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# European grapevine moth, *Lobesia botrana*

## Part II: Prevention and management

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With 1 figure

**Abstract:** *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), commonly known as the European grapevine moth (EGVM), is a primary pest of vineyards. This article provides an updated review of its monitoring, modelling, and management tools. EGVM management strategies analysed here include insecticide-based control, insecticide resistance, side-effects (particularly those caused by the exposure to sublethal doses of pesticides), cultural control, sterile insect technique, pheromone-mediated control strategies (with special reference to pheromone-based mating disruption), biological control, and area-wide control programs. Lastly, we outline significant challenges for future EGVM research and sustainable control implementation.

**Keywords:** area-wide pest management; biological control; entomopathogens; Integrated Pest Management; mating disruption; modelling; monitoring; parasitoids; predators; sex pheromones; tortricids

## 1 Introduction

Benelli et al. (2023) recently reviewed the biology, ecology, and invasiveness of the European grapevine moth (EGVM) *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), outlining new research advances. Strategies for its control have a long history, starting from the pioneer work of Götz (1939), who firstly showed that EGVM females were able to attract males for mating. He preconized the concept of pheromone-based control before the first sex pheromone was chemically identified by Karlson & Butenandt (1959). Even before, Silvestri (1912), Feytaud (1913) and Marchal (1912) performed the first key natural history studies on EGVM biology and natural enemies. Noteworthy, several biocontrol issues were already treated in their studies as future efficient control options. Interestingly, a few decades before the onset of synthetic insecticides, the egg parasitoids *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), entomopathogenic fungi and the efficient larval parasitoid, *Campoplex capitator* Aubert (Hymenoptera: Ichneumonidae) were studied by several authors (Coscollá 1997; Ioriatti et al. 2012; Reineke & Thiéry 2016; Thiéry et al. 2018).

Herein, we analyze current knowledge on EGVM monitoring, modelling, and management. The management section includes insecticide-based control, insecticide resistance, side-effects, sterile insect technique (SIT), pheromone-mediated control strategies, biological control, cultural control, and functional biodiversity as Integrated Pest Management (IPM) tools and area-wide control programs. Finally, a research agenda highlighting challenges for forthcoming studies is proposed.

## 2 Prevention

### 2.1 Monitoring

Prediction of phenological stages of EGVM is critical to time control operations in IPM and to reduce the use of pesticides to comply with the EU Directive 128/2009. Monitoring also serves to determine if control methods are working and to obtain the diel activity periods and optimise the synthetic pheromone release through automated aerosol devices.

Egg counting is a reliable sampling method, but it takes a trained eye to find eggs because EGVM lays them singly, and it is rather difficult on inflorescences. Egg colour changes as the larva develops, allowing more precise insecticide timing. Markheiser et al. (2018; 2020) and Rid et al. (2018; 2019) explored oviposition monitoring in vineyards with the help of dispenser cards delivering attractive plant volatiles on surfaces that were physically appropriate for oviposition. So far, the project has failed, technically because it was not possible to produce a dispenser for complex but defined fragrance bouquets.

Larval monitoring is pivotal during the first generation, when the larvae build visible nests on the inflorescences and the damage is limited. In the second and third generations, monitoring the eggs is crucial to prevent the damage, whereas monitoring the larvae is of relevance only to assess infestation levels. Searching for pupae, which hide in the leaves, bark or soil, is not a feasible method to estimate populations. Artificial pupation substrate, such as corrugated cardboard, has been used to sample and control EGVM pupae (e.g., Catoni & Schwangart 1914).

The adult is the only stage that can be sampled passively using traps lured with pheromones. Because it is highly specific, reliable and easy to use, the main component of the EGVM sex pheromone (*E,Z*-7,9-12:Ac) is the most widely used attractant in the traps. If the latter are used in untreated plots there is no clear correlation between male catches and later grape infestations (Roehrich & Boller 1991), especially with low catches.

Sex pheromone does not attract females and its attractiveness to males decreases a lot under mating disruption (MD), so alternatives are needed. The so-called “food” or “host” lures are effective alternatives to pheromone lures under MD conditions or when females need to be estimated (Ioriatti et al. 2011). “Food” lures are based on fermentation of sugar-containing solutions. The nature and amount of the volatiles released is largely unknown because it depends on substrate, microorganisms, temperature, and time (Davis et al. 2013; Beck & Vannette 2017; Tasin et al. 2018), and thus it is likely to show regional and seasonal variation. Despite these limitations, EGVM food lures based on wine (Bagnoli et al. 2013; Pinto et al. 2020) and fruit juices (Thiéry et al. 2006a, b) are gaining interest. The ratio of

males and females, and of mated and unmated females caught with food-baited traps varies widely among reports (Henrici 1941; Thiery et al. 2006a, b; Bagnoli et al. 2013; Pinto et al. 2020). Using food traps based on concentrated apple juice in two French vineyards, Thiery et al. (2006a, b) observed in both study sites a higher prevalence of captured females (i.e., 60% or more) than males. Similar results were obtained by Bagnoli et al. (2013) using wine-based food traps. The drawback associated with these traps is the stickiness of the sugar solution, the poor selectivity, and the need of periodic refilling.

In moths, the innate preference of unmated female towards “food” volatiles shifts to “host” volatiles upon mated, to help them finding oviposition sites (Saveer et al. 2012). Therefore, the proportion of unmated and mated females in food traps may not represent the actual proportion of unmated and mated females in the field.

Host volatiles that attract EGVM in the laboratory and in the field have been identified (reviewed in Ioriatti et al. 2011). Field tests demonstrate that the mix of phenylacetonitrile (an HIPV) and acetic acid is more attractive to both sexes of EGVM than acetic acid alone (El-Sayed et al. 2019). Similarly, a blend of 2-phenylethanol and acetic acid was attractive in a dose-dependent manner (Larsson-Herrera et al. 2020). In all these host-plant VOC studies the number of males and females captured was similar.

Ever more accessible and efficient electronics and software are paving the way to develop automated monitoring devices (Ünlü et al. 2019; Lima et al. 2020). They provide two benefits over traditional hand-operated traps: high temporal resolution and network access. High temporal resolution indicates the time of the day when the pest is active and serves to time automated pheromone dispensers (i.e., puffers) cutting pheromone costs. EGVM males fly for a 1–3 h period starting at sunset as shown by using live females (Götz 1943) and image traps (Lucchi et al. 2018a). Images are relatively large (byte-wise) and the camera and accessory night-time illumination consume energy, so the commercial models offer low time resolution (1–2 pictures/day) (Lucchi et al. 2018a). Traps that encode the wingbeat of a moth crossing a LED-optical-sensor array may be the future of automated monitoring because they are real-time and less demanding in terms of energy and bytes than image-sensor traps (Burks 2022). “Wing-beat” traps, like image traps (Faria et al. 2021), require learning algorithms to discriminate among species, but this should not be a problem for the highly specificity of the pheromone lures. In addition, “wing-beat” traps do not retain the insects and so they spare “trap cleaning” visits.

Light may increase the attractiveness of pheromone and plant-volatile traps. The retina of the EGVM has two response peaks, one at 460–540 nm (blue-green region of visible spectrum), and a smaller one at 340–420 nm (ultraviolet) which is the region of the spectrum considered behaviourally maximal in night flying insects (Crook et al. 2022).

