed automatically, the authors were able to access all images on a computer (through a web application, http://www.trapview.com/en) and visually review and verify the collected insects.

Data collection

The sticky plate pictures were revised on daily basis by one of us through the web application (http://www.trapview.com/en). The high-resolution images, taken every 30 min by the traps, were checked for a visual identification of the insects caught to confirm or refuse the identification performed by the automated image processing data provided by Trapview. The number of adult male and the time of the day when they were caught was registered. In addition, the traps were inspected in the field every week to re-confirm the record achieved with the web application (total *L. botrana* weekly catches) while cleaning the sticky plate.

155

156 Statistical analysis

157

158 Goodness of fit tests evaluating the distribution of *L. botrana* male catches (n) per trap
159 every 30 minutes for 24 hours a day showed that data were not normally distributed (Shapiro-
160 Wilk test, P>0.05), due to the high abundance of zero values during daylight hours. Thus, we
161 employed non-parametric statistics, analysing the significance of differences in *L. botrana*
162 male catches per trap every 30 minutes for 24 hours a day (Steel-Dwass test), vineyards,
163 generations and years (Wilcoxon test). The same statistics was carried out on male catches per
164 trap every 30 minutes from 21:00 to 23:00, since we noted that >90 % of the catches falls in
165 that time frame. P=0.05 was selected as threshold to assess significant differences.

166

167 **Results**

168

169 In our experiments, significant differences in *L. botrana* male catches per trap every
170 30 minutes for 24 hours a day were detected (χ^2=3442.763; *d.f.*=47; *P*<0.001) (Figure 1).
171 Analysing data from field trials conducted in 2016 and 2017, it was observed that EGVM
172 male catches were significantly higher from 21:00 to 23:00, if compared to early morning
173 (from 6:00 to 12:00) (*Z*=1.896; *P*<0.001), early afternoon (from 12:00 to 16:00) (*Z*=9.842;
174 *P*<0.001) and late afternoon (from 16:00 to 21:00) (*Z*=11.904; *P*<0.001), with the only
175 exception of catches recorded during the first flight period of 2017 (*P*>0.05) (Figure 1).
176 Significant differences among EGVM catches over the three annual flights were detected
177 (χ^2=127.030; *d.f.*=2; *P*<0.001). Male catches per trap every 30 minutes were higher in the
178 third flight, if compared to those of flights 1 and 2. EGVM catches were higher in 2016 over
179 2017 (*Z*=14.356; *P*<0.001) (Figure 1).

Since 90% of *L. botrana* male catches were recorded from 21:00 to 23:00, we focused on this time interval to analyse our data. Figure 2a showed significant differences in EGVM male catches per trap every 30 minutes over hours of the main period of male activity (2016: χ^2=68.604, *d.f.*=4, *P*<0.001; 2017: χ^2=53.314, *d.f.*=4, *P*<0.001). In 2016, we observed a significant difference between catches recorded at 21:00 over those achieved at 21:30 (*Z*=4.108; *P*<0.001), as well as between catches at 22:30 over those at 23:00 (*Z*=-3.891; *P*<0.001). On the other hand, male catches per trap every 30 minutes did not differ significantly between 21:00 and 23:00 (*Z*=-1.637; *P*=0.473) as well as between 21:30 and 22:30 (*Z*=-2.202; *P*=0.179), outlining a rather constant male activity in the central period of the selected time frame (Figure 2a). In 2017, the overall abundance of *L. botrana* male catches was lower if compared to 2016. However, we still observed a significant difference between catches recorded at 22:00 over those achieved at 21:30 (*Z*=4.112; *P*<0.001) and 23:00 (*Z*=-4.107; *P*<0.001) (Figure 2a).

