Chapter

# Integrated Pest Management of *Lobesia botrana* with Microorganism in Vineyards: An Alternative for Clean Grapes Production

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## Abstract

The moth *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae) is one of the principal pests of the grapevines (*Vitis vinifera L.*). His larvae feeds from grape, reducing production and increasing susceptibility to fungal infections. This makes it one of the most economically important pest insects in wine and table grape exporting countries. This chapter will describe the distribution, biology, and behavior of *L. botrana* regarding its host, the grapevine, along with its control via the use of natural enemies, entomopathogenic microorganisms, MD (mating disruption) and chemical control. Finally, we will describe an integrated management strategy based on monitoring, MD, and biological control using entomopathogenic microorganisms. This strategy could be useful as a basis for integrated pest control plans in various regions worldwide.

**Keywords:** *Lobesia botrana* (Denis & Schiffermüller), grapevine, integrated pest management, biological control, ethological control and chemical control

## 1. Introduction

*L. botrana* was first scientifically described in 1775 by Denis and Shiffermüller in Austria. This pest is endemic to the Palearctic Region, but is economically more important in southern Europe and South America [1–3]. In Europe it principally affects southern France, central and southern Spain, Portugal, Greece, Italy and the Mediterranean islands [2, 4], while in South America it affects Argentina and Chile [5]. Its broad range is partly attributable to its ability to adapt to climate changes, characteristic of lepidopterans [6] causing a lack of synchronization with its natural parasites and predators and contributing to significant short-term increases in *L. botrana*. Its nature as a polyphagous pest also contributes to its swift establishment in any geographic region it reaches. In its larval stage, it has been reported to eat grapes along with 40 other plant species belonging to 27 families. These host plants generally grow in warm and dry environments, and include *Olea europea* L., *Zizyphus vulgaris* L., *Rosmarinus officinalis* L., *Clematis vitalba* L., *Cornus* spp.,

*Lonicera xylosteum* L., *Viburnum lantana* L, *Ligustrum vulgare* L., *Ribes* spp., and *Hedera helix* L, among others [7–10]. To develop an integrated *L. botrana* management strategy, we must (1) adequately identify and monitor this pest in its different development stages and its natural enemies. (2) determine the economic damage thresholds at which to begin controlling. (3) Take management decisions according to information from monitoring. (4) Do natural, cultural and biological follow-ups along with the use of selective chemical insecticides, where necessary.

## 2. Life cycle of *L. botrana* on grapevines

*L. botrana* is a multivoltine species with a facultative diapause (physiological state of inactivity). The number of generations depends on latitude, photoperiod, humidity, temperature, climate, microclimate and food type [11]. In Europe, two generations per year are common in Germany, Switzerland, Austria and northern France, while three generations (and sometimes four) have been reported in southern France, Spain, Portugal, Greece and Italy [12, 13]. In Chile at least three and possibly four annual generations are known [14].

The eggs of the first generation are deposited separately or in groups of two or three on grapevine buds, pedicels and flowers [15]. Their shape is elliptical, flat and slightly convex, and they measure between 0.65–0.90 mm long by 0.45–0.75 mm wide. Recently laid eggs are translucent and creamy white in color (**Figure 1A**), turning pale yellow with time (**Figure 1B**). They then turn black, with the head of the developing larva visible (**Figure 1C**) [16]. Finally, the egg hatches 7–11 days after laying, depending on temperature and humidity conditions (**Figure 1D**) [8, 15]. Once the larva emerges from the egg, only the shell or the round and nacreous mark of the shell remains (**Figure 1E**).

*L. botrana* larvae have five development stages (**Figure 2**): I (L1: 0.9–1-0 mm), II (L2: 1.9–3.0 mm), stage III (L3: 4.5–5.0 mm), stage IV (L4: 6.0–7.0 mm) and stage V (L5: 10.0–11.0 mm). Larval development concludes after 20 to 30 days in optimal conditions of 26.7°-29.4°C and 40–70% relative humidity [14].