## 2.2 Modelling

Today, phenological, physiological and predictive models are prevalent in IPM. By combining the simplicity of temperature-driven models and tools (e.g., sex-pheromone traps and weather stations), the application of phenological models on Decision Support Systems (DSS) can contribute to the advance of IPM programs. The relationship between EGVM pheromone trap catches and degree-day (DD) accumulations using phenological models to determine the best time for insecticide spraying it has been assessed (Del Tio et al. 2001; Milonas et al. 2001; Gallardo et al. 2009; Ortega-Lopez et al. 2014; Heit et al. 2015). Many models have been developed to monitor EGVM adult flight, including process-based ones (Logan et al. 1976; Touzeau 1981; Gabel & Mocko 1984; Caffarelli & Vita 1988; Milonas et al. 2001; Gallardo et al. 2009; Caffarra et al. 2012). Other models use biological responses to temperature (Moravie et al. 2006; Ainseba et al. 2011).

As reviewed by Carlos et al. (2018), several researchers have proposed predictive models for the development of EGVM based on the relationship between temperature and developmental rate (Gabel & Mocko 1984; Baumgärtner & Baronio 1988; Cravedi & Mazzoni 1994; Savopoulou-Soultani et al. 1999; Brière & Pracros 1998; Del Tio et al. 2001; Milonas et al. 2001; Gallardo et al. 2009; Heit et al. 2015). In general, humidity does not play a major role in temperature-based phenological models, but it can be limiting under particularly dry (Torres-Vila et al. 1996) and humid conditions (Bovey 1966). According to Bovey (1966) the combination of both parameters influences mortality, so the application of a model from one geographic area may need adjustments to the local conditions of a different one (Allen 1976; Gallardo et al. 2009; Amo-Salas et al. 2011; Flores & Azin 2016). Only a few EGVM models have been applied to large geographical scales (Baumgärtner et al. 2012; Ortega-López et al. 2014; Castex et al. 2020).

Physiological models including both environmental and life history parameters have been developed (Schmidt et al. 2003; Moravie et al. 2006; Amo-Salas et al. 2011; Gutierrez et al. 2012; Ortega-Lopez et al. 2014; Gilioli et al. 2016). However, DD models have the advantage of being simpler to develop and easier to use by growers, after being validated locally because emergence of EGVM adults varies with location, climate, and year (Gallardo et al. 2009). The development of phenological models based on temperature either for grapevine, as well as for EGVM has been studied (Reineke & Thiéry 2016; Castex et al. 2020; Reis et al. 2021a, b). Simulations with phenological models can help understanding the impact of climate change on the phenology of both trophic levels (EGVM and grapevine). Their use would not replace common monitoring programs or economic thresholds. An adequate sampling technique must be adopted to accurately determine egg and larval population structure variability in space and time (Ioriatti et al. 2011). Knowledge of the spatial distribution of EGVM populations is important for developing efficient sampling programs that



enable a more accurate assessment of damage and determine an intervention threshold. Direct plant inspections and intervention thresholds help farmers to decide if and when using insecticides. Second and third EGVM generations are particularly difficult to control since larvae quickly penetrate the berries or the innermost parts of the grape cluster (Ioriatti et al. 2005; Carlos et al. 2018) and the canopy growth acts as a barrier for sprays. As economic damage to grapes occurs when larvae feed on grape clusters, control measures against the second and third generation should primarily target EGVM eggs, reducing the trophic activity of the emerging larvae to a minimum. Therefore, precise timing of sprays with ovicides (e.g., IGRs), before eggs hatch is particularly important to avoid damage. Ensuring effective strategies for EGVM control requires the development of tools to support sampling programs and timing of insecticide sprays. In this scenario, extension services or consultants should still carry out egg and larval assessments to estimate the actual level of infestation. The need of spraying-related DSS taking into account the actual vineyard infestation led to several approaches, some of which are not yet practicable, e.g., DSS by cage station under local field conditions (Polesny et al. 2000) where survival and development of overwintered EGVM is monitored year around.

Models estimating the mortality of different stages under certain weather conditions based on long-term field assessment (Pasquali et al. 2022) are the most promising approaches for forecasting infestation if they deliver reliable quantitative results everywhere or are easily adjustable to other local conditions.

### 3 Management

#### 3.1 Insecticide-based control

Despite the widespread application of pheromone MD (Ioriatti & Lucchi 2016; Benelli et al. 2019) and recent pesticide restrictions (Daane et al. 2018a), EGVM is still managed using insecticides on wide surfaces worldwide. In IPM programs, timing of sprays is based on trapping (Vacas et al. 2011), on forecasting models (Castex et al. 2020; Lessio & Alma 2021), and on visual inspections (Bažok & Diklić 2016). The number of applications per year depends on population density, cultivar (i.e., wine or table grapes), and related economic injury levels (Moschos 2005; Vassiliou 2011). A high economic threshold is established for the 1<sup>st</sup> generation, especially on cultivars with abundant inflorescences, while 2<sup>nd</sup> and 3<sup>rd</sup> generation are considered the most destructive, with a lower injury threshold (i.e., 1–15% of infested clusters), depending on the cultivar, bunch tightness, and harvest time (Ioriatti et al. 2008).

Insecticide treatments are generally timed at the vine phenological phase of pre-closure and post-veraison in bunches targeting eggs or the 1<sup>st</sup> instar larvae depending on the insecticide mode of action (Coscollá 1997; Voigt & Sárospataki

2000; Thiéry 2008; Ioriatti et al. 2012; Linder et al. 2016; Altimira et al. 2021). EGVM 3<sup>rd</sup> generation is generally considered the most harmful due to its closest relationship with the development of grey mold and sour rot (Pavan et al. 1998; Ioriatti et al. 2005).

A wide variety of insecticides with different modes of action are available worldwide. Most of them are registered for grape pest control allowing resistance management through rotating chemical classes in successive spray applications (Roditakis 2003; Bostanian et al. 2012). Since the 1980s, organophosphorus insecticides were widely used due to their effectiveness and inexpensiveness, in particular chlorpyrifos-ethyl and chlorpyrifos-methyl (Ioriatti et al. 2008), both recently withdrawn by the EU regulation. In the IPM frame, the side effects of organophosphates, carbamates, and pyrethroids on non-target beneficial arthropods, e.g., Phytoseiidae mites, is crucial (Tirello et al. 2013; Pozzebon et al. 2015; Schmidt-Jeffris et al. 2021). Chlorpyrifos-methyl is harmful for other biocontrol agents (BCAs) in the vineyard. Mansour et al. (2011) reported that this organophosphate caused 100% mortality 24 h post-treatment on the mealybug parasitoid *Anagyrus vladimiri* Triapitsyn (Hymenoptera: Encyrtidae) exposed to contact toxicity assays.

In the early 1990s, more selective insecticides like the insect growth regulators (IGRs) became available, including chitin synthesis inhibitors (CSIs), juvenile hormone agonists (JHAs) and molting hormone agonists or molting accelerator compounds (MACs). Being effective at lower concentration levels and scarcely hazardous to non-target species such as mammals and predatory mites, they have been considered as an alternative to several conventional compounds (Tirello et al. 2013; Pozzebon et al. 2015). CSIs, such as flufenoxuron and lufenuron, targeting the 1<sup>st</sup> larval generation of EGVM, reduced the second generation and caused significant larval mortality for up to 45 days post-treatment (Pavan et al. 2014). JHAs as pyriproxyfen target the endocrine hormonal system and disturb the pupation process and the embryonic development in freshly laid eggs (Dalla Montà & Pavan 1990). MACs as methoxyfenozide are effective after ingestion or contact on larvae, inducing a premature moult and having ovicide activity (Dorn et al. 1981). The non-steroidal ecdysone agonist methoxyfenozide was more effective on older EGVM larvae over younger ones; when administered orally to adults it reduced EGVM fecundity and fertility, but not longevity (Sáenz de Cabezón Irigaray et al. 2005). Methoxyfenozide provided a higher larvicidal efficacy than tebufenozide in laboratory (Pasquier & Charmillot 2000). The commercial product at a dose of 0.03% was very effective in controlling larval infestation and showed a good rain fastness under a simulated rainfall of 30 or 60 mm. Being an effective ovicide, it boosted stakeholders to review the classic chemical control strategy based on neurotoxic insecticides targeting EGVM larvae. Thus, a standard biological efficacy test method and a forecasting system taking into

account the biological stages of EGVM were developed (Pasquier & Charmillot 2000). IGR residues reduce fecundity and fertility of adult moths in contact with treated surfaces (Charmillot et al. 1994). Early treatments targeting freshly laid eggs could reduce the reproductive potential of adults and therefore crop damage (Charmillot et al. 1994).