Furthermore, Figure 2b showed significant differences among *L. botrana* catches per trap from 21:00 to 23:00 in the three study vineyards (2016: χ^2=138.109; *d.f.*=2, *P*<0.001; 2017: χ^2=25.922; *d.f.*=2, *P*<0.001). In both experimental years, EGVM catches were higher in the vineyard Testigo, if compared to El Navarro (2016: *Z*=5.108; *P*<0.001; 2017: *Z*=2.453; *P*=0.038) and La Noria (2016: *Z*=11.769; *P*<0.001; 2017: *Z*=5.039; *P*<0.001). The difference between catches recorded in El Navarro and La Noria was also significant (2016: *Z*=-7.277; *P*<0.001; 2017: *Z*=-2.762; *P*=0.016).

Figure 3 provides an overall analysis of *L. botrana* catches per trap every 30 minutes in the main period of male activity (21:00-23:00). EGVM catches showed significant differences among the three flight periods (2016: χ^2=122.109; *d.f.*=2, *P*<0.001; 2017: χ^2=46.516; *d.f.*=2, *P*<0.001). In the vineyard El Navarro, catches during 2016 were more abundant during the third flight over the first (*Z*=8.884; *P*<0.001) and second one (*Z*=9.031;

205 $P<0.001$). This trend was not confirmed in the vineyards La Noria and Testigo (Figure 3). On
206 the other hand, in all study vineyards, no differences in male catches were noted between the
207 first and second flight ($Z=0.641$; $P=0.797$). Both in El Navarro and Testigo, most of the 2016
208 male catches occurred between 21:30 and 23:00, with peaks reaching 69 male catches per trap
209 every 30 minutes (Figure 3). In 2017, catches in El Navarro and Testigo were lower during
210 the first flight over the second ($Z=5.111$; $P<0.001$) and third one ($Z=6.896$; $P<0.001$),
211 whereas no differences were noted between the second and third flight ($Z=1.958$; $P=0.122$).
212 No differences were detected analysing catches from La Noria vineyard (Figure 3).
213
214 **Discussion**
215
216 Moth species show specific daily activity rhythms in their sexual activities. It has been
217 pointed out that some species are sexually active early at night, while others are sexually
218 active late at night (e.g., about Tortricidae, Bovey 1966; Batiste 1970; Batiste et al. 1973a,b;
219 see Groot 2014 for a recent review). It has been argued that the differentiation in daily activity
220 rhythms of sexual activities has been probably developed to reduce communication
221 interference among closely related moth species (Roelofs and Cardé 1974; Haynes and Birch
222 1986; Byers 2006; Groot 2014). When searching information about the effect of the hour of
223 the day on EGVM male flight, we experienced a severe lack of literature. Indeed, even if it
224 has been reported that EGVM females call during the first hours of the scotophase (Harari et
225 al. 2011, 2015; Navarro-Roldán and Gemeno 2017) and the circadian flight activity of
226 females in laboratory was mainly concentrated in the six hours around the onset of scotophase
227 (Hurtrel and Thiéry 1999), precise field data on flight daily activity of *L. botrana* males are
228 scarce. Substantiating a preliminary evidence by Götz (1943), our field experiments using
229 camera traps showed that male flights are mostly comprised between 21:00 and 23:00 h, with

significant differences over the study year, EGVM flight and vineyard. This substantiate earlier studies showing that the *L. botrana* female calling activity is mainly concentrated in these hours (Harari et al. 2011, 2015; Muller et al. 2016). Concerning the effect of the annual flight period in Spanish vineyards on daily male catches, we observed a slight advance of the daily flight period in the first flight (i.e., adults coming from the wintering generation) compared to the second and third flights. This was due to the reduced length of daylight time in late spring if compared to that of summer.

