

# Successful controlling of *Lobesia botrana* (Lepidoptera: Tortricidae), using meso-dispensers for mating disruption in urban areas

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## ABSTRACT

The European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller), is a severe pest of grapes, since detected in Chile in 2008 has been subjected to an official control program by the Chilean Department of Agriculture, mainly in vineyards and orchards. *Lobesia botrana* has also been found in urban areas, mostly on backyard grapes, those have become important refuges for large *L. botrana* populations and significant sources for both dispersal and re-infestation to agricultural settings, thus the need for control. Chemical sprays are not allowed for intensive pest management in residential areas; therefore, the mating disruption technique has been the main tool to control *L. botrana* in cities. However, it is not always feasible to evenly deploy the required amount of dispenser ha<sup>-1</sup> in urban areas using conventional formulations. A new meso-dispenser (MeD), loaded with 10x the regular amount of pheromone of standard dispensers, and recommended at 50 units ha<sup>-1</sup>, was evaluated in three consecutive seasons (2013-2016), in four cities in central Chile. This new dispenser yielded significantly lower male captures in traps in comparison with untreated areas. Cumulative male captures per individual flights per season, ranged between 292-2043 trap<sup>-1</sup> (MeD) and 15 795-28 403 trap<sup>-1</sup> (untreated), and significantly declined in the second and third seasons of MeD usage. Disruption index ranged between 68.9% and 98.9% considering flights individually, and above 88.0% considering whole seasons. The presence of eggs, larvae, and pupae infesting clusters, also significantly declined with the number of seasons treated with MeD.

**Key words:** Disruption index, immature fruit infestation, (*E,Z*)-7,9-dodecadienyl acetate, source-point density, urban pest control.

## INTRODUCTION

The European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), a severe pest in vineyards and table grapes (*Vitis vinifera* L.) (Ioriatti et al., 2011), was first detected in Chile in 2008, and it is now distributed along ~1100 km in the country, from Coquimbo (29°95' S) to Temuco (38°45' S) (SAG, 2021). *Lobesia botrana* spreads diseases like *Botrytis cinerea* Pers.: Fr., reduces yields and restricts trade because of its quarantine status in several important Chilean fresh fruit markets (SAG, 2021). Because of that, EGVM has been, for over a decade, subjected by the Agriculture and Livestock Service (SAG) from the Chilean Department of Agriculture to an official control program on table grapes and vineyards (*V. vinifera*, main host), plum (*Prunus domestica* L. and *Prunus salicina*

Lindl.) and blueberry (*Vaccinium corymbosum* L.) orchards (blueberries and plums considered minor hosts in Chile), using either synthetic insecticides, mating disruption (MD), or both.

Mating disruption is a pheromone-based control technique developed for containment (Ioriatti et al., 2011), and it is also a key component for management in areas where *L. botrana* has been introduced and eradicated, as in California (Lance et al., 2016; USDA, 2020), or where on-going efforts are currently conducted for containment, as in Argentina and Chile. Among other important advantages, MD entails minimal health and environmental risks (Lance et al., 2016); high acceptance by people in residential communities (Suckling et al., 2017); viability to be used in conventional and organic orchards (Kutinkova et al., 2019); specific activity, and an effectiveness equivalent to conventional pest management when used properly. Described MD mechanisms in Lepidoptera include two main categories: non-competitive (e.g., sensory adaptation) and competitive (e.g., false-trail following) (Miller and Gut, 2015). Non-competitive mechanisms have not been considered an important contributor to mating disruption on *L. botrana* (Miller et al., 2006; Harari et al., 2015). On the other hand, competition between calling females and sources of synthetic pheromone seem to be of significance to achieve mating disruption in several moth species. Specifically, Ioriatti et al. (2004) highlighted the “false-trail following” as the most important mechanism explaining MD on male *L. botrana*. Thus, MD can occur by competition when high densities of synthetic sources release pheromones at rates greater than those emitted by females, which leads males toward the dispensers deviating instead of calling females.

In Chile, the agricultural area treated with MD has significantly increased over time. Last season (2020-2021) over 107 000 ha were covered by this technique (~20%-100% of the orchard/vineyard area founded by the Chilean government depending on the species and area planted), reaching close to 50% of the total area covered with vineyards, table grapes, blueberries, and plums (ODEPA, 2021; SAG, 2021). Due to *L. botrana* active migration and passive transportation on infested materials (fruits, plants, and pruned wood), the pest has also been frequently found in urban areas in plant hosts, mostly vines which are very common in Chilean backyards. Thus, male captures in pheromone-baited traps and damage in fruits, are quite significant in some cities (Curkovic and Ferrera, 2010; SAG, 2021) that have become a place of refuge for large populations, that will later migrate back to vineyards and orchards in the surrounding areas. In fact, anthropogenic factors have been highlighted as a crucial cause in *L. botrana* dispersal (Schartel et al., 2019). Thus, the need for *L. botrana* control in cities and towns.

Synthetic insecticides are not allowed for massive pest management in residential areas, because of public concern (Lance et al., 2016). In that light, MD formulations have been the main tool used against invasions of Lepidopteran pests in the few urban situations where a pest has been addressed by this technique (Soopaya et al., 2015; Suckling et al., 2017; Trematerra and Colacci, 2019). Eleven MD formulations (including emulsified wax, puffers, flowable, polyethylene tubes, etc.) authorized against *L. botrana* are available to Chilean growers (SAG, 2021), but the most commonly used is the single polyethylene tube (ISONET® L, Shin-Etsu Chemical, Tokyo, Japan), usually recommended at 500 dispensers ha<sup>-1</sup> (equivalent to 86 g (*E,Z*)-7,9-dodecadienyl acetate ha<sup>-1</sup> as minimum) that should be deployed homogeneously in the field (SAG, 2021). This rate provides a long-lasting and efficient emission of pheromone to the atmosphere, a main requirement for the MD technique to work efficiently. In Chile, the authority (SAG) requires the dispensers designed for a single application during the season cover effectively (causing male disruption) for at least 150-180 d (SAG, 2021). This type of dispensers has been used in Chilean cities since 2010 due to its availability and proven efficacy, reaching between 2000 to 6000 ha treated in urban areas in some seasons (SAG, 2021). Unfortunately, the presence of buildings and homes frequently make very difficult to reach either the recommended dispenser rate or a homogeneous distribution. Besides, the installation requires an important investment both of labor and time to deploy them in the field (Chouinard et al., 2016). As a result, meso-dispensers (MeD) having a greater pheromone load per unit (Welter and Cave, 2006) than standard ones (as ISONET® L) but recommended at a much lower density have been in development mainly since the mid 2000's and tested and used against lepidopteran pests, mostly less than a 100 ha<sup>-1</sup>, depending on the dispenser and the release rate (Baker et al., 2016). However, the pheromone load per dispenser and the number of sources per hectare are proven key factors for MD efficiency.