Chlorantraniliprole, belonging to the selective ryanodine receptor agonist's class, achieved a high level of toxicity to EGVM neonate larvae in laboratory. In vineyards, chlorantraniliprole applied against the larvae at 35 mg AI L<sup>-1</sup> was as effective as the commercial standard reference, chlorpyrifos-ethyl, and had a long-lasting effect. Chlorantraniliprole at the same field rate caused >20% egg mortality (Ioriatti et al. 2009). This active ingredient had no side-effects on eggs, nymphs, and adults of the predatory phytoseiid mite *Amblyseius (Neoseiulus) californicus* (McGregor) using the spray tower-leaf disk method (Kaplan et al. 2012). It was harmless when applied at the maximum recommended field dose and at twice the field dose on *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) (Zotti et al. 2013). The new mode of action of chlorantraniliprole and its safety to non-target organisms are useful in IPM, as well as for managing insecticide resistance. The macrocyclic lactone emamectin benzoate is a neurotoxic compound active through ingestion, effective at very low rates and on all instar stages (Boselli et al. 2008). Due to its rapid photodegradation on leaf surface it is considered harmless for beneficials, and it has a favourable residue profile requiring a short preharvest interval (Liguori et al. 2010). Spinosad is widely used in organic vineyards and fruit orchards to control various pests, including EGVM (Duso et al. 2022). In the laboratory, it demonstrated toxicity to several arthropod natural enemies, but in vineyards this effect is mitigated by its short persistence (Dripps et al. 2011). Nevertheless, based on field observations, spinosad was reported as harmful to predatory mites and to egg parasitoids of leafhoppers (Tirello et al. 2013; Duso et al. 2022). Spinetoram is a new insecticide derived from spinosad, through a chemical modification to improve its insecticidal activity, and a longer residual activity that in turn may led to higher toxicity to hymenopteran parasitoids (Varela et al. 2010). Spinetoram was applied in vineyards to control the 2<sup>nd</sup> larval generation and its efficacy was comparable with that of methoxyfenozide and chlorantraniliprole (Forte et al. 2014).

*Bacillus thuringiensis* (Bt)-based products are used commercially against EGVM both in IPM and organic viticulture. The efficiency of different Bt ssp. on different grapevine cultivars has been studied (Roditakis 1986; Ifoulis & Savapoulou-Soultani 2004; Ruiz de Escudero et al. 2007; Thiéry et al. 2018). Exploring new natural products generated by the symbionts of entomopathogenic nematodes, i.e., the bacteria species belonging to *Xenorhabdus* and *Photorhabdus* genera, may contribute to develop new tools for EGVM control (Vicente-Díez et al. 2021), pending proper field evaluation.

Botanical insecticides are of interest for future EGVM management. *Carlina acaulis* L. (Asteraceae) root essential oil and its highly stable aqueous nanoemulsion has been reported as effective against 1<sup>st</sup> instar larvae (LC<sub>50</sub> = 7.29 and 9.04 µL/mL, respectively) feeding on an insecticide-sprayed diet (Benelli et al. 2020a). Later, Dasenaki et al. (2022) reported the larvicidal effect of *Pistacia lentiscus* L. (Anacardiaceae) fruit, leaf, and bark extracts on EGVM, highlighting that oleic and linoleic acids from the triglyceride mixture of the fruit hexane extract were responsible for the insecticidal activity observed towards the 5<sup>th</sup> instar larvae through topical application (oleic acid LD<sub>50</sub> 112.89–172.33 g/cm<sup>3</sup>, linoleic acid LD<sub>50</sub> 157.26–201.48 g/cm<sup>3</sup>).

Kaolin is effective in limiting the EGVM oviposition and larval settlement, also showing its good selectivity towards beneficial insects (Pease et al. 2016; Tacoli et al. 2019). A significant effect against EGVM was also detected through sulphur applications, widely used in viticulture against powdery mildew (Tacoli et al. 2020).

Regardless of the active ingredient used, its effectiveness depends on its formulation and related chemical-physical properties, as well as on the dosage of the insecticidal mixture applied per unit of surface area, or rather per unit of vegetation volume, the coverage/wetting of the target organs, the size of the droplets, and the persistence/penetration of the substance applied on or into the vegetation to be protected (Bostanian et al. 2012). In this scenario, the new sensory technologies can contribute to a precision viticulture where insecticide-based EGVM control can be carried out according to “when needed and only enough” (Pertot et al. 2017).

### 3.2 Insecticide resistance

There are very few insecticide resistance reports for EGVM, including indoxacarb resistance in Italy (Civolani et al. 2014) and Turkey (Durmusoğlu et al. 2015; Hatipoğlu et al. 2015), and pyrethroid and spinosad resistance in EGVM populations from Greece, and recently from Turkey (Vontas & Roditakis pers. comm.). To evaluate EGVM insecticide efficacy and insecticide resistance levels, bioassay protocols that facilitate exposure of larvae to the insecticide via artificial diet are used. They include incorporation of the active ingredient in the artificial diet, and incubation of individual larvae in ventilated cells for selected time intervals (Civolani et al. 2014, Durmusoğlu et al. 2015, Vontas & Roditakis pers. comm.). These protocols were originally designed for another tortricid, the codling moth *Cydia pomonella* (L.) and have been validated by IRAC (method 017 or modified protocols, <https://irac-online.org/methods/cydia-pomonella-larvae/>) (Civolani et al. 2014).

One of the major difficulties in performing standard dose-response bioassays with EGVM is the slow and elaborate rearing of field-collected strains. The establishment of EGVM strains in the laboratory is not always successful. An

adaptation period is often required, aggressive fungal contaminations have been observed, and minor alterations in the rearing conditions may reduce the moth reproduction rate. These issues may explain the limited published laboratory insecticide bioassays for EGVM in comparison with other lepidopteran species.

Insecticide-resistance mechanisms have been elucidated at the molecular level in several lepidopteran species. These include modifications of target sites and/or enhancement of the detoxification/metabolism process (Ffrench-Constant 2013; Nauen et al. 2022). Target site mutations have been associated with striking insecticide resistance phenotypes against several insecticide classes in key moth species (Wang et al. 2016; Roditakis et al. 2017a, b; Zhang et al. 2017). Detoxification-based resistance, either by overexpression or (less often) by mutations that increase enzyme-substrate affinity, have been also reported in many lepidopterans (Katsavou et al. 2022; Nauen et al. 2022). Molecular characterisation of insecticide-resistance mechanisms in the EGVM have not kept pace with other lepidopteran pests due to the lack of genome information. However, a *de novo* transcriptome was recently assembled for the EGVM containing the complete sequence of 84% of the Insecta BUSCO (Vontas & Roditakis pers. comm.).