From an applied point of view, the findings presented here can be useful to optimize MD technique, identifying selected time intervals in correspondence of which the release of EGVM synthetic pheromones can be restricted. Indeed, even if most of the EGVM mating disruption dispensers spread continuous sex pheromone plumes, new automatic devices releasing puffs of sex pheromones at selected time intervals (e.g., Checkmate Puffer® LB, Suterra) were recently registered and commercially used in vineyards (Ioriatti and Lucchi, 2016). These aerosol formulations can be easily tuned to release pheromone plumes during the hours where males really flight, searching for mates, providing a cost-effective alternative to hand-applied dispensers. This may help boosting MD efficacy against EGVM, currently recognized as a key pest of vineyards worldwide, and minimizing the release of synthetic sex pheromone molecules in the environment.

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Conflict of Interest

The authors declare no competing interests. The mention of trade names or commercial products in this publication is solely to providing specific information and does not imply recommendation or endorsement by the University of Pisa.

Author contributions

All authors designed the research and conducted the experiments. AL and GB analysed data. AL and PS contributed new reagents and/or analytical tools. All authors wrote and approved the manuscript.

References

Amo-Salas M, Ortega-Lopez V, Harman R, Alonso-Gonzalez A (2011) A new model for predicting the flight activity of *Lobesia botrana* (Lepidoptera: Tortricidae). Crop Prot 30:1586-1593

Anfora G, Baldessari M, De Cristofaro A, Germinara GS, Ioriatti C, Reggiori F et al (2008) Control of *Lobesia botrana* (Lepidoptera: Tortricidae) by biodegradable Ecodian sex pheromone dispensers. J Econ Entomol 101(2):444-450

Batiste WC (1970) A timing sex-pheromone trap with special reference to codling moth collections. J Econ Entomol 63(3):915-918

Batiste WC, Olson, WH, Berlowitz A (1973a) Codling moth: influence of temperature and daylight intensity on periodicity of daily flight in the field. J Econ Entomol 66(4):883-892

279 Batiste WC, Olson WH, Berlowitz A (1973b) Codling moth: diel periodicity of catch in
280 synthetic sex attractant vs. female-baited traps. Environ Entomol 2(4):673-676
281 Bovey P (1966) Super-famille des Tortricoidea. In: Balachowsky AS (ed) Entomologie
282 appliqué à l'agriculture. Tome II. Lépidopterés, premier volume. Masson et Cie, Paris,
283 pp 461-486, pp 859-887
284 Brockerhoff EG, Suckling DM, Kimberley M, Richardson B, Coker G, Gous S, Kerr JL,
285 Cowan DM, Lance DR, Strand T, Zhang A (2012) Aerial application of pheromones
286 for mating disruption of an invasive moth as a potential eradication tool. PLoS ONE
287 7(8): e43767, doi: 10.1371/journal.pone.0043767
288 Byers JA (2006) Pheromone component patterns of moth evolution revealed by computer
289 analysis of the Pherolist. J Anim Ecol 75(2):399–407
290 Cooper M, Varela LG, Smith RJ, Whitmer DR, Simmons GA, Lucchi A, Broadway R,
291 Steinhauer R (2014) Growers, Scientists and Regulators collaborate on European
292 Grapevine Moth program. Calif Agric 4:125-133
293 De Lame FM, Epstein D, Gut LJ, Goldfarb H, Miller J R (2010) Effect of varying dispenser
294 point source density on mating disruption of *Grapholita molesta* (Lepidoptera:
295 Tortricidae). J Econ Entomol 103(4):1299-1305
296 El-Sayed AM, Gödde J, Arn H (2000) A computer-controlled video system for real-time
297 recording of insect flight in three dimensions. J Insect Behav 13:881-900
298 El-Sayed A, Gödde J, Witzgall P, Arn H (1999) Characterization of pheromone blend for
299 grapevine moth, *Lobesia botrana* by using flight track recording. J Chem Ecol 25:389-
300 400
301 Gabel B., Stockel J (1988) Studies of the flight behaviour of the European vine moth, *Lobesia*
302 *botrana* Den. et Schiff. (Lep., Tortricidae). J Appl Entomol 105:205-211