Accordingly, the modifications in both factors, when using MeD, need to be evaluated to verify disruption. In some studies, competitive mechanisms seem to operate when MeD are used in the field against the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) (McGhee, 2014). Some available MeD formulations are the battery-powered aerosol spray type (Benelli et al., 2019; Gavara et al., 2020), programmable devices that periodically and actively release pheromone from

pressurized cans (used at 2.5 units ha<sup>-1</sup>) at certain times of the day; and the large polyvinyl chloride polymers (Light, 2016) or the polyethylene rings (Light et al., 2017), both acting as passive-dispensers (pheromone is released constantly [Gavara et al., 2020] over time depending mainly on temperature). The latter formulations have been successfully tested for MD against several tortricid pests in orchards, at low densities, reaching satisfactory percentage of disruption (Hedstrom et al., 2014; USU, 2020). However, only a few reports on passive (Hummel, 2017: only reporting results for 7 wk) or active (Benelli et al., 2019) MeD have been published regarding *L. botrana*; and, as far as we know, none under urban situations. Thus, all these data support the idea to develop a similar approach for practical and affordable urban control against *L. botrana*. Our goal was to evaluate the efficacy of a new MeD device for *L. botrana* mating disruption, measuring both adult male disruption and densities of eggs, larvae, and pupae in grape clusters, in residential areas in several infested cities in central Chile, during three consecutive seasons (2013-2016).

## MATERIALS AND METHODS

### Locations and sampling stations

The study was conducted in four urban areas in central Chile: Lontué, Molina, Rauco, and Sagrada Familia (34°06' to 34°56' S, 71°18' to 71°17' W), all in the Maule Region. Field trials were set as detailed in Table 1. All four cities are surrounded frequently by vineyards and fruit orchards and were severely infested (at least 85% of clusters damaged) by European grapevine moth (EGVM), *Lobesia botrana* (Denis & Schiffmüller), the season before our studies started. These cities are very close to each other (no more than ~ 20 km between the farthest ones) and share geographic and climatic conditions. The urban areas ranged between 89 to 250 ha, with at least 80% of homes with vegetation (backyard gardens). A selection of sampling sites was made by using a 2500 m<sup>2</sup> (50 × 50 m) grid on each city map, discarding non eligible ones (e.g., industrial lots, large buildings), numbering grid sites, and sorting 16 sites per city by using a random number table. After visiting each selected site, three houses having vines in the yard were chosen as sampling stations for clusters (n = 48 houses per city). One of those houses per site was selected for EGVM male trapping (n = 16 traps per city). In the selected cities, *L. botrana* had not previously been managed by mating disruption (MD). No insecticides were used during the study seasons.

### Monitoring of traps and sampling of clusters

In each selected city, one white delta trap (Pherocon VI, Trécé, Adair, Oklahoma, USA) with removable liner (replaced once a month or when cumulative captures exceeded 100 individuals) was set, at 1.8-2.0 m height, in vines or other plants in each house backyard, at least 50 m apart from each other. A septum, containing 1 mg (*E,Z*)-7,9-dodecadienyl acetate (E7Z9-12:OAc, Pherocon EGVM, Trécé Inc., Adair, Oklahoma, USA), was used per trap and replaced every 6-8 wk. The *L. botrana* traps were placed in the houses along the installation of MeD (made early in the season as required by the governmental authority) in the respective city. Traps were checked weekly for male counts and, when found, the specimens were removed from the liner. The first season (2013-2014), both trap and cluster sampling were conducted only for 17 wk because the trial was set once the meso-dispensers arrived to Chile, by mid-December (*ca.* in the middle of second flight). For the 2<sup>nd</sup> (2014-2015) and 3<sup>rd</sup> seasons (2015-2016), monitoring and cluster sampling (floral or fruits) were conducted for 30 wk, completely covering the three typical flights occurring in the area. Cluster review (n = 30 house<sup>-1</sup>) was also conducted weekly, registering the total numbers of eggs, larvae, and pupae observed on the grapes. Flight extensions, in each season, were estimated based on *L. botrana* forecast model from the Chilean Department of Agriculture (SAG, 2021).

**Table 1. Seasons and cities treated/untreated with meso-dispensers (MeD) for *Lobesia botrana* mating disruption.**

Seasons	Lontué	Molina	Rauco	Sagrada Familia
2013-2014	Treated (T1)	Untreated (T0)	N/T	N/T
2014-2015	Treated (T1)	Treated (T1)	Untreated (T0)	N/T
2015-2016	Treated (T1)	N/T	Treated (T1)	Untreated (T0)

T1: Treated with MeD; T0: untreated: used as control in that particular season; N/T: city not tested/sampled in that season.