Alignment and analysis of the target site fragments indicate the absence of known target-site resistance mutations in EGVM populations from Turkey. The EGVM CYPOME (cytochrome P450s), which was annotated and phylogenetically classified, and the transcriptome and cytochrome P450 analysis will facilitate further molecular studies on the metabolism-based insecticide resistance in EGVM (Vontas & Roditakis pers. comm.). Resistance monitoring is rarely implemented in EGVM (Pasquini et al. 2018), but this is expected to change because fewer insecticides are becoming available for control.

The identification of genes potentially involved in metabolic resistance, and the functional characterization of resistance mechanisms, including the understanding of their evolutionary features (e.g., intensity and fitness cost) will facilitate the development of molecular tools, in line with other pests, which can be used in combination with toxicological bioassay data to allow the implementation of the most appropriate evidence-based control strategies (Van Leeuwen et al. 2020).

### 3.3 Side and sublethal effects of insecticides

Insecticides, with special reference to carbamates, neonicotinoids and pyrethroids, have unexpected effects on the fitness of arthropods directly or indirectly through the trophic chain (e.g., James & Xu 2012; Mahmood et al. 2016). The effect of neonicotinoids on semiochemical communication of EGVM adults has been studied in the laboratory (Navarro-Roldán & Gemeno 2017; Navarro-Roldán et al. 2017; 2019). Dose-mortality curves with an organophosphate, a pyrethroid, and a neonicotinoid insecticide were made to estimate the

dose at which 50% of the population died (LD<sub>50</sub>) (Navarro-Roldán et al. 2017). Unexpectedly, female larvae were more susceptible to the organophosphate chlorpyrifos than male larvae, despite females being larger, and this difference was not explained by sex differences in detoxification enzymes (Navarro-Roldán et al. 2020).

To study the effect of the neonicotinoid thiacloprid on pheromone production and release, and on male detection of and orientation to sex pheromone, lethal concentrations LC<sub>0.001</sub>, LC<sub>1</sub>, LC<sub>10</sub> and LC<sub>20</sub> were applied to the thorax of CO<sub>2</sub>-anesthetized moths. Calling behaviour decreased starting at LC<sub>1</sub> in the EGVM (Navarro-Roldán & Gemeno 2017). Thiacloprid delayed and reduced the percentage of EGVM males responding to pheromone in a wind tunnel without alteration of electrophysiological antennal responses (i.e., electroantennography) (Navarro-Roldán et al. 2019). During navigation along an odour plume, treated males exhibited increased susceptibility to wind-induced drift. Further insights on the impact of sublethal doses of insecticides on semiochemical monitoring and control should be achieved under semi-field and field conditions.

MD is competitive when males are attracted to MD pheromone dispensers, and it is non-competitive when detection of sex pheromone is altered (Miller & Gut 2015). We do not know yet if MD is competitive or non-competitive in EGVM. Determining this requires demanding large-field-cage experiments (Miller & Gut 2015), but prolonged exposure to large pheromone concentration reduces antennal and flight responses, so non-competitive MD is likely in EGVM (Gavara et al. 2022). A reduction in calling behaviour by sublethal doses could make artificial pheromone lures more attractive than females, and this could increase the efficiency of monitoring traps and of competitive MD. Thus, sublethal insecticide doses may synergize MD, but simultaneously may overestimate population counts. On the other hand, a deterioration of pheromone detection and response in males by sublethal insecticide doses could boost non-competitive MD, and could also increase their response to synthetic lures, which probably have higher emission rates than females.

Sublethal insecticide effects could affect other IPM control methods, such as adult emergence phenology, protandry (Thiéry et al. 2014a, b), and parasitoid resistance via the larval immune system and other defence strategies (Vogelweith et al. 2014; Thiéry et al. 2018). The experiments required to demonstrate the potential effects of sublethal insecticide doses on other IPM control methods in the field are technically challenging but could shed light on the interaction among control methods and improve IPM implementation.

### 3.4 Development of the Sterile Insect Technique (SIT)

There is increasing interest in developing the sterile insect technique (SIT) against EGVM due to its increased worldwide pest status and the need to eradicate it from newly invaded regions (Saour 2014; FAO-IAEA 2018; Simmons



et al. 2021c). Particularly, when EGVM occurs close to residential areas, it is necessary to develop tools that are acceptable to the public (FAO-IAEA 2018; Simmons et al. 2021b). Over the last ten years several efforts have been initiated to develop the SIT, which includes determining the effect of radiation on the biology and mating behaviour, developing mass-rearing methods, and making field releases to evaluate sterile moth competitiveness, dispersal, longevity, and population suppression with season-long, small-scale pilot projects to evaluate operational use (Saour 2016; Simmons et al. 2021c; Taret et al. 2021).

#### 3.4.1 Radiation biology for SIT development

Complete female sterility was achieved by irradiating adults with 150 Gy, with 400 Gy achieving near 100% male sterility (Saour 2014), and 150 Gy was suggested as the dose to use for an F<sub>1</sub> sterility program (or inherited sterility program, see Marec et al. 2021), allowing a trade-off between field performance and higher residual male fertility (Saour 2016). At 150 Gy, the frequency of F<sub>1</sub> male progeny increased, and these had high sterility levels when crossed with fertile females (Saour 2014). Steinitz et al. (2015) found a similar level of sterility at 150 Gy in EGVM females when irradiated as pharate pupae and crossed to fertile males, though they found somewhat lower sterility levels with less than 4% residual fertility. Crosses of fertile females with 150-Gy-irradiated males had higher levels of fertility than what Saour (2014) reported but had similar sterility levels in the F<sub>1</sub> generation (Steinitz et al. 2015; Simmons et al. 2021c).

#### 3.4.2 Impact of the mating biology on SIT development

EGVM males, like all lepidopterans, produce two types of sperm, eupyrene, the fertilising sperm, and apyrene, the non-fertilising sperm cells (Friedländer et al. 2005), which have a role in sperm competition when females remate (Cook & Gage 1995). EGVM is unique among most moth species, having a monandrous mating system with about 80% of females mate once and only 20% mate more than once (Torres-Vila et al. 2002a; 2002b). In the EGVM the ratio of apyrene to eupyrene sperm is about 2:1, which is different from the typical 10:1 ratio of most polyandrous species (Cook & Wedell 1996; Watanabe et al. 1998). Sperm precedence is about 0.5, with no advantage to either first or second mating males (Sadeh & Harari 2015). Monandry and lack of precedence is advantageous for the SIT approach because it increases mating competitiveness of sterile males over wild males (Lance & McInnis 2021). EGVM males cease to produce fertile (eupyrene) sperm at the pupal stage (Sadeh 2016), thus, there is no risk of producing new viable sperm at the adult stage after irradiation.

#### 3.4.3 Pilot projects for SIT development

In Chile, the Servicio Agrícola y Ganadero (SAG), an Agency of the Agricultural Department (Ministry of Agriculture), working with partners in the fruit industry (Fundación para el

Desarrollo Frutícola, FDF), started a pilot project to develop the SIT for EGVM. The first steps were based on methods adapted from mass-rearing moths for other SIT programs and adapting existing artificial diets (Nadel et al. 2018; Simmons et al. 2021b). After optimizing an artificial diet using ingredients available at low cost in Chile, a mass-rearing capable of producing 100,000 or more moths per week was developed. SAG also developed quality control procedures and a system for adult collection and irradiation based on the pink bollworm and codling moth programs (Simmons et al. 2021c), while FDF developed collection and irradiation of pharate adults (S. Izquierdo pers. comm.). A smaller scale mass-rearing and pilot release program has been started in Argentina in cooperation with researchers from Chile (Taret et al. 2021).