303 Gallardo A, Ocete R, Lopez MA, Maistrello L, Ortega F, Samedo A, Soria FJ (2009)
304 Forecasting the flight activity of *Lobesia botrana* (Denis & Schiffermuller)
305 (Lepidoptera, Tortricidae) in Southwestern Spain. J Appl Entomol 133:626–632
306 Götz B (1939) Untersuchungen über die Wirkung des Sexualduftstoffes bei den
307 Traubenwicklern *Clysia ambiguella* und *Polychrosis botrana*. Z. Angew Entomol
308 26:143-164
309 Götz B (1943) Freiland- und Laboratoriums-Untersuchungen über Ausschlüpfen, Eiablage
310 und Nahrungsaufnahme bei den Traubenwicklern *Clysia ambiguella* und *Polychrosis*
311 *botrana*. Wein und Rebe 25:135-153
312 Groot AT (2014) Circadian rhythms of sexual activities in moths: a review. Front Ecol Evol
313 2:43
314 Gut LJ, Stelinski L L, Thomson DR, Miller JR (2004) Behaviour-modifying chemicals:
315 prospects and constraints in IPM, In: Integrated Pest Management: potential,
316 constraints, and challenges. Eds. Koul, Dhaliwal and Cuperus, CABI Publishing,
317 Cambridge, MA, pp. 73-121.
318 Harari AR, Zahavi T, Steinitz H (2015) Female detection of the synthetic sex pheromone
319 contributes to the efficacy of mating disruption of the European grapevine moth,
320 *Lobesia botrana*. Pest Manag Sci 71:316–322
321 Harari AR, Zahavi T, Thiéry D (2011) Fitness cost of pheromone production in signaling
322 female moths. Evolution 65(6):1572–1582.
323 Haynes KF, Birch MC (1986) Temporal reproductive isolation between 2 species of plume
324 moths (Lepidoptera, Pterophoridae). Ann Entomol Soc Am 79:210–215
325 Hummel HE (2017) A brief review on *Lobesia botrana* mating disruption by mechanically
326 distributing and releasing sex pheromones from biodegradable mesofiber dispensers.
327 Biochem Mol Biol J 3:1-4.

328 Hurtrel B, Thiéry D (1999) Modulation of flight activity in *Lobesia botrana* Den & Schiff.
329 (Lepidoptera: Tortricidae) females studied in wind tunnel. J Insect Behav 12:199-211
330 Ioriatti C, Lucchi A (2016) Semiochemical strategies for tortricid moth control in apple
331 orchards and vineyards in Italy. J Chem Ecol 42(7):571-583
332 Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P, Lucchi A (2011) Chemical
333 ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). J Econ
334 Entomol 104(4):1125-113
335 Ioriatti C, Lucchi A, Bagnoli B (2008) Grape Areawide Pest Management in Italy. In: Koul et
336 al. (Eds.) Areawide Pest Management: Theory and Implementation, CAB
337 International, pp. 208-225
338 Ioriatti C, Lucchi A, Varela L G (2012) Grape berry moths in Western European vineyards
339 and their recent movement into the New World. In: N.J. Bostanian et al. (Eds.)
340 Arthropod Management in Vineyards: pests, approaches, and future directions, doi:
341 10.1007/978-94-007-4032-7_14, Springer Science + Business Media B.V. 2012
342 Lance DR, Leonard DS, Mastro VC, Walters ML (2016) Mating disruption as a suppression
343 tactic in programs targeting regulated lepidopteran pests in US. J Chem Ecol
344 42(7):590-605
345 Lucchi A, Benelli G (2018) Towards pesticide-free farming? Sharing needs and knowledge
346 promotes Integrated Pest Management. Environ Sci Poll Res doi: 10.1007/s11356-
347 018-1919-0
348 Lucchi A, Ladurner E, Iodice A, Savino F, Ricciardi R, Cosci F, Conte G, Benelli G (2018)
349 Eco-friendly pheromone dispensers – a green route to manage the European grapevine
350 moth? Environ Sci Poll Res, doi: 10.1007/s11356-018-1248-3
351 Metcalf RL (1962) Destructive and useful insects, their habits and control. 4th edition,
352 McGraw-Hill Book Co., New York. pp. 403-404