First generation larvae are called the anthophagous generation, since they attack the plant in or near its flowering season, feeding on flower buttons, flowers and occasional small recently formed fruits. First generation larvae form "nests" or glomerules before and during flowering (**Figure 3**) [14]. These glomerules are formed by various flower buds joined together by silk threads spun by the larvae [8]. Damage caused by first generation larvae on the vines have minimal repercussions [17]. However, larvae in the second generation cause decreased vine productivity, since they attack developing grapes, perforating the skin and feeding on their pulp. Finally, these grapes are scared (**Figure 4**), dry out, fall or rot, depending on their size and the ambient humidity. Third generation larvae, by comparison with second generation larvae (both called carpophagous generations) produce greater damage to vine productivity, since the grapes are matured or in the maturation process [14]. Therefore, larval action exposes their sugary juices, favoring the entry,

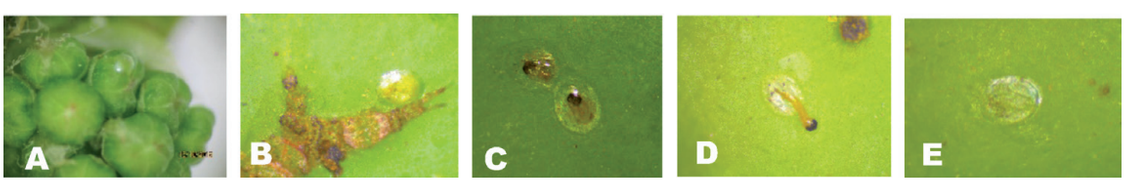


**Figure 1.**
*L. botrana eggs. A, creamy white egg. B, yellow eggs. C, black head egg. D, larva hatching, E) round and nacreous mark.*

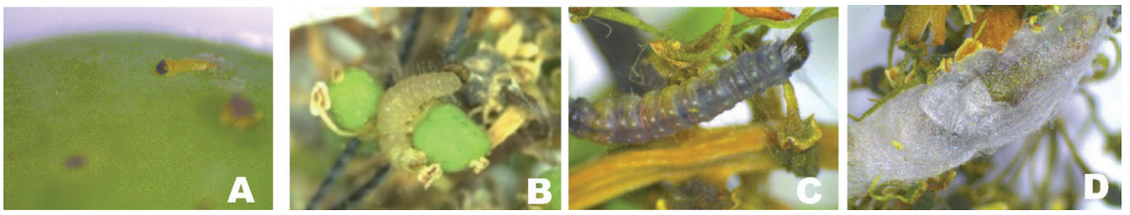


**Figure 2.**
*L. botrana larvae. A, newborn larva. B, young larva. D, mature larva. E, stage V larva spinning a grayish-white silk cocoon for the pupation process.*

**Figure 3.**
L. botrana *glomerules on grape bunches.*

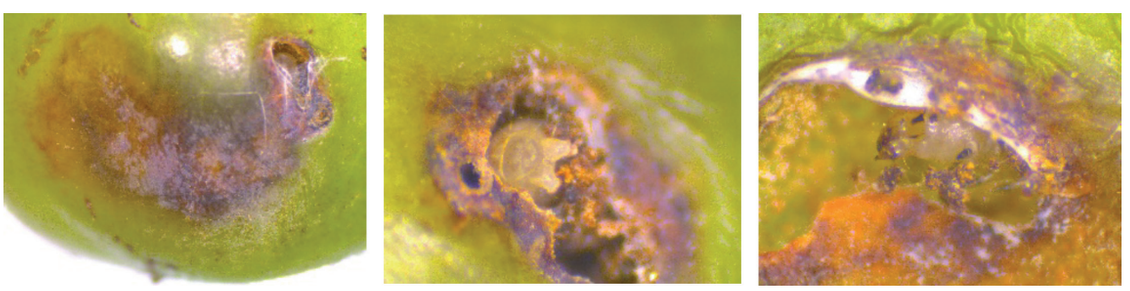

**Figure 4.**
*Grape damage from* L. botrana *larvae.*

establishment and proliferation of microorganisms responsible for diseases including *Botrytis cinerea* (Persoon: Fries) (*Sclerotiniaceae)* [18] and black *Aspergillus* (*Aspergilus niger* and *Aspergilus carbonarius*) which produces ochratoxin A [1].