#### 3.4.4 SIT field studies

A field dispersal experiment in Syria demonstrated a reduction in the percentage of male recapture for moths irradiated at 350 Gy compared to moths irradiated at 150 Gy, while there was no difference between 150-Gy irradiated and unirradiated moths (Saour 2016). Starting in 2016, several field studies were conducted to evaluate the potential of the SIT to control EGVM infesting grapevines in urban gardens adjacent to vineyards and fruit production areas in Chile. Initial field cage and mark-recapture experiments of moths and pupae irradiated at 150 Gy showed that the average dispersal and longevity of irradiated males was 58 m, with a 62% survival after 3 days (Simmons et al. 2021c).

During the 2019–2020 growing season, a season-long field release experiment was conducted in a 25-ha urban plot in a small city next to grape production areas in central Chile. A release of 752,353 moths was made for 37 weeks for an average rate of 813 moths/ha/week. High overflowing ratios were achieved for the first weeks of the experiment, but because of the high wild moth population in this area, the release of sterile moths did not reach large enough ratios of sterile versus wild moths to achieve effective control. Yet high moth quality and field performance of sterile moths was observed, with some flying as far as 800 m and living as long as 10 days (Simmons et al. 2021c). The project demonstrated that high-quality sterile moths could be produced and released but it was clear that additional IPM tactics were needed, such as fruit stripping or treatments with Bt to lower the overall population for sterile release to be effective (Simmons et al. 2021c.)

## 4 Pheromone-mediated control strategies

One of the most effective biocontrol methods used against EGVM over large areas and in the long term is MD (Ioriatti et al. 2011; Ioriatti & Lucchi 2016), which is characterized by high selectivity, and very low environmental impact. The first MD tests against EGVM were carried out in France,



under laboratory (Roehrich et al. 1977) and field conditions (Roehrich et al. 1977; 1979; Roehrich & Carles 1981). Despite heterogeneous results, they established basic points and procedures to improve the efficiency of this method, including adult dispersal, plot shape, minimum area treated, edge area, number and dosage of pheromone dispensers, initial population density, habitat details, global pest species spectrum, and other compatible control measures. Improved knowledge of the insect species combined with technical and economic enhancements allowed to optimise the use of MD against EGVM (e.g., Stockel et al. 1994; Charmillot et al. 1995; Schmitz et al. 1995; Torres-Vila et al. 1997; Ioriatti et al. 2005).

The case of the Trento province (northern Italy) can be cited as a model example for effective and large-scale application of this control strategy (Ioriatti & Lucchi 2016). This territory indeed is recognized as a pioneer in Italy in the application of MD against Lepidoptera Tortricidae such as EGVM, *Eupoecilia ambiguella* (Hübner) and, in some areas, *Argyrotaenia ljungiana* (Thunberg), for which three-purpose dispensers have been developed. Currently, the vine-growing area in the Province of Trento involved in pheromone MD represents about 95% of the total viticulture (about 9,700 hectares), and together with the area where the control of the codling moth in the apple orchards is carried out with MD, represents the largest area treated with pheromones in Italy.

Nowadays, MD against EGVM is applied on approximately 300,000 ha of European vineyards, i.e., about 6–7% of the total grapevine-growing area. To date, no resistance to MD has been detected under field conditions, even if there is heritable variation in the ability of males to locate females in commercial pheromone-treated areas (Torres-Vila et al. 1997). MD area-wide applications have been recently conducted in Chile, Argentina, and California where EGVM was accidentally introduced (Ioriatti et al. 2012; Cooper et al. 2014). The most common hand-applied “passive” dispensers available on the market for EGVM are Shin-Etsu twist-ties ropes (Isonet® L, L plus, L E and L A plus in Europe; Isomate® EGVM in the USA), BASF twin ampoules (RAK® 2 MAX and RAK® 1+2), and ShinEtsu Isonet® L TT and BIOOTwin® L twin ropes (Lucchi et al. 2018b). The active ingredient in these dispensers is the main EGVM pheromone compound, (*E,Z*)-7,9-dodecadienyl acetate, rarely mixed with other minor compounds. Depending on the formulation, 200 to 500 dispensers per hectare (the number of dispensers may vary depending on manufacturer formulation and EGVM population level) must be deployed in the vineyards before the onset of the first seasonal flight, because late deployment will cause control failures. In urban areas of Chile, important refuges for large EGVM populations and frequent sources for vineyards re-infestation, the use of as low as 50 mesodispensers ha<sup>-1</sup> (each containing 1.72 g (*E,Z*)-7,9-dodecadienyl acetate) for three consecutive seasons (between 2013–2016) have shown an asymptotic reduction in trap captures overtime (Ceballos et al. 2022).

Dispensers must be evenly distributed in the vineyard and should be attached to vine shoots covered with foliage for protection against direct sun exposure and high temperatures. Twice as many dispensers must be hung along the vineyard edges to compensate for the loss of pheromone concentration in those areas. Border effects are remarkably reduced when MD is applied in area wide projects, as in certain growing regions of Germany, France, Switzerland, northern Italy, and Spain (Kast 2001; Ioriatti et al. 2008; Ioriatti & Lucchi 2016). Depending on the vineyard layout and trellising system, the time to deploy the dispensers in the vineyards may vary between 1 and 2.5 h/ha. Currently, the surface area of vineyards in Europe under EGVM MD control remains limited, despite intensive research and substantial experience with practical applications during the past two decades. This is due to socio-cultural and economic conditions existing in the different vine-growing areas where interest and trust for innovative methods is often low. Recently, increasing quality standards for wine and table grapes with respect to pesticide residues, and improved pest control in high-pressure areas, are creating new opportunities for extensive adoption of MD in IPM programmes.

Further research is needed to improve the efficacy of MD technology, and to manage invasive and secondary pests without compromising the value of the pheromone-based approach for the management of the target tortricid pests (Nieri et al. 2022). For example, it remains critical to develop and commercialise novel and/or better formulations that are more effective, cheaper, and easier to deploy. In this sense, aerosol technologies may provide a cost-effective alternative to hand-applied passive dispensers (Benelli et al. 2019).

Automatic aerosol devices can actively release pheromone puffs at programmed time intervals (Suterra CheckMate Puffer® LB and ShinEtsu Isonet® Mister L). These active devices require a much lower number of units per hectare (3 to 5) compared to hand-applied dispensers because they release liquid micro-droplets that float in the air and evaporate, differing from the slow releasing vaporized molecules of the passive dispensers. The reduced number of units can save labour costs, at least after the first year of application, and contribute, to some extent, to reduce plastic disposal in agricultural settings (McGhee et al. 2016; Lucchi et al. 2018b c).

Aerosol formulations can be easily tuned to release pheromone plumes during the hours when males really fly searching for mates, providing a cost-effective alternative to passive dispensers. As above mentioned, Lucchi et al. (2018a) reported that EGVM flight mainly occurs between 21:00 and 23:00 h along the three generations. These findings are useful in optimizing the MD technique, identifying selected time intervals when the release of synthetic pheromones can be concentrated, boosting MD efficacy against this important pest, reducing the release of synthetic sex pheromone molecules, and potentially reducing application costs.

Further research to develop aerosol dispensers with reduced pheromone content and finely tuned release programmes is ongoing with the aim of producing highly effective, economic, and easy-to-manage aerosol devices. This research will undoubtedly have to be linked with the measurement of pheromone concentration in the air via research on sensors (Ivaskovic et al. 2021). Harari et al. (2011) showed that females can detect their synthetic pheromone and may adjust several behavioural traits and their spatial distribution. Hence, the optimal efficiency of MD will depend on the uniformity of the pheromone cloud and its spatial distribution in time.