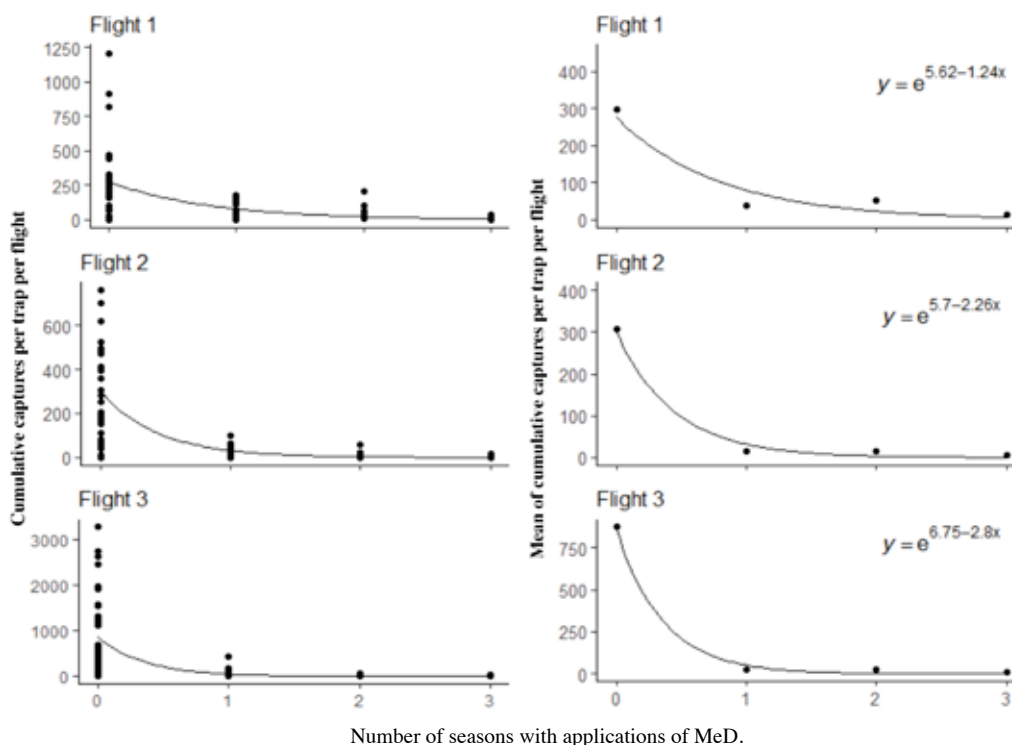
## Meso-dispensers

Mating disruption meso-dispensers, ISONET® L ring (Shin-Etsu Chemical, Tokyo, Japan), consist of 1 m-length polyethylene twin tubing. A similar dispenser has also been developed for codling moth (USU, 2020). One ISONET® L ring is equivalent to 10 pieces of standard ISONET® L, which consist of a 10 cm-length single tube (E7Z9-12:OAc is 67 wt.% as minimum, loaded with 172 mg E7Z9-12:OAc dispenser<sup>1</sup>, recommended at 500 units ha<sup>-1</sup>), i.e., 1 m ISONET® L ring dispenser was loaded with 1.72 g (E7Z9-12:OAc) each as minimum. The installation of MeD in the field was made by the end of August and the beginning of September in 2014 and 2015, and by mid-December in 2013. Meso dispensers were placed in the field at a rate of 50 MeD ha<sup>-1</sup> (equivalent to 86 g ha<sup>-1</sup> as minimum) in every selected city, covering all its urban area. MeD were tied around branches and trunks of the trees along road sides, parks, and yards of houses, and utility poles usually at 1.8-2.0 m height.

## Experimental design and data analysis

The contrasts between cities treated with MeD (treatment 1, T1) and the respective untreated cities (T0) were conducted taking into consideration either cumulative male trap captures or either, eggs, larvae, and pupae densities, per flight, for the 16 sites per city (considered pseudo replicates) within locations (replicates). In the first season (2013-2014) only the third flight was contrasted between treatments (due to the delay in MeD installation), whereas all three flights were evaluated in the following seasons. A complete randomized block design was used to contrast MeD and untreated locations using the seasons as blocks (2013-2014 block 1, 2014-2015 block 2, and 2015-2016 block 3) and sites inside cities as sub-samplings. Data of the three flights were only evaluated in seasons 2014-2015 and 2015-2016, so in this case a complete randomized block design with factorial structure was considered, whose levels were the flights (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup>) and treatments (T1 and T0), both considered as factors. Treatments were a city with just one application of MeD (T1) vs. the untreated city (T0), therefore in block 1 (season 2013-2014) T1 was Lontué and T0 was Molina; Block 2 (season 2014-2015) T1 was Molina and T0 was Rauco, and, finally, in block 3 (2015-2016) T1 was Rauco and T0 was Sagrada Familia. Lontué was treated with MeD all three consecutive seasons but considered as T1 only in block 1. Generalized linear mixed models were adjusted using a negative binomial distribution and log link function. Block and sites were considered both as random effects where

**Figure 1. Cumulative individual values (left graph) and mean (right graph) of captures per flight of *Lobesia botrana* males for 1, 2, or 3 seasons consecutively receiving meso-dispenser for mating disruption (MeD) vs. untreated controls (0), in one or more cities (Lontué, Molina, Rauco, Sagrada Familia) in central Chile.**



sites were nested to block, in a hierarchical structure. Wald's test was used to verify differences between treatments. The mean values presented per treatment were obtained through the application of the inverse link function of linear predictors, and their respective standard errors were calculated through the Delta Method (Agresti, 2013). Significant differences between linear predictors were separated using the Tukey test for multiple comparisons. Besides, in cities with 1 (Lontué, Molina, Sagrada Familia), 2 (Lontué), or 3 (Lontué) consecutive MeD applications and the respective untreated cities (0 applications), the number of cumulative male *L. botrana* catches per trap per site were correlated with the number of MeD treatments (Figure 1 where each point represents a trap within a location with that number of MeD applications), using a Generalized Linear model with Poisson distribution and log link function to create a Poisson regression. The disruption index (DI) for male captures was estimated (per cities, seasons, and individual flights) as  $DI = 100 \times (1 - MeD/U)$ , where MeD is captures in sites treated with meso-dispensers, and U is captures at the respective untreated sites. All the analyses were done using the R programming language (R Core Team, 2017).