*L. botrana* pupae are elongated, with a green to dark brown color. The average length of a male and female pupa is 5.5 mm and 7.0 mm, respectively, while the average width is 1.6–1.7 mm (**Figure 5A**). Males have 4 abdominal segments, and


**Figure 5.**
L. botrana *pupae. A, left – female and right - male. B, pupae in diapause with cocoon.*

females have 3. Their eyes, antennae, wings and abdominal segments can be seen in their structure. Pupae are covered by a silky, white, fused and continuous cocoon.

In vineyards, *L. botrana* hibernates in the pupal stage principally beneath the grapevine bark, in trunk cracks, soil and fallen leaves. During this period the pupae are in diapause, presenting a thick, highly hydrophobic cocoon. This tissue protects the pupa from low temperatures and water (**Figure 5B**) [19]. Full pupal development in diapause takes around 90 days while the pupal state during spring and summer is around 12–14 days, or 130°C days [14, 16].

In springtime, when temperatures rise, adults emerge from pupae in diapause. They emerge in stages, beginning before grapevine budding or extending over several weeks. The first adults to emerge are generally males, but in the later part of the flight period females predominate.

Adult *L. botrana* specimens are 6.0–8.0 mm long with a wingspan of 11.0–13.0 mm. Both sexes have a dorsal design with a cross-sectional band on the front wing pair, which can be seen with the wings laid to rest over the body. Male lack a side fold in their front wings; their back wings are whitish with a brown edge, while female rear wings are completely brown [16]. They can live from one to three weeks. Their activity is crepuscular, remaining inactive during the day and hiding in leaves and bunches. They mate in flight (1 to 6 days after emerging), females generally mate once in their lives. Egg laying begins one or two days after mating, and each female can lay between 80 and 160 eggs [16].

Regarding the dispersion capacity of *L. botrana* moths, males can fly several meters above vegetation and use air currents for longer migrations, while females generally spread over small areas and cannot go beyond 100 m [20]. This indicates that *L. botrana* colonization in new territories occurs mainly due to transferring pest-infested materials.

## 3. Chemical control

Insecticides are applied according to economic damage level, which can vary depending on generation, cultivar susceptibility to subsequent infection by *B. cinerea* and the grape product target market (wine production or fresh consumption). Chemical control of the first generation is only applied when pest population density reaches 50% of buds infested. The apparent greater flexibility of the damage threshold for controlling the first generation lies in the fact that during the flowering and harvest periods, the reduction of flowers and grapes is compensated by increased size and weight of healthy grapes. For following larval generations, the damage threshold varies between 1% and 5% or between 10% and 15% of bunches damaged, depending on the cultivar, bunch rigidity and harvest time [21].

Neurotoxic insecticides are mainly used for controlling *L. botrana* populations, including chlorantraniliprole, abamectin, indoxacarb, chlorpyrifos, methyl chlorpyrifos, anthranilic diamides, emamectin and spinosad. Growth regulators are also used, including fenoxycarb, methoxyfenozide, and tebufenozide. All the insecticides mentioned are larvicides; however, methoxyfenozide, chlorantraniliprole and indoxacarb are also ovicides.

To be effective, these substances must be applied when the pest is in its most vulnerable development stage, which makes predicting the *L. botrana* development cycle fundamental for determining optimal treatment programs. Selective insecticide programs along with population monitoring via pheromone traps and field monitoring for eggs generally provide adequate *L. botrana* control [22].

## 4. Ethological control: pheromones and their use in mating disruption (MD)

Pheromones are volatile chemical messengers released into the environment which can influence the behavior of other individuals of the same species at a distance. They are secreted by individuals via their exocrine glands. They are highly specific at the species level, affecting insects' aggregation, dispersion, alarm and sexual behavior [23].

In the exocrine glands of female *L. botrana* specimens, a linear hydrocarbon chain of 15 carbons have been identified which present acetate and alcohols as functional groups. The principal pheromone compound among these is (E, Z) -7,9-dodecadienyl acetate. *L. botrana* can sense and respond to this compound in a wide range of concentrations between 0.1–2500 ng [24, 25].