Other novel pheromone application systems as auto-confusion, lure and kill, microencapsulated sprayables and nanofibers are still under investigation and can hopefully represent future opportunities for grapevine moth control (Underwood et al. 2002; Charmillot et al. 2005; Ioriatti & Lucchi 2016). Investment in fundamental research is however still critical for an effective improvement in semiochemical applications. Research should address the reproductive, physiological and behavioural mechanisms by which pheromone affects the target insects, as well as explain how these compounds are involved in tritrophic interactions. For steep slope regions of countries located in the south of Europe, research about new dispensers is still needed, to avoid exhaustion of pheromone during the 3<sup>rd</sup> flight. Under such situations, and particularly on windy slopes, aerosol dispensers can show a limited efficacy.

In some wine-growing areas where MD is applied, the reduction in insecticide use against the key pests is surprisingly one of the main limitations to the extensive use of MD. In fact, the outbreaks of resident secondary pests or the invasion of new alien species allowed by the lower quantity of insecticides applied because of MD application can sometimes occur. Therefore, in order not to jeopardise the adoption of MD, it is crucial to develop environmentally safe control methods against these secondary or invasive pests (Pertot et al. 2017; Nieri et al. 2022).

## 4.1 Biological control

The occurrence of EGVM natural enemies varies on the season and strictly depends on the features of the ecological niche in which the moth lives (Thiéry et al. 2001; Bagnoli & Lucchi 2006).

### 4.1.1 Predators

The predators associated with EGVM include insects, other arthropods, and vertebrates (Coscollá 1997; Ioriatti et al. 2011; Thiéry et al. 2018). The latter are represented by birds and bats, which occasionally feed on EGVM adults (Thiéry et al. 2018; Baroja et al. 2021; Charbonnier et al. 2021; Chaperon et al. 2022). Among arthropods, Clubionidae, Theridiidae, Thomisidae, Linyphiidae and Salticidae spiders (Araneae) are considered EGVM predators in Mediterranean

vineyards (Marchesini & Dalla Montà 1994; Addante et al. 2008; Ioriatti et al. 2011; Thiéry et al. 2018). Several insect species in the orders Dermaptera, Hemiptera, Neuroptera, Diptera and Coleoptera can prey on EGVM, providing a moderate contribution to control their populations (Coscollá 1997; Ioriatti et al. 2011).

### 4.1.2 Parasitoids

A number of studies have been carried out to describe parasitoids, specifically or occasionally associated with EGVM (Thiéry et al. 2006c; 2011; Xuéreb & Thiéry 2006; El-Wakeil et al. 2009; Moreau et al. 2010; Loni et al. 2016; Scaramozzino et al. 2017; 2018; Carlos et al. 2022; Di Giovanni et al. 2022). For a correct association between host and parasitoid, dedicated breeding protocols by expert taxonomists and carefulness in the selection and management of the field-collected material are required. To this regard, PCR-RFLP analysis on EGVM larvae can be a reliable tool to shed light on parasitoid species identity and parasitization rates (Papura et al. 2016).

From a physiological point of view, larval immune functions depend on host plant quality and are positively correlated to parasitism, suggesting that parasitism selects for greater investment into immunity in the EGVM (Vogelweith et al. 2013a). Larvae exposed to parasitoids develop faster, which provides added defence to immunity (Vogelweith et al. 2013b). Feeding on several host plants in addition to grapevines increases the immune response, which could explain the maintenance of polyphagy in the EGVM (Muller et al. 2015). During the night-time EGVM larvae spend more time eating, have higher phenoloxidase activity and experiences changes in immunity-related haemocytes, all of which points towards adaptations to enhance the immune response to parasitoids (Iltis et al. 2021).

As a general trend, egg and larval parasitisation rate is higher in the first two EGVM generations and decreases in the overwintering generation, which is mainly affected by larval and pupal parasitoids (Ioriatti et al. 2011). Most of the species associated with EGVM (>95%) are either parasitic Hymenoptera belonging to the families Braconidae, Chalcididae, Elasmidae, Eulophidae, Ichneumonidae, Pteromalidae and Trichogrammatidae, or belong to Tachinidae (Diptera). The numbers of Ichneumonidae species associated with EGVM have been recently recorded at 120 (Scaramozzino et al. 2018). In Italy, 63 taxa have been reported, the most abundant belonging to Cryptinae (20), Campopleginae (11), Pimplinae (10), Ichneumoninae (9), Metopiinae (7), Anomaloniinae (3), Tryphoninae (2) and Cremastinae (1) (Scaramozzino et al. 2018). Moreover, in the Douro Demarcated Region (DDR) (Portugal), 16 parasitoid taxa have been identified, most of them belongs to Hymenoptera (Carlos et al. 2022; Di Giovanni et al. 2022). Among all EGVM parasitoids, only two of them have been considered suitable BCAs to be mass-reared for

subsequent release in the vineyard: *Trichogramma* spp. (Castaneda-Samayoa et al. 1993; Hommay et al. 2002; Pizzol et al. 2012; Lucchi et al. 2016; Thiéry & Desneux 2018) and *Campoplex capitator* Aubert (Bagnoli & Lucchi 2006; Xuéreb & Thiéry 2006; Lucchi et al. 2018d; Moreau et al. 2019; Scaramozzino et al. 2018; Benelli et al. 2020b) (Fig. 1). Castaneda-Samayoa et al. (1993) evaluated three *Trichogramma* species (*T. cacaeciae* Marchal, *T. embryophagum* (Hartig) and *T. dendrolimi* Matsumura) in the laboratory and in the field for the management of EGVM and *E. ambiguella*. Depending on the tested parasitoid species, different egg parasitisation rates ranging from 22.5% to 83.3% were achieved. In addition, eggs older than two days were parasitized significantly less than newly laid eggs. Lucchi et al. (2016) explored the parasitism of *T. euproctidis*, *T. cordubense*, *T. evanescens* and *T. cacaeciae* on eggs of EGVM feeding on *Daphne gnidium* L. (Thymelaeaceae). During the spring-summer season, the parasitisation rate increased and peaked at 55% in the third EGVM generation. The contrasting results concerning parasitisation rates achieved by the different *Trichogramma* spp. led to a failure in using these natural enemies as an effective and reliable strategy for controlling EGVM. On the other hand, research on the most suitable and reliable *Trichogramma* species for EGVM biocontrol is still ongoing.

*C. capitator* showed interesting EGVM parasitisation rates, depending on the context (Moreau et al. 2019); it has

a host range limited to a few grapevine-feeding tortricids (Villemant et al. 2011), and the woodland deciduous tortricid *Ancylis mitterbacheriana* (Aubert) (Scaramozzino et al. 2018). Its rearing is difficult (Xuéreb & Thiéry 2006; Benelli et al. 2020b), because, as in other ichneumonids, mating failures rapidly drifts the sex ratio towards males. However, a joint project between University of Pisa (Italy) and SAG (Chile), led to the mass-rearing of *C. capitator*, followed by its release for EGVM management purposes in Chile (Lucchi et al. 2018d; Navarro 2021). Results still need to be fully evaluated, since the covid-19 emergence precluded an immediate assessment of the parasitisation rates directly after the release of the parasitoid. However, this species is still considered one of the most promising parasitoids for EGVM management.