353 Millar JG (2007) Insect pheromones for integrated pest management: promise versus reality.
354 Redia 90:51-55
355 Miller JR, Gut L J (2015) Mating Disruption for the 21st Century: Matching technology with
356 mechanism. Environ Entomol 44(3):427-453
357 Miller J R, Gut L J, De Lame F M, Stelinski L L (2006) Differentiation of competitive vs.
358 non-competitive mechanisms mediating disruption of moth sexual communication by
359 point sources of sex pheromone (Part 2): case studies. J Chem Ecol 32(10):2115-2143
360 Muller K, Arenas L, Thiéry D, Moreau J (2016) Direct benefits from choosing a virgin male
361 in the European grapevine moth, *Lobesia botrana*. Animal Behav 114:165-172
362 Navarro-Roldán MA, Gemeno C (2017) Sublethal effects of neonicotinoid insecticide on
363 calling behavior and pheromone production of tortricid moths. J Chem Ecol
364 43(9):881-890
365 Reineke A, Thiéry D (2016) Grapevine insect pests and their natural enemies in the age of
366 global warming. J Pest Sci 89:313–328
367 Roelofs WL, Cardé RT (1974) Sex pheromones in the reproductive isolation of lepidopterous
368 species. In: Pheromones (M. C. Birch Ed.), Amsterdam: North Holland Publishing, pp.
369 96–114
370 Ting D, Eya DB (2010) Human Health Risk Assessment of Isomate®-EGVM. Report
371 reviewed by the Department of Pesticide Regulation, California Environmental
372 Protection Agency and the California Department of Public Health, 39 pp.
373 Welter S, Pickel C, Millar J, Cave F, Van Steenwyk R, Dunley J (2005) Pheromone mating
374 disruption offers selective management options for key pests. Calif Agric 59(1):16-22
375 Witzgall P, Arn H (1990) Direct measurement of the flight behavior of male moths to calling
376 females and synthetic sex pheromones. Zeitsch Naturforsch 45:1067-1069

377 Witzgall P, Kirsch P, Cork A (2010) Sex pheromones and their impact on Pest Management.
378 J Chem Ecol 36(1):80-100.
379

Figure 1. *Lobesia botrana* male catches (n) per trap every 30 minutes for 24 hours a day; data from three study vineyards collected during the three flights in 2016 and 2017 were analysed. Box plots are given in red; quantiles and outliers are indicated by blue T-bars and black dots, respectively. 90% of male catches were from 21:00 to 23:00. Asterisk indicates significant differences in male catches over 24 hours a day (Steel-Dwass test, P<0.05); n.s.=not significant.