The chemical attractant capacities of this pheromone lead to its use as a tool for monitoring adult male *L. botrana* specimens. Monitoring is done via counting captured males which are trapped on the sticky surfaces of female pheromone traps (**Figure 6**). Female pheromone use also allows us to control pest populations via MD. This strategy consists of interfering with insects' olfactory chemical communication via mass distribution of synthetic pheromones in the field with MD dispensers. This creates a pheromone cloud which disorients and confuses the males and keeps them from finding females, thereby impeding mating and reducing pest populations [23]. The MD strategy relies on two different mechanisms: one is competition between females and MD dispensers in attracting males; and the other is based on camouflaging the olfactory track which have on females. Commercial MD dispensers, carry the compound (E, Z) -7,9- dodecadienyl acetate, which is progressively sprayed into the farming environment for a determined period of time. The release rate for each unit is generally 50–60 μg/h [26].

When applying this method, pest population density must be considered, as it is more effective with a lower adult population density. Above a certain density, mating is not interrupted regardless of ambient pheromone concentrations; the critical density for *L. botrana* is 4000 couples per hectare, and beyond this population density, the effectiveness of MD drops drastically [20]. Furthermore, when bunches are infested at a rate of 5–10% during the first generation, the effectiveness of MD in following generations is greatly reduced [21, 27, 28].

For MD to be effective, 500 sexual MD dispenser per hectare must be installed in vineyards before the first seasonal flight begins. MD dispenser must be uniformly distributed around the vineyard and attached to shoots so that foliage protects them from direct sunlight exposure and high temperatures [23]. To compensate for atmospheric pheromone dilution around lot perimeters, twice as many MD dispenser must be placed along property edges [29].


**Figure 6.**
*Ethological control. A, traps baited with synthetic lures. B, MD emitter for* L. botrana *control.*

MD efficacy evaluation is done by checking the presence of adults and larvae via field monitoring and follow-up. Catching males in traps baited with synthetic lures is considered the easiest way to evaluate MD effectiveness. Capturing no males in traps is considered a "necessary but insufficient" indicator of effective MD, since the pheromone quantity necessary to interrupt males' orientation towards traps baited with synthetic lures is lower than the amount needed to disrupt mating [30]. Thus, capturing a few males in the same trap indicates a high risk of MD control strategy failure. The reliability of traps for monitoring adults might be increased by the use of high-dose lures. In other hand, monitoring of this pest and its damages can be done in the vineyard to determine infestation rate. For this, the following variables must be considered: percentage of bunches infested, number of larvae per inflorescence, number of eggs, larvae and damaged grapes per bunch. The mean number of larvae per bunch gives the most precise evaluation of meting disruption effectiveness, while the number of larvae per inflorescence (i.e., the number of first-generation larvae) can be very quickly evaluated in the field. Precise larval population estimates during the second and third generation require destructive sampling and dissection which take significant time, especially for varietals with compact grape bunches. Sexual confusion evaluations based on final crop damage can be deceptive because this damage, especially primary and secondary rotting, may be due to factors apart from larva feeding [30].

Finally, it must be noted that employing MD has many advantages, including being an ecologically clean method which leaves no wastes, is targeted and does not alter the ecosystem. Finally, it has a cumulative effect through the years, along with being comfortable to apply [23].

## 5. Biological control: natural enemies

An alternative to chemical control is using natural enemies such as "parasites and predators". Around 21 species have been described as preying on *L. botrana*, belonging to the following orders: Neuroptera, Coleoptera (coccinelids, carabids, clerids, malachiinae), Dermaptera, Hymenoptera, and Hemiptera. In laboratory tests, the predator *Chrisoperla defreitasi* (Neuroptera*:* Chrysopidae) has been observed eating eggs, larvae and pupae of *L. botrana* [23].