## RESULTS

### Trap captures

The average of male captures trap<sup>-1</sup> during the third flight (the only one with monitoring data in all three seasons) was 100 (untreated) and 10 (MeD), being both treatments significantly different. When considering the three flights jointly, per season (only data from 2014-2015 and 2015-2016), the interaction between flights and treatments was nonsignificant, i.e., there was no influence on captures per treatment by the flight number; the average per treatment was 300 (untreated) and 15 (MeD), both highly significantly different, whereas the average trap per flights were 100 (1<sup>st</sup>) and 100 (3<sup>rd</sup>), both not significantly different, but significantly greater than the 2<sup>nd</sup> (50).

Cumulative captures (in 16 traps per city) per flight are presented in Table 2. The disruption index (DI) ranged between ca. 69 and ca. 99 (by single flights) considering all cities and seasons. In seasons when the three flight cycles were evaluated jointly, a trend for lower DI values during the first one was observed. The DI values, considering all three flights, ranged from ca. 88 to ca. 98. Molina and Sagrada Familia, cities used as control one year and treated with MeD the next one, always presented a notable reduction in captures after MD was used the previous season: i.e., in Molina, in the 3<sup>rd</sup> flight, cumulative captures decreased from 28 403 in 2013-2014 (when it was the untreated city) to 470 in 2014-2015 (the treated city), and in Sagrada Familia, cumulative captures for the three flights all together, decreased from 17 538

**Table 2. Cumulative *Lobesia botrana* male captures per treatment with meso-dispensers for mating disruption (MeD) and untreated control, and disruption index (DI), by cities, seasons, and flights within seasons, and the respective whole season (N = 16 traps per city).**

Cities	Seasons	Flight number	Captures		DI
			MeD	Untreated <sup>1</sup>	
Lontué	2013/2014	3 <sup>rd</sup>	303	28 403	98.9
		1 <sup>st</sup>	837	3581	76.6
	2014/2015	2 <sup>nd</sup>	201	6247	96.8
		3 <sup>rd</sup>	367	7710	95.2
		Whole season	1405	17 538	92.0
	2015/2016	1 <sup>st</sup>	245	5964	95.9
		2 <sup>nd</sup>	65	3628	98.2
3 <sup>rd</sup>		203	6203	96.7	
Whole season		513	15 795	96.8	
Molina (Mo)	2014/2015	1 <sup>st</sup>	1113	3581	68.9
		2 <sup>nd</sup>	460	6247	92.6
		3 <sup>rd</sup>	470	7710	93.9
		Whole season	2043	17 538	88.4
Rauco (RA)	2015/2016	1 <sup>st</sup>	93	5964	98.4
		2 <sup>nd</sup>	48	3628	98.7
		3 <sup>rd</sup>	151	6203	97.6
		Whole season	292	15 795	98.2

<sup>1</sup>Mo in 2013/2014, RA in 2014/2015, and Sagrada Familia in 2015/2016.

(untreated city) in 2014-2015 to 292 in 2015-2016 (treated city). When the whole set of data (captures) per trap per flight was modelled through a Poisson regression to the number of seasons with consecutive applications of MeD, all were highly significant, where an inverse and asymptotic trend occurred (Figure 1).

### Cluster infestation

Within flights, considering all three consecutive seasons (2013-2016), the respective city treated with MeD presented a significantly lower number of eggs (than the untreated one) in the first and second cycles, but nonsignificant differences occurred in the third one (Table 3). However, between the 2014-2015 and 2015-2016 seasons, a greater number of eggs was always (in all three flights) found in the untreated cities. In the same period there were highly significant differences between flights and the Treatment × Flight interaction.

Larval densities on clusters, considering only the third flight in three consecutive seasons, were significantly lower in the MeD treatment. When comparing the number of larvae during the whole seasons (2014-2015 and 2015-2016), highly significant differences occurred between treatments, flights and the Treatment × Flight interaction. Similar to our results on eggs, the mean of pupae on clusters, considering the third flight in three consecutive seasons, was not significantly different between treatments. When comparing pupae densities between treatments, considering the three flights in the two seasons evaluated entirely, results matched those obtained for eggs and larvae, i.e., significant differences occurred between treatments, flights, and the Treatment × Flight interaction.

Cumulative densities of eggs, larvae, and pupae of *L. botrana* on clusters, per flights or the whole season, in all cities receiving MeD treatments, are presented in Figures 2, 3 and 4, showing in all immature stages a decreasing trend.

## DISCUSSION

*Lobesia botrana* male captures reveal huge populations in the urban areas selected as controls (untreated) for the study, as reported previously in other cities in central Chile (Curkovic and Ferrera, 2010), being much greater than those observed in agricultural situations in the country. In general, our results (at local level) showed similar captures during the first and third flights, being significantly lower during the second one, like historical reports for Chilean populations at regional level (SAG, 2021). Data also agrees with the three generations described for most of central Chile and Argentina, except for some specific localities, where a 4<sup>th</sup> flight has been informed (Dagatti and Becerra, 2015; SAG, 2021), but not including the cities used in our study.

In terms of seasonal disruption, our results agree with most data on MD trials against *L. botrana*, where captures tend to be significantly lower in treatments with pheromone whereas the untreated (control) presented much greater captures, showing the normal *L. botrana* phenology in orchards (as results shown by Louis and Schirra, 2001; Varner et al., 2001; Ioriatti et al., 2011). However, most previous results come from trials in agricultural areas, with much smaller initial populations (than those observed in the Chilean cities) and very low captures along the season when MD is implemented, reaching either total or very high disruption (but in a few of those reports) on *L. botrana* managed by MD, disruption has been lower than our findings: e.g., 83% found by Vacas et al. (2011), 77% by Arioli et al. (2014). At this respect, it is broadly accepted that MD success strongly depends on moth density at the application site (Louis and Schirra, 2001; Ioriatti et al., 2004), even when MeD have been used (Welter et al., 2011). Thus, the level of seasonal disruption obtained in our study (> 88%), despite the large *L. botrana* populations, is considered high for severe infestations, specially based on the threshold reported by Ioriatti et al. (2011) of 4000 pairs (ha season<sup>-1</sup>), under which MD was dramatically