Among the better-performing parasitoids, three other candidates emerged, i.e., *Dibrachys microgastri* (Bouché) (= *Dibrachys cavus* (Walker)) (Hymenoptera: Pteromalidae) (Coscollá 1980; Chuche et al. 2006), *D. affinis* Masi, 1907 (Coscollá 1980; Bagnoli & Lucchi 2006) and *Phytomyptera nigrina* (Meigen) (Diptera: Tachinidae) (Bagnoli & Lucchi 2006). Coscollá (1980) identified *D. microgastri* as a suitable parasitoid of EGVM in the Valencian wine-growing area. The tachinid fly *P. nigrina* represents an important polyphagous endoparasitoid for the natural control of EGVM in Tuscany (central Italy) especially during the anthophagous generation (Bagnoli & Lucchi 2006).



**Fig. 1.** (A) *Lobesia botrana* eggs parasitized by *Trichogramma* spp. (Photo: P. L. Scaramozzino). (B) *Campoplex capitator* female (lateral view) (Photo: R. Ricciardi).



Overall, given the difficulties in finding and breeding effective BCAs, it is important to support those already present in the agroecosystem, boosting conservation biological control (CBC) and IPM. This can be achieved through the adoption of selective, low-persistence (bio)insecticides, or by the use of non-insecticide-based techniques, but also through a smart management of the vineyard agro-ecosystem, e.g., allowing the presence of flower strips in the inter-row or by ensuring the presence of hedges or shrubs around the vineyard to provide food and refuges to BCAs, boosting biodiversity and agroecosystem stability (Segoli et al. 2020). Natural surrounding habitats are also crucial for maintaining and providing resources for potentially EGVM natural enemies.

#### 4.1.3 Entomopathogens

Several studies focused on entomopathogenic fungi (EPF) in the order Hypocreales, given their extensive expansion and persistence in various agroecosystems (Bidochka et al. 2001). López Plantey et al. (2019) showed that *Beauveria bassiana* and *Metarhizium robertsii* are capable of infecting EGVM larvae and that both species were adapted to the specific conditions of the studied vineyard. Further, Altimira et al. (2019, 2022) proposed the use of *B. pseudobassiana* strains during the winter season to control the overwintering pupae with the aim to reduce the number of adults in the following flight. Aguilera-Sammaritano et al. (2021) assessed the use of *M. anisopliae* for managing different developmental stages of EGVM, showing 85% pupal and adult mortality and 75% larval mortality in the laboratory. In the field, larval mortality ranged from 64 to 91%, with a correlation between the EPF strain and the pupal stage.

Differences between laboratory and field tests do not represent *a priori* an impediment to the use of these organisms in agricultural systems (Roy & Pell 2000). Some authors have focused on alternatives such as the application of *B. bassiana* and *Paecilomyces fumosoroseus* together with *B. thuringiensis*, which shows a synergistic effect in EGVM larval mortality (Beris & Korkas 2021).

Regarding entomopathogenic nematodes (EPN), laboratory studies proved that *Steinernema feltiae* (Filipjev), *S. carpocapsae* Weiser, *S. riojaense* n. sp. and *Heterorhabditis bacteriophora* (Poinar) were able to kill larvae and pupae of EGVM, with *S. carpocapsae* being the most efficient, killing in 2 days ~50% of L1 and >75% L3 and L5. At the pupal stage, a concentration 5 times higher than that used in the larval tests was required to reach ~50% mortality (Vicente-Díez et al. 2021). Ongoing experiments are showing the compatibility with regular adjuvants used in viticulture, the co-formulation with other chemical products applied in vineyards to manage fungal diseases, and the compatibility at 10–15 °C that can facilitate their application to target overwintering pupae at the stem under the bark (Campos-Herrera et al. unpublished data).

## 5 Cultural control, functional biodiversity, and conservation biological control as tools of integrated pest management

The cultivation of less susceptible varieties is a tool to modulate damage in EGVM hot spots (Esmenjaud et al. 2008). It is unclear whether these longstanding observations on the susceptibility of grape varieties to EGVM are based on observed egg-laying behaviour or on different degrees of symptom expression with the same infestation (Rid et al. 2019). However, these questions are of outstanding relevance for breeding research. The effect of grape varieties was reported by several authors as having an important impact in EGVM development and fecundity (e.g., Moreau et al. 2006; Thiéry et al. 2014a, b; Carlos et al. 2018). Moreover, the size and shape of the grape bunches in the different varieties, as well as other characteristics of the larval micro-habitat, may influence EGVM developmental time (Thiery et al. 2014a, b), suggesting that the difference of compactness of clusters among varieties could infer differences in sunlight exposure and temperature having possibly an impact on the insect development.

Traditional ways of controlling EGVM without insecticides include piling vineyard soil around the stems during winter to cause mold development on the overwintering pupae (Hoffmann 2021). Removal of the bark with metal mesh gloves, which can kill overwintering pupae is another possible option (Schwangart 1913). Both methods require too many hours of manual labor but could represent an incentive for designing new machines automatically performing these tasks.

Leaf removal in the bunch zone of the canopy is an efficient way to reduce cluster size and compactness, making them less susceptible to EGVM attack and subsequent rot. Another positive effect is that the direct sunlight increases berry temperature in a way that EGVM eggs can be killed. As demonstrated by Moosavi et al. (2018) bunch-zone leaf removal (BZLR) reduced the carpophagous generation by about 50%. Temperatures from 37–40 °C increased the mortality of eggs and newly hatched larvae. In the field, the berry temperatures of sun-exposed bunches exceeded the temperatures that were lethal in the laboratory reducing egg hatching and larval settlement, even if the number of eggs laid by females on these bunches was not affected. BZLR can be considered as a valid cultural practice in the context of Integrated Pest Management and combined with optimised grapevine-row orientations, can improve EGVM control.

In addition to the agronomic techniques just mentioned, the management of the wild flora in or around the vineyard can play an important role in fostering EGVM natural enemies. The timing and frequency of weed control by herbicide applications or tillage of inter-row strips determines the diversity of the plants and arthropods that can inhabit these strips (Hall et al. 2020). It is difficult to cultivate green cover



crops at all in rainfall-deficient vineyards, especially in climates like the Mediterranean (Winter et al. 2018).

The idea that intercropping or mixing different crops can improve moth natural regulation is old (Silvestri 1912) but was temporarily forgotten after the introduction onwards of synthetic insecticides from the 1950s. Several studies have pointed out a positive influence of landscape diversity on the abundance and diversity of natural enemies at the field level (Bianchi et al. 2006; Tamburini et al. 2020), but the impact of this diversity in enhancing biological control has not always been demonstrated (Begg et al. 2017; Lichtenberg et al. 2017).

The importance of non-crop vegetation or soil cover vegetation for providing resources to parasitoids, such as shelter, overwintering habitat, nectar, pollen, and alternate hosts or preys, is well documented (e.g., Thomson et al. 2010; Daane et al. 2018b; Shapira et al. 2018; Segoli et al. 2020; Möller et al. 2021). Semi-natural elements in the surrounding landscape and vineyard inter-row management are necessary to foster natural pest control (Judt et al. 2019) and to execute a successful CBC program based on the implementation of habitat management strategies. The use of plants native to a region in the habitat management to support natural enemy populations has several other advantages (Landis et al. 2012). Because these plants are locally adapted, they require, in general, little management after establishment and can persist within the agroecosystem for decades. The incorporation of such habitats into the vineyard, in addition to biocontrol, can provide other benefits such as conserving wildlife, protecting water quality, and reducing erosion and runoff (Tillman et al. 2012). Furthermore, the use of native plants contributes to their conservation, which is especially important in the Mediterranean Basin, since this is one of the world's richest places in terms of plant diversity (Cuttelod et al. 2009), being considered a prime candidate for conservation support (Myers et al. 2000).