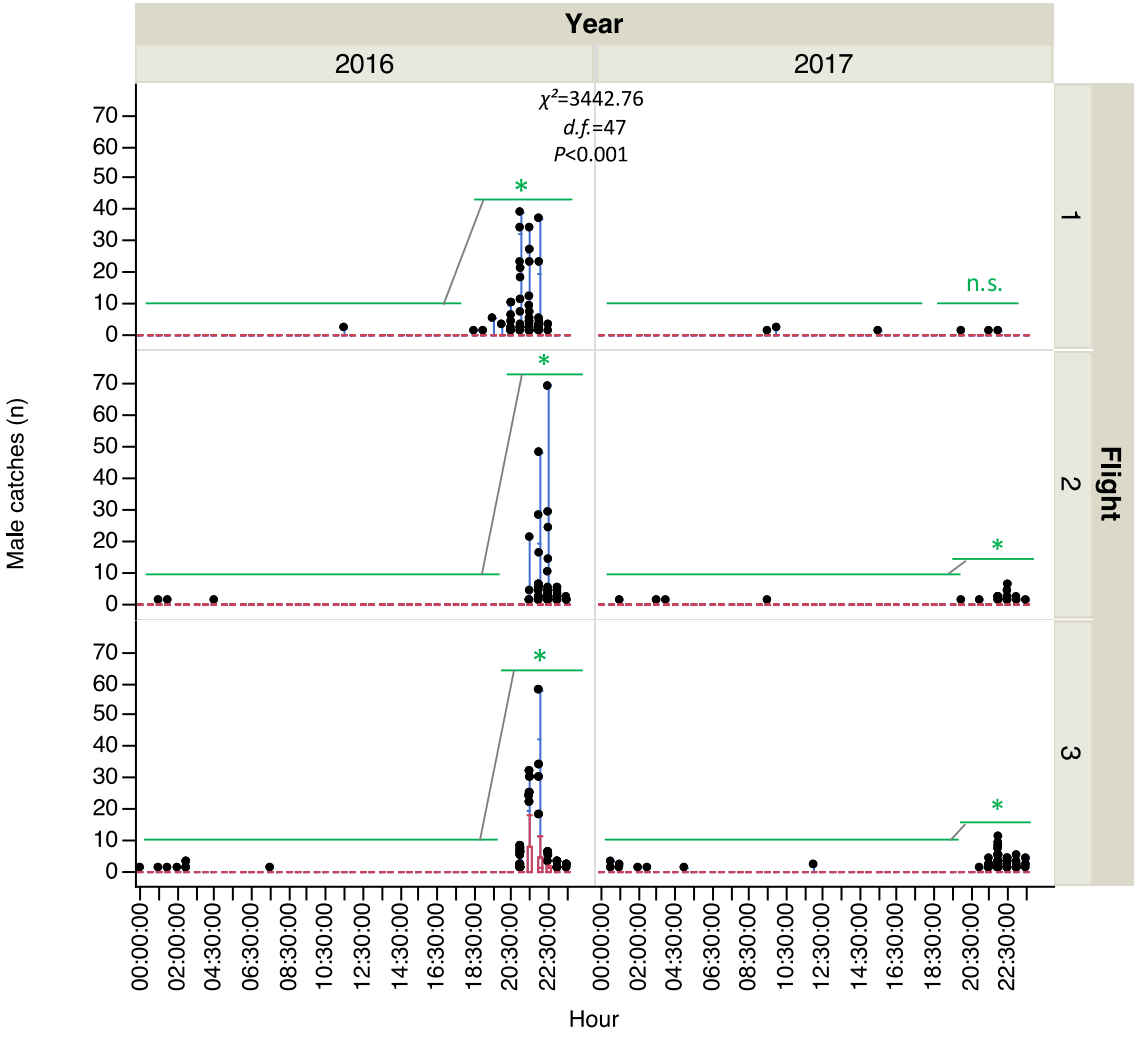

Figure 2. (a) *Lobesia botrana* male catches (n) per trap every 30 minutes in the main period of male activity (21:00-23:00, >90% of catches). Data from three study vineyards collected during the three flights in 2016 and 2017 were analysed. **(b)** Male catches (2016 and 2017) from 21:00 to 23:00 analysed separately for the three study vineyards. Box plots are given in red; quantiles and outliers are indicated by blue T-bars and black dots, respectively. Within each year, different letters above boxplots indicate significant differences (Steel-Dwass test, P<0.05).