**Table 3. Mean cumulative densities (± SE) per flight of *Lobesia botrana* eggs, larvae, and pupae found on clusters sampled from vines in sites (n = 16 sites × 3 houses per site) treated with meso-dispenser for mating disruption (MeD) vs. untreated controls.**

Flight	Eggs		Larvae		Pupae	
	MeD	Untreated	MeD	Untreated	MeD	Untreated
1 <sup>st</sup>	7.3 ± 3.6a	27.7 ± 13.6b	7.2 ± 3.6a	46.3 ± 23.0b	2.9 ± 1.1a	17.3 ± 6.3b
2 <sup>nd</sup>	17.6 ± 8.7a	51.7 ± 25.5b	27.6 ± 13.7a	92.9 ± 42.2b	10.1 ± 3.7a	35.6 ± 12.9b
3 <sup>rd</sup>	11.7 ± 5.8a	7.9 ± 3.9a	26.4 ± 13.1a	74.5 ± 37.0b	9.5 ± 3.5a	13.5 ± 4.9b

Different letters between treated (MeD) and untreated sites, within immature stages and flights, indicate significant differences according to the Tukey comparison test (p < 0.05).

Figure 2. Cumulative densities of eggs of *Lobesia botrana* on clusters per individual flight (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) or the whole flight season, in 48 houses per city (Lontué, Molina, Rauco, Sagrada Familia), receiving meso-dispenser for mating disruption (MeD) either 1, 2, or 3 consecutive seasons vs. untreated controls (0).

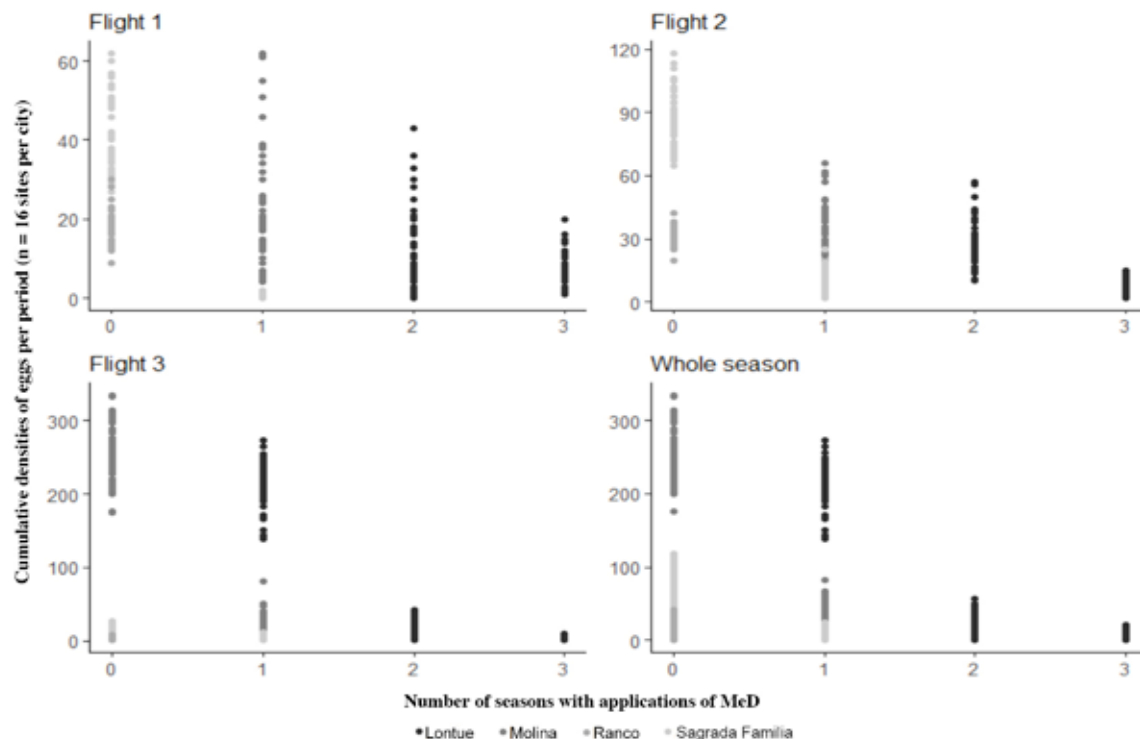
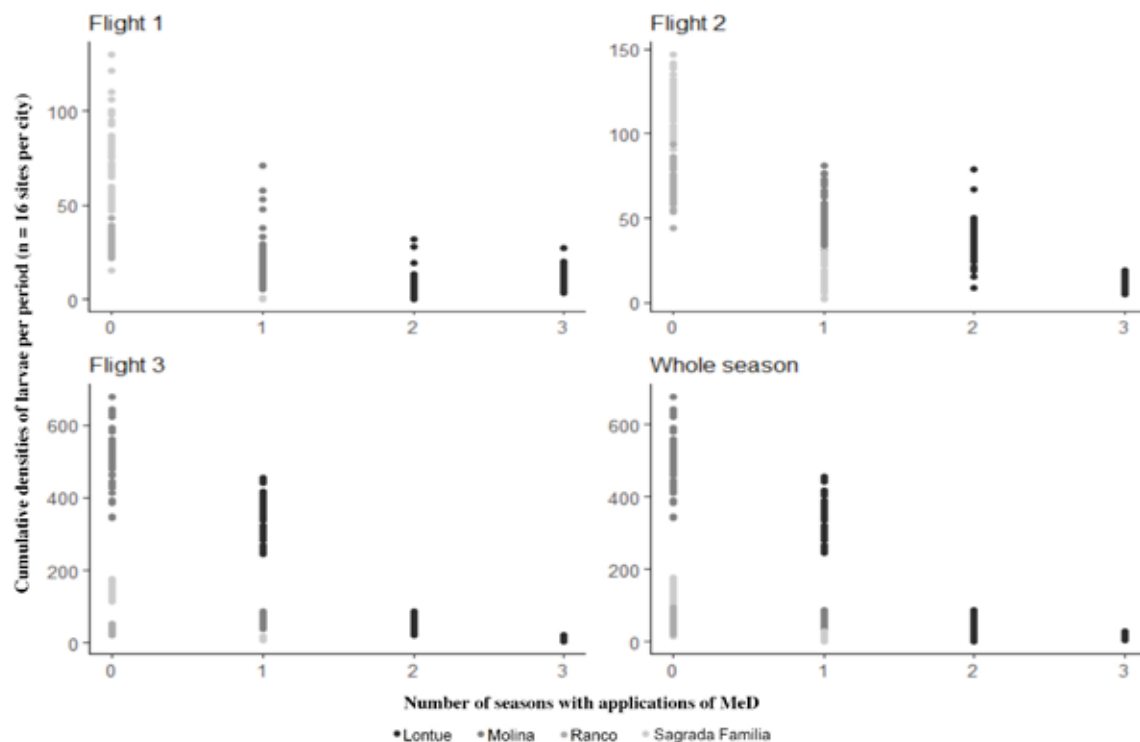