97 species of insects can parasitize *L. botrana* [31], belonging to the families Tachinidae (**Figure 7A**), Ichneumonidae, Pteromalidae and Chalcididae, among others. Among the ichneumenoid parasites, *Campoplex capitator* stands out due to its natural efficiency, density and wide geographic distribution. It has been regularly found in most European vineyards (Italy, Spain, Switzerland and France). *C. capitator* parasitizes *L. botrana* pupae in diapause. Freeing them en masse at the start of the season could reduce reproduction of later generations of this pest. *Trichogramma* spp. are microhymenopteras which act on eggs (egg-eating parasites) (**Figure 7B** and **C**). Their action has the advantage of controlling this pest before it can cause harm. In laboratory tests, 95% parasitism has been achieved. Freeing them en masse (thousands of micro-wasps per week) in the field could be useful for egg control. To use these parasites, it is important to monitor adult moths present in the field in order to effectively control eggs. Similarly, Ichneumonidae (**Figure 7D** and **F**) can be a good alternative for controlling *L. botrana*, as they attack larvae and pupae of a wide variety of insects. *Dibrachys affinis* Masi, which belongs to the Pteromalidiae family, also acts upon *L. botrana* chrysalises, reaching parasitism rates of 88%. The ectoparasite *Apanteles* sp. has been noted in the larval stage of *L. botrana.* (**Figure 6**). It has the advantage of global distribution [23].

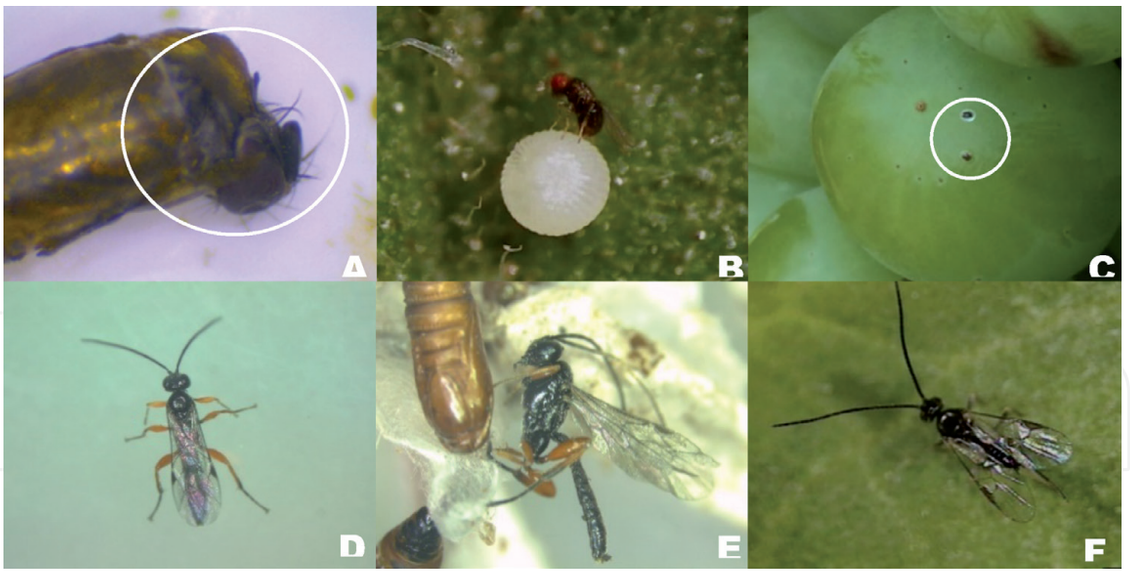


**Figure 7.**
*Natural enemies for controlling* L. botrana. *A, adult* Phytomyptera nigrina *(Diptera:Tachinidae) emerging from* L. botrana *pupa. B,* Trichogramma sp. *parasitizing egg. C,* L. botrana *eggs parasitized by* Trichogramma. *D, adult Ichneumonidae. E, adult Ichneumonidae parasitizing* L. botrana *pupa. F, adult* Apanteles *sp.*

## 6. Biological control: *Bacillus thuringiensis*

Within the biological control market, biopesticides based on *Bacillus thuringiensis* are the most used worldwide due to their toxicity towards a wide range of pest insects from different orders and harmlessness to humans [32].