In DDR, a strong impact of the ecological infrastructure (EI) on the parasitism of EGVM was found (Carlos et al. 2022), being five times higher in areas with a higher percentage of EI, when compared with areas with a lower percentage of EI. It was also found that native ground cover increased parasitism by 50%, compared to vineyards with bare soil. In Andalusian vineyards, surrounding landscapes influence EGVM infestations on farms (Paredes et al. 2021). At harvest, pest outbreaks increased four-fold in simplified vineyard-dominated landscapes compared to complex landscapes in which vineyards are surrounded by semi-natural habitats. Similarly, insecticide applications doubled in vineyard-dominated landscapes but declined in vineyards surrounded by shrubland.

Thomson et al. (2010) observed a positive impact of woody vegetation surrounding vineyards on Eulophidae parasitoids. On the other hand, temporal dynamics and disturbance regimes of perennial and annual crops greatly differ, and these differences may modify the effect of landscape composition on natural enemies, pest communities, and bio-

control services. Consequently, it is not known if landscape heterogeneity positively affects pest control over time or if antagonistic relationships between guilds emerge over time, neutralising the effects of landscape heterogeneity (Rusch et al. 2017). The general variability of the results shows that the regional context can strongly influence the local effects of the habitat characteristics (Begg et al. 2017).

It is clear that the habitat management systems must be developed and evaluated regionally, according to the natural characteristics of the context (Fernández-Mena et al. 2021) and that it is not possible to standardise the intervention methods in different contexts. New management systems have to be harmonised with the behaviour of growers at local or regional scales (Yang et al. 2021). This means that the research on the functional biodiversity in vineyards will probably become one of the main tasks for future plant protection research. At this regard, the use of sentinel traps for standardised measurement of insect parasitisation and predation (Hoffmann et al. 2017; Pennington et al. 2018; Reiff et al. 2021) and the use of molecular markers and metabarcoding for ecosystem analysis could be useful. Using sentinel traps, which consist of cards with previously counted instars of EGVM temporarily exposed in the field, both predation- and parasitisation rates can be assessed in various contexts, independently from the actual occurrence of the pest (Hoffmann et al. 2017). These authors observed that flowering green cover crops did not enhance parasitism of EGVM compared to spontaneous vegetation in a given EI, but they increased the activity of unspecific egg and pupal predators. Pennington et al. (2018) found that reduced applications of plant protection products, by planting mildew resistant varieties, increased egg removal by predators. Reiff et al. (2021) did not find egg and pupal parasitism at all in Austrian vineyards but found that predation of EGVM pupae was negatively influenced by organic management and positively influenced by spontaneous vegetation. According to these authors, the landscape and the management have no effect on the egg predation. These results were found in an environment where insecticides were not used but where copper and sulphur applications in organic viticulture appear to be the most important bottleneck for biodiversity.

From a molecular ecology point of view, Papura et al. (2020) used EGVM-specific primers for food web research. Kaczmarek et al. (2022) firstly used metabarcoding of the whole vineyard/malaise trap fauna to analyse and manipulate entire vineyards on a whole ecosystem level.

## 6 Area-wide control programs (experiences from USA, Argentina, Chile)

As mentioned above, EGVM was introduced into the Americas region with the first detections occurring in Chile in 2008, in California (USA) in 2009 and in Argentina in 2010 (Gilligan et al. 2011; Ioriatti et al. 2012; Altimira et al. 2022b). Based

on its status as a significant Palearctic grape pest, its establishment in these regions presented significant production and export issues for grapes, as well as for other fresh market agricultural commodities. In response, the national and regional plant protection authorities in each country implemented official control programs where area-wide integrated pest management and regulatory control measures were established to eradicate or contain the spread of the pest.

### 6.1 Program response in Chile

In April 2008, the first confirmed South American detection was made in the Linderos area of Maipo province in the Metropolitan region in Chile (FAO-IAEA 2020). In response, the SAG, in cooperation with the Instituto de Investigaciones Agropecuarias (INIA), the grape and fruit industries and university scientists, began a national control program for the management of EGVM and reduction of damage caused by the pest. This was accomplished by deployment of a pest monitoring program in grape production areas, the area-wide application of MD, insecticidal treatments and regulatory controls for grape production areas (Ioriatti et al. 2012; SAG 2015; Simmons et al. 2021a). Mandatory treatments were applied to areas within 500 m of a detection with a quarantine established within 3 km of the find. The program paid for all or part of the treatments depending on the size of the farm. The regions most impacted were wine grape production areas near Santiago in the metropolitan region, and to the south in O'Higgins and Del Maule regions. There were also significant infestations in residential and urban gardens due the popularity of growing wine grapes in Chilean gardens, also requiring monitoring and control tactics (Ceballos et al. 2022). Because of incidents of detections in plums and blueberries in areas where these crops were grown near infested vineyards, efforts are ongoing to protect these crops from EGVM impacts, especially to valuable export markets for fresh fruit. To help coordinate national activities, SAG and INIA developed a nation-wide alert system which included a phenology model, temperature monitoring stations and weekly EGVM pheromone trap capture, which were used to predict optimal timing for applications of MD and pesticide treatments.

Infestations in urban areas adjacent to agricultural production areas were a significant challenge for control due to the difficulties in setting up effective control measures in residential areas (Ioriatti et al. 2012; FAO-IAEA 2020). Applications of MD were made to residential areas using regular dispensers, as well as mesodispensers (Ceballos et al. 2022) and SAG launched a program to develop additional control tools to include development of the sterile insect technique (SIT) and a classical biological program to rear and release the European parasitoid *C. capitator* to improve control in urban areas (Lucchi et al. 2017; SAG 2018; 2019; FAO-IAEA 2020).

The peak period of EGVM detections was in the 2015–2016 growing season and since that time there has been a

significant decline in adult moth detections in grapes above 90%, along with a decrease in the area under regulation (A. Alvarez pers. comm.) and the establishment of regions of low pest prevalence.

### 6.2 Program response in the United States

In 2009, a year after its detection in Chile, EGVM was reported in the Napa Valley in California; the first record in the United States (Varela et al. 2010; Gilligan et al. 2011). To react to this plant pest emergency, a cooperative response and control program was established among the California Department of Food and Agriculture, several offices of county Agricultural Commissioners, the University of California, the United States Department of Agriculture – Animal and Plant Health Inspection Service and grape producers (Ioriatti et al. 2012; Cooper et al. 2014). An eradication program was established that included a surveillance program, restrictions on the movement of plant material and equipment, multiple control measures, research and methods development and an extension and outreach program. The surveillance program consisted of a state-wide network of pheromone-baited traps to delimit the scope of the EGVM invasion, with 39 traps per km<sup>2</sup> deployed in the central quarantine areas and 10 traps per km<sup>2</sup> in grape producing areas outside of the quarantine area (Simmons et al. 2021a). Insecticide and mating disruption treatments were made in a 500 m radius around detection spots. Coordinated insecticide treatments using degree-day models to target eggs and young larvae were implemented at the start of the first and second flights (Varela et al. 2010; Cooper et al. 2014). MD applications were made during at least the first two flights after a detection (Simmons et al. 2021a). Coordinated treatments were also made in residential areas with grape plantings within quarantine areas in Napa County. These properties were either treated by stripping flowers and fruit or by applications of *Bt* with some limited uses of MD (Cooper et al. 2014). Before its detection and the establishment of an official control program, movement of infested grapes and in one case, recycled vineyard stakes, led to EGVM spreading to several other California counties as far as 300 km from where it was first detected. However, the control program successfully caused populations to decline sharply over time. In 2016, after two years without detection, the pest was declared eradicated (Simmons et al. 2021a).

### 6.3 Program response in Argentina