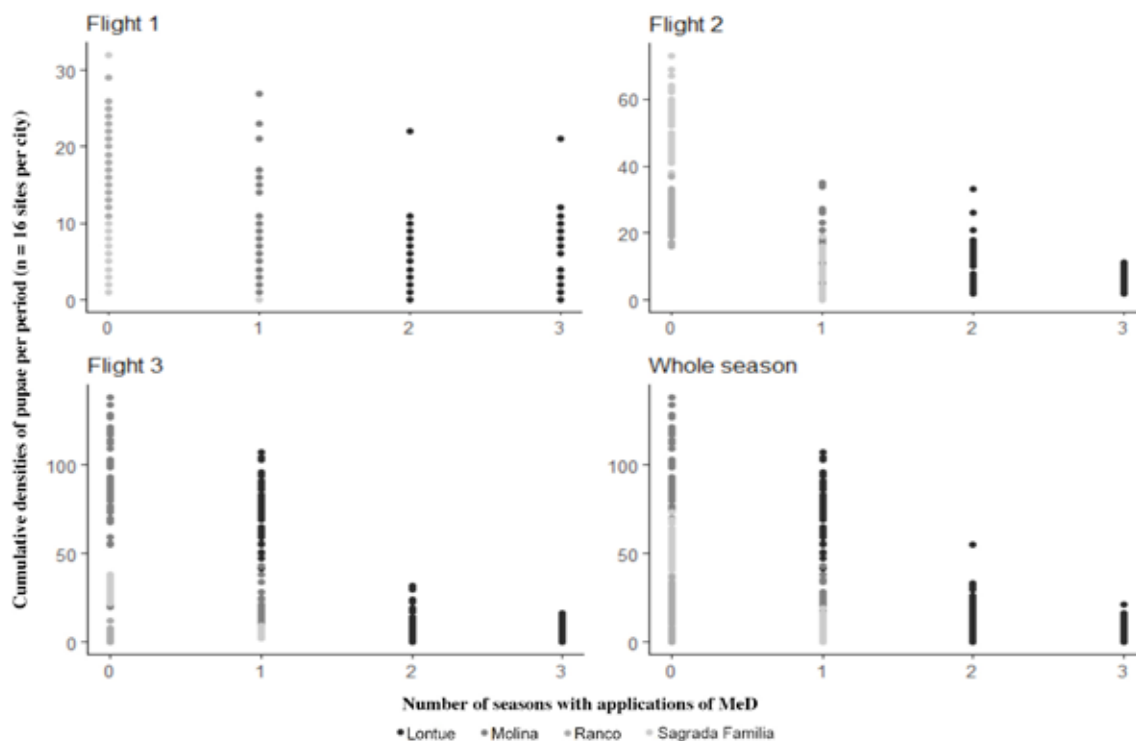
Figure 3. Cumulative densities of larvae of *Lobesia botrana* on clusters per individual flight (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) or the whole flight season, in 48 houses per city (Lontué, Molina, Rauco, Sagrada Familia), receiving meso-dispenser for mating disruption (MeD) either 1, 2, or 3 consecutive seasons vs. untreated controls (0).



reduced. Consequently, both trap shutdown (trap captures ceasing immediately after MD is applied; Light, 2016) and, consequently total disruption (DI = 100%), were not achieved in the MD trials we conducted in cities in central Chile. Despite that, an asymptotic trend for lower captures was observed (regarding the number of seasons that the MD was implemented) particularly in Lontué, where MeD was used for three consecutive seasons. As herein, MeD used for MD against other species (not *L. botrana*) has been promissory in several studies in orchards. For instance, Bulgarian researchers demonstrated MeD applied once a season at 20 units ha<sup>-1</sup> was as effective as insecticide management in controlling codling moth in apple orchards, and oriental fruit moth, *Grapholita (Cydia) molesta* Busck (Lepidoptera: Tortricidae) in peach orchards (Kutinkova et al., 2019), obtaining damage below the economic threshold in both cases. In another study on the Filbert worm, *Cydia latiferrana* Walsingham (Lepidoptera: Tortricidae) in field tests using as low as 24 dispensers ha<sup>-1</sup> for three consecutive seasons in hazelnut orchards, the authors obtained relatively low moth density over time and disruption above 50% in most cases (Hedstrom et al., 2014). Similarly, Grant et al. (2012) found that MeD (using about 60 ha<sup>-1</sup>) was effective for codling moth MD even in relatively small orchards.