The insecticidal activity of most *B. thuringiensis* subspecies is due to their producing a cytoplasmic inclusion called δ-endotoxin, which is synthesized during the sporulation process [33]. The δ-endotoxins of the two *B. thuringiensis* subspecies *kurstaki* and *aizawai* are insecticidal against *L. botrana* larvae. This insecticidal action occurs when spores and endotoxins are ingested by the larvae, and then solubilized and turned into active toxins with lower molecular mass by insect proteases in the alkaline pH of larvae midgut. Active toxins bond to specific receptors and induce pore formation in the membrane of intestinal cells, causing membrane integrity loss and cellular lysis that allows bacteria to enter the hemocoel (insect circulatory system), finally leading to larval death due to starvation and sepsis [34]. *L. botrana* larval stage 1 is the most susceptible to δ-endotoxin action, so it is recommended to monitor grape bunches and apply this strategy to eggs in the black head development stage. In this way, emerging L1 larvae will have direct contact with the biopesticide.

The lethality of δ-endotoxins from *Bacillus thuringiensis* groups Cry1, Cry2 and Cry9 which presented activity against Lepidopterae was evaluated on L1 stage *L. botrana* larvae [35]. The toxins with the greatest insecticidal activity were Cry9Ca, Cry2Ab and Cry1Ab, with LC50 values of 0.09, 0.1 and 1.4 μg/ml, respectively. Cry9Ca and Cry1Ab do not share affinity with the same receptor, so combining both δ-endotoxins together with *B. thuringiensis* would allow for better control of L1 stage *L. botrana* larvae [35].

## 7. Biological control: entomopathogenic fungi

Entomopathogenic fungi (EPF) are microorganisms able to infect and naturally control arthropod populations, allowing them to be used as an alternative to chemical insecticides for pest control. In the microbial pest killer market, around

80% of available EPF products are based on species from the *Metarhizium* and *Beauveria* genera, since both have a wide range of hosts and are easy to mass-produce [36]. *Metarhizium* and *Beauveria* include different species which over time have expanded, due to new types being isolated worldwide and the use of molecular techniques which allow for conclusive and certain identification.

EPF form complex relations with plants, apart from naturally controlling arthropod populations. Studies have shown that EPF species *M. robertsii* and *B. bassiana* provide plants part of the nitrogen which they absorb during insect parasitization [37, 38], promoting plant growth [39]. *Beauveria bassiana* has also been shown to act as an endophyte (colonizing plant interiors) in around 25 plant species, contributing to control of pests and phytopathogenic fungi [38, 40, 41]. It colonizes leaves, buds and roots, allowing plants to be more resistant to insect attacks [38, 42].

The action mechanism developed by EPF to parasitize insects requires EPF to differentiate into morphologically different cellular structures: conidium, germ tube, appressorium, hypha and blastospores. These structures participate in the insect infection and parasitizing process: conidia adhesion to the host cuticle (**Figure 8A**), formation and differentiation of the germinal tube in a structure called appressorium along with its penetration inside the insect cuticle (**Figure 8B**). Hemocoel colonization by blastospores (**Figure 8C**). Emergence of EPF hyphae from inside the insect and EPF sporulation on the corpse (**Figure 8D**), thereby promoting conidia dispersion and the start of new infections.

Although the action mechanism of EPF is known and interest in adopting biological pest control strategies is high, there are few scientific studies which have evaluated EPF effectiveness on *L. botrana* in field conditions. To this end, the study by Cozzi et al. [1] determined the lethality of 6 EPF isolates in an *in vitro* test on *L. botrana* larvae. The best strain, *B. bassiana* ITM 1559, showed a mortality rate of 55% of individuals of this pest. Furthermore, in field tests the incidence of bunches harmed by *L. botrana* larvae was significantly reduced via treatment with this strain, by comparison with the untreated control. In the study by Altimira et al. [19] 100%