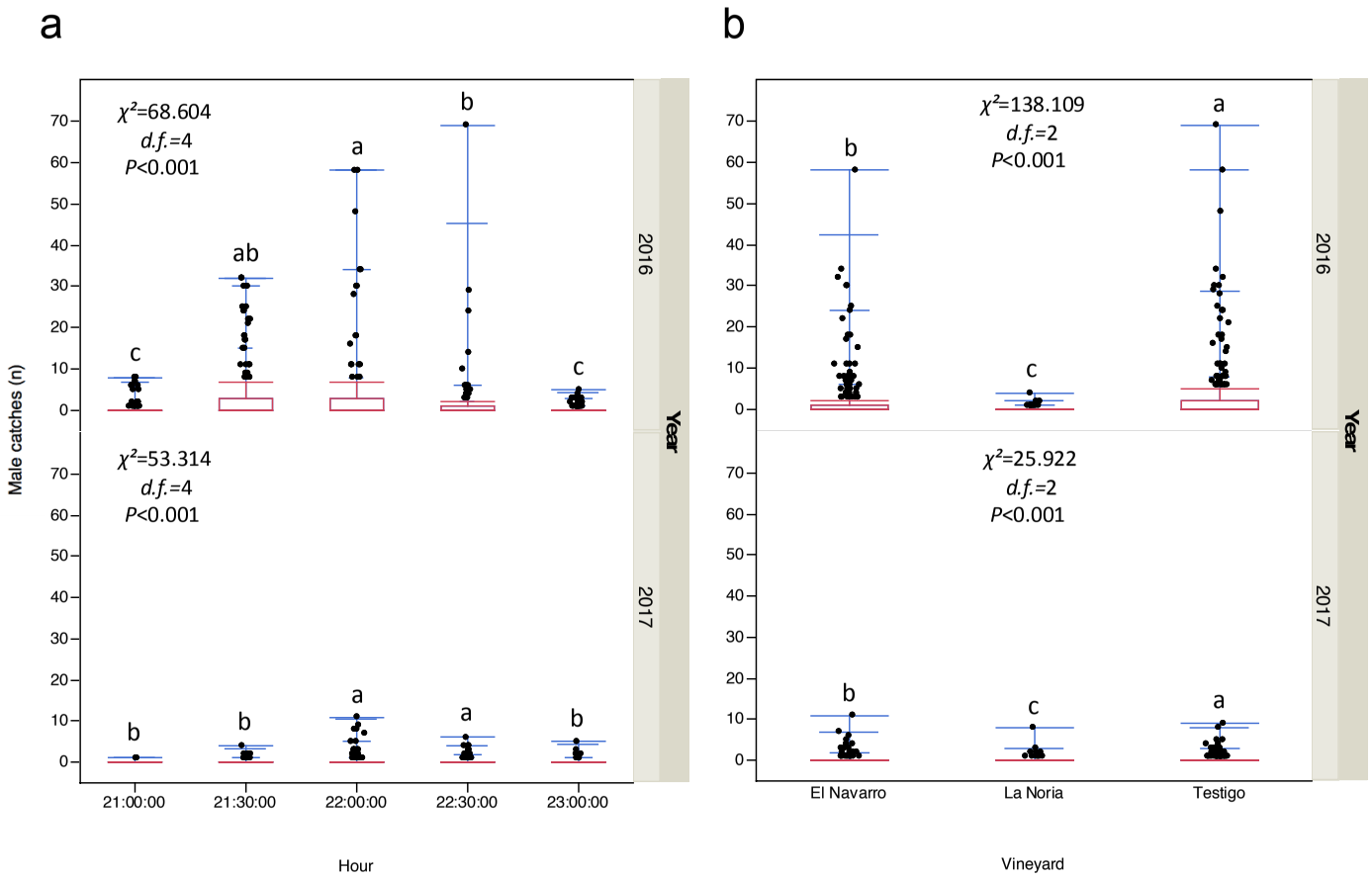

Figure 3. (a) *Lobesia botrana* male catches (n) per trap every 30 minutes in the main period of male activity (21:00-23:00, >90% of catches) analysed separately to show differences among the three flight periods over two years and three study sites. Differences between years and study sites are significant ($P<0.001$). Box plots are given in green (flight period) or violet (hour of the day). Quantiles are indicated by blue (flight period) and red (hour of the day) T-bars. Outliers are indicated by black dots. Within each year, different letters above boxplots indicate significant differences among flight periods (green) and hours of the day (violet) (Steel-Dwass test, P<0.05).