Regarding immature *L. botrana* found in clusters, only a few reports on successful MD tested against lepidopteran pests have measured effects on densities of juvenile stages, none evaluating MeD. Additionally, our results were sorted by the three stages (eggs, larvae, and pupae) and the flight number, showing in eight out of nine cases (3 flight × 3 stages) immature densities were significantly greater in the untreated sites, and no differences were found in the 9<sup>th</sup> case. The literature review showed less detailed results. For instance, Varner et al. (2001) found no *L. botrana* larvae after five seasons using two types of regular dispensers (Rak from BASF and Isonet from Shin-Etsu, both used at 500 ha<sup>-1</sup>) for MD in vineyards in Italy. Within a particular season, Louis and Schirra (2001) found significant reduction on *L. botrana* larval infestation on vine clusters during the first generation but increasing notably during the second one after being treated by regular (Rak) dispensers designed to control both *L. botrana* and the sympatric species *Eupoecilia ambiguella* Hbn. (Lepidoptera: Tortricidae). In previous publications on *L. botrana* egg density after MD treatments, only a report showing data on the sum of eggs + larvae infestations on clusters in vineyards (Gordon et al., 2005) was found, but the study was

**Figure 4. Cumulative densities of pupae of *Lobesia botrana* on clusters per individual flight (1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>) or the whole flight season, in 48 houses per city (Lontué, Molina, Rauco, Sagrada Familia), receiving meso-dispenser for mating disruption (MeD) either 1, 2, or 3 consecutive seasons vs. untreated controls (0).**





conducted during a single season. Our results, particularly on eggs, have shown *L. botrana* densities from the first and second flights did match the expected reduction in densities, following the decrease on male captures because of the MeD treatment, but not on the third flight. It is possible that the delay on the onset of the MeD application the first season in Lontué (flights 1 and 2 were not covered by MeD) allowed many females to mate and lay viable eggs on clusters before any disruption on males, influencing later the analysis of the pooled data for all three seasons. On the other hand, MeD treatments in our study significantly decreased both larval and pupal densities in all cases. Reports on *L. botrana* pupae densities after MD treatments over time were not found. Results with all three immatures densities obtained under a different number of seasons with MeD treatments, also showed an asymptotic trend (as observed for male catches). Our results agree with population dynamics effects described by Liebhold and Tobin (2008), when the use of techniques as MD facilitate eradication programs. As far as we know, this is the first report of MeD used against *L. botrana* in urban areas.

## CONCLUSIONS

The meso-dispenser (MeD) treatment significantly reduced captures of *Lobesia botrana* adult males in traps despite the initial elevated populations observed in urban areas in central Chile. In fact, high values of disruption were obtained in most cases, and an asymptotic trend in male captures is predicted over time if mating disruption (MD) treatments were to be conducted during enough successive seasons. These results demonstrate that the increase (10 times) in initial pheromone load per MeD, and the consequent 10x reduction on point sources, was enough to obtain a high level of male disruption. In addition, the effects on adult male *L. botrana* disruption leads to a significant reduction on immature densities over time without using any other pest control, including chemical sprays. To our best knowledge, this is the first successful trial to control *L. botrana* in urban areas with MeD for MD technique, thus, the new formulation is suitable for MD treatments in cities.

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## REFERENCES

- Agresti, A. 2013. Categorical data analysis. 3<sup>rd</sup> ed. John Wiley & Sons, Hoboken, New Jersey, USA.
- Arioli, C.J., Pastori, P.L., Botton, M., Garcia, M.S., Borges, R., and Mafra-Neto, A. 2014. Assessment of SPLAT formulations to control *Grapholita molesta* (Lepidoptera: Tortricidae) in a Brazilian apple orchard. Chilean Journal of Agricultural Research 74:184-190. doi:10.4067/S0718-58392014000200009.
- Baker, T.C., Myrick, A.J., and Park, K.C. 2016. Optimizing the point-source emission rates and geometries of pheromone mating disruption mega-dispensers. Journal of Chemical Ecology 42:896-907. doi:10.1007/s10886-016-0769-9.
- Benelli, G., Lucchi, A., Thomson, D., and Ioriatti, C. 2019. Sex pheromone aerosol devices for mating disruption: Challenges for a brighter future. Insects 10(10):308. doi:10.3390/insects10100308.
- Chouinard, G., Firlj, A., and Cormier, D. 2016. Going beyond sprays and killing agents: Exclusion, sterilization and disruption for insect pest control in pome and stone fruit orchards. Scientia Horticulturae 208:12-27. doi:10.1016/j.scienta.2016.03.014.
- Curkovic T., y Ferrera, C. 2010. Autoconfusión: una nueva tecnología en base a feromonas para el control de *Lobesia botrana* en Chile. Aconex 105:5-11.
- Dagatti, C.V., y Becerra, V.C. 2015. Ajuste de modelo fenológico para predecir el comportamiento de *Lobesia botrana* (Lepidoptera: Tortricidae) en un viñedo de Mendoza, Argentina. Revista de la Sociedad Entomológica Argentina 74:3-4.
- Gavara, A., Vacas, S., Navarro, I., Primo, J., and Navarro-Llopis, V. 2020. Airborne pheromone quantification in treated vineyards with different mating disruption dispensers against *Lobesia botrana*. Insects 11(5):289. doi:10.3390/insects11050289.
- Gordon, D., Zahavi, T., Anshelevich, L., Harel, M., Ovadia, S., Dunkelblum, E., et al. 2005. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae): Effect of pheromone formulations and concentrations. Journal of Economical Entomology 98:135-142. doi:10.1093/jee/98.1.135.
- Grant, J., Pickel, C., Light, D., Goldman Smith, S., and Lowrimore, J. 2012. Pheromone-based codling moth and navel orangeworm management in walnuts. Walnut Research Report 2012:223-235.