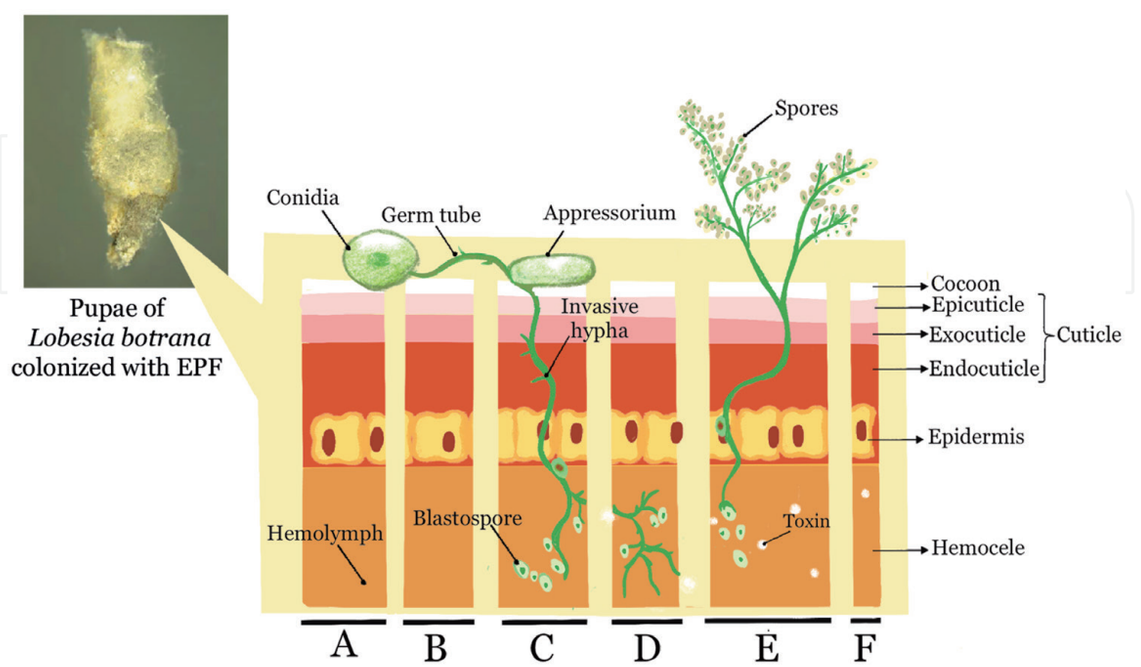


**Figure 8.**
*Infection and development cycle of entomopathogenic fungus (EPF) on an insect pupa. Panel A: Conidium adhesion; panel B: Spore germination; panel C: Appressorium differentiation and cuticle penetration; panel D: Hemocoel colonization; panel E: Hyphae emergence and sporulation; panel F: Strata which EPF must cross to colonize the hemolymph.*

effectiveness was obtained against un-cocooned *L. botrana* pupae via using a wettable powder formulation of the strain *B. pseudobassiana* RGM 1747. This field test was done with a controlled infestation of *L. botrana* in ´Red Globe' *V. vinifera* during autumn (average temperature 9.1°C). In natural infestation trials, an effectiveness rate of 51% was achieved in different *V. vinífera* varieties with an average temperature of 8.4°C. During this period, the adhesion, germination and colonization of *B. pseudobassiana* in cocooned pupae was achieved, demonstrating its effectiveness in climate conditions with low temperatures, rain and high humidity present in this time of year in the Metropolitan Region of Chile [19]. Subsequently, Tapia [43] achieved 80% effectiveness with the inverse emulsion formula of the *M. robertsii* RGM 678 strain against *L. botrana* pupae in field tests, along with achieving a significantly lower percentage of male *L. botrana* captures compared to the control treatment.

## 8. Proposal for integrated *Lobesia botrana* management in Chile

Chile is the main global table grape exporter. One major challenge for grapevine cultivation is controlling *L. botrana*, which has been declared a quarantining pest in this country, due to the economic damages it generates to grapevines and in table grape exportation. The presence of any individual of this species (egg, larva, etc.) on fruit causes the full lot to be rejected for exportation to target markets without *L. botrana*.