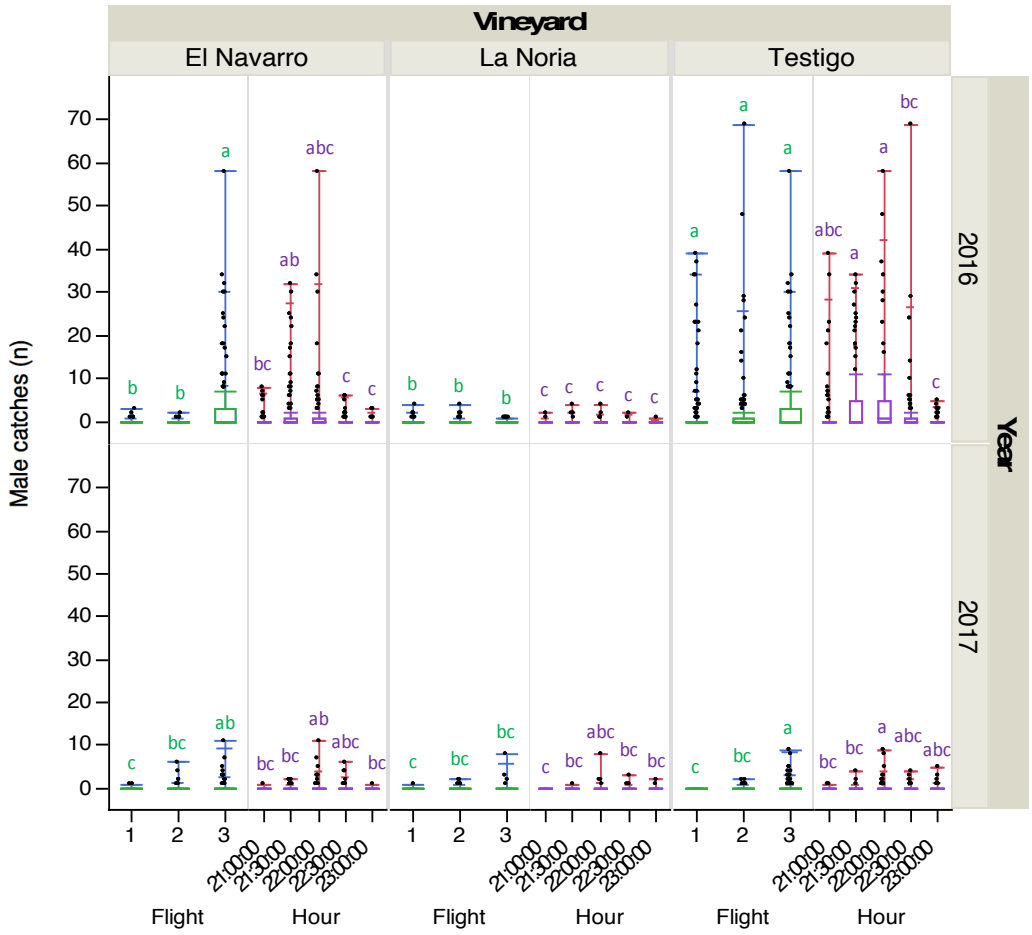

Table 1. Dates and the duration of the three flight periods of *Lobesia botrana* in the study vineyards located in Aragon region (Spain) over two consecutive years (2016 and 2017).

Lobesia botrana flights	2016		2017	
	Beginning of flight	End of flight	Beginning of flight	End of flight
1	April 26th	May 21th	April 11th	May 29th
2	June 11th	July 8th	June 5th	June 28th
3	July 29th	September 5th	July 18th	August 30th