- Harari, A.R., Zahavi, T., and Steinitz, H. 2015. Female detection of the synthetic sex pheromone contributes to the efficacy of mating disruption of the European grape vine moth, *Lobesia botrana*. *Pest Management Science* 71:316-322. doi:10.1002/ps.3830.
- Hedstrom, C.S., Olsen, J., Walton, V.M., and Chambers, U. 2014. Pheromone mating disruption of filbertworm moth (*Cydia latiferreana*) in commercial hazelnut orchards. *Acta Horticulturae* 1052:253-262. doi:10.17660/ActaHortic.2014.1052.34.
- Hummel, H.E. 2017. A brief review on *Lobesia botrana* mating disruption by mechanically distributing and releasing sex pheromones from biodegradable mesofiber dispensers. *Biochemistry and Molecular Biology Journal* 3:1. doi:10.21767/2471-8084.100032.
- Ioriatti, C., Bagnoli, B., Lucchi, A., and Veronelli, V. 2004. Vine moths control by mating disruption in Italy: Results and future prospects. *Redia* 87:117-128.
- Ioriatti, C., Anfora, G., Witzgall, P., and Lucchi, A. 2011. Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). *Journal of Economical Entomology* 104:1125-1137. doi:10.1603/ec10443.
- Kutinkova, H., Dzhuvinov, V., Stefanova, D., Andreev, R., Palagacheva, N., and Lingren, B. 2019. Control of oriental fruit moth, *Cydia molesta* Busck and peach twig borer *Anarsia lineatella* Zell. using reduced rate of pheromone dispensers. *IOBC-WPRS Bulletin* 146:47-54.
- Lance, D.R., Leonard, D.S., Mastro, V.C., and Walters, M.L. 2016. Mating disruption as a suppression tactic in programs targeting regulated lepidopteran pest in US. *Journal of Chemical Ecology* 42:590-605. doi:10.1007/s10886-016-0732-9.
- Liebhold, A.N., and Tobin, P.C. 2008. Population ecology of insect invasions and their management. *Annual Review of Entomology* 53:387-408. doi:10.1146/annurev.ento.52.110405.091401.
- Light, D.M. 2016. Control and monitoring of codling moth (Lepidoptera: Tortricidae) in walnut orchards treated with novel high-load, low-density “meso” dispensers of sex pheromone and pear ester. *Environmental Entomology* 45:700-707. doi:10.1093/ee/nvw017.
- Light, D.M., Grant, J.A., Haff, R.P., and Knight, A.L. 2017. Addition of pear ester with sex pheromone enhances disruption of mating by female codling moth (Lepidoptera: Tortricidae) in walnut orchards treated with meso dispensers. *Environmental Entomology* 46:319-327. doi:10.1093/ee/nvw168.
- Louis, F., and Schirra, K.J. 2001. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards with very high population densities. *IOBC-WPRS Bulletin* 24:75-80.
- McGhee, P.S. 2014. Impact of high releasing mating disruption formulations on (male) Codling moth, *Cydia pomonella* L., behavior. PhD dissertation. Michigan State University, Lansing, Michigan, USA.
- Miller, J.R., and Gut, L.J. 2015. Mating disruption for the 21st century: matching technology with mechanism. *Environmental Entomology* 44:427-453. doi:10.1093/ee/nvv052.
- Miller, J.R., Gut, L.J., de Lame, F.M., and Stelinski, L.L. 2006. Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone: (Part 1) theory. *Journal of Chemical Ecology* 32:2089-2114. doi:10.1007/s10886-006-9134-8.
- ODEPA. 2021. Superficie de frutales por región. Oficina de Estudios y Políticas Agrarias (ODEPA), Santiago, Chile. Available at <https://www.odepa.gob.cl/superficie-de-frutales-por-region-2> (accessed April 2021).
- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at <http://www.r-project.org/>.
- SAG. 2021. *Lobesia botrana* o polilla del racimo de la vid. Servicio Agrícola y Ganadero (SAG), Santiago, Chile. Available at <https://www.sag.gob.cl/ambitos-de-accion/lobesia-botrana-o-polilla-del-racimo-de-la-vid> (accessed April 2021).
- Schartel, T.E., Bayles, B.R., Cooper, M.L., Simmons, G.S., Thomas, S.M., Varela, L.G., et al. 2019. Reconstructing the European grapevine moth (Lepidoptera: Tortricidae), invasion in California: insights from a successful eradication. *Annals of the Entomological Society of America* 112:107-117. doi:10.1093/aesa/say056.
- Soopaya, R., Woods, B., Lacey, I., Viridi, A., Mafra-Neto, A., and Suckling, D.M. 2015. Feasibility of mating disruption for agricultural pest eradication in an urban environment: light brown apple moth (Lepidoptera: Tortricidae) in Perth. *Journal of Economical Entomology* 108:1930-1935. doi:10.1093/jee/tov142.
- Suckling, D.M., Conlong, D.E., Carpenter, J.E., Bloem, K.A., Rendon, P., and Vreysen, M.J. 2017. Global range expansion of pest Lepidoptera requires socially acceptable solutions. *Biological Invasions* 19:1107-1119. doi:10.1007/s10530-016-1325-9.
- Trematerra, P., and Colacci, M. 2019. Recent advances in management by pheromones of *Thaumetopoea* moths in urban parks and woodland recreational areas. *Insects* 10:395. doi:10.3390/insects10110395.
- USDA. 2020. European grapevine moth (*Lobesia botrana*). United States Department of Agriculture (USDA), Washington, D.C., USA. Available at [https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/european-grapevine-moth/ct\\_european\\_grapevine\\_moth](https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/european-grapevine-moth/ct_european_grapevine_moth) (accessed April 2020).
- USU. 2020. Codling moth mating disruption. Utah Pest Fact Sheet, January 2020. Utah University Extension and Utah Plant Pest Diagnostic Laboratory (Ent-137-10), Utah State University (USU), Logan, Utah, USA.

- Vacas, S., Alfaro, C., Primo, J., and Navarro-Llopis, V. 2011. Studies on the development of a mating disruption system to control the tomato leafminer, *Tuta absoluta* Povolny (Lepidoptera:Gelechiidae). *Pest Management Science* 67:1473-1480. doi:10.1002/ps.2202.
- Varner, M., Lucin, R., Mattedi, L., and Forno, F. 2001. Experience with mating disruption technique to control grape berry moth, *Lobesia botrana*, in Trentino. *IOBC-WPRS Bulletin* 24:81-88.
- Welter, S., and Cave, F. 2006. Evaluation of alternative pheromone dispensing technologies for codling moth. Walnut Research Report. University of California, Fruit and Nut Research and Information Center, Davis, California, USA.
- Welter, S., Casado, D., Cave, F., Elkins, R., Grant, J., and Pickel, C. 2011. Efficacy of modified pheromone application methods for codling moth management in walnuts: I. Low emission rate puffer application II. New aerosol emitter III. Modified hand-applied dispensers. *Walnut Research Reports* 2011. p. 195-208. California Walnut Board, Folsom, California, USA.