In Chile *L. botrana* has three annual generations, with a diapausal pupal state in the autumn-winter period. In this condition *L. botrana* lives under grapevine bark and has a highly hydrophobic cocoon impeding agrochemicals' penetration, making control difficult. However, EPF strains adapted to low temperatures have shown their ability to infect *L. botrana* in this state [19], with greater control efficacy in early autumn [43], since *L. botrana* cocoons in the start of the season are less dense and hydrophobic, facilitating EPF action. Controlling this pest in autumn and winter allows for reducing individuals in the first flight. In spring, we recommend monitoring black head eggs to apply *B. thuringiensis*. Tapia [43] achieved efficacy rates or 55–85% with various commercial products on 'Red Globe' *V. vinifera* crops. The impacts of EPF and *B. thuringiensis* are shown in **Figure 9** [43]. Based on these studies we propose an integrated control program with EPF-based biopesticide applications from early autumn to late winter, complementing these applications with *B. thuringiensis* from early spring to late summer, according to black head egg

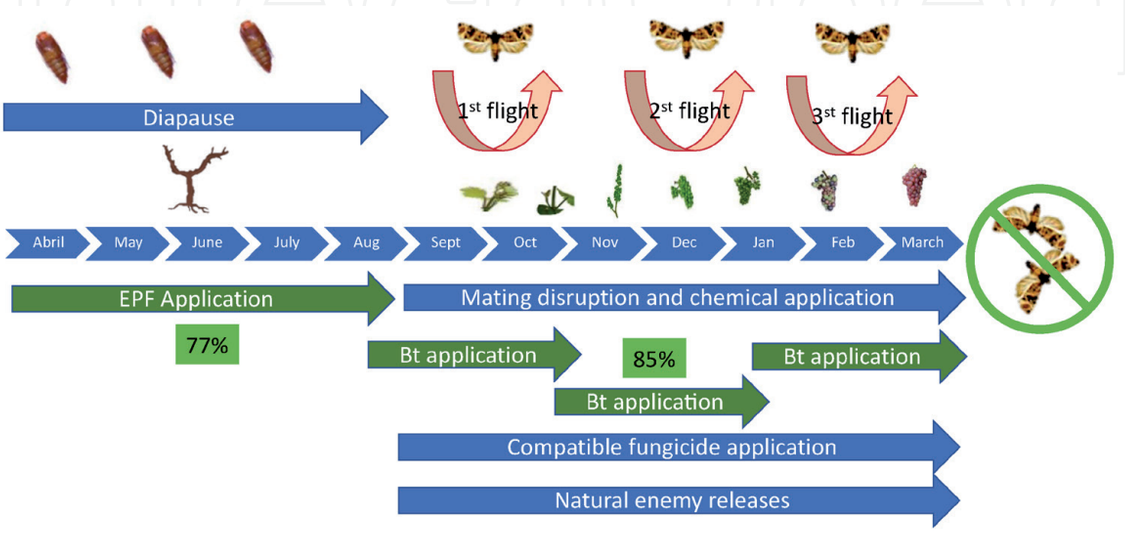


**Figure 9.**
*Integrated* Lobesia botrana *management plan with biopesticides and other control tools.*

monitoring. The integrated management plan must consider the MD strategy in vineyards and releasing natural enemies in urban zones with pest concentrations, along with applying synthetic chemical products -preferably green label-after moth flight alerts (**Figure 9**).

## 9. Conclusion

*L. botrana* is a pest economically important in southern Europe and South America. Despite the wide host range recorded, grapevine is the major host crop in which damage is really significant. To develop an integrated *L. botrana* management strategy, we must (1) adequately identify and monitor this pest in its different development stages and its natural enemies. (2) determine the economic damage thresholds at which to begin controlling. (3) Take management decisions according to information from monitoring. (4) User different biological tools together with MD allows for reasonable use of synthetic chemical molecules to control *L. botrana*, achieving a sustainable and environmentally friendly production and ultimately, a healthier grape for eating or winemaking.

## Acknowledgements

We gratefully acknowledge to the farmers and the National Program of *Lobesia botrana*, SAG. Special thanks to FIA ("Fundación para la Innovación Agraria" in Spanish) for supporting the research project PYT-2017-0182 on *L. botrana* and all the members of the Laboratory of Entomology and Biotechnology of INIA La Platina.

## Conflict of interest

The authors declare no conflict of interest.

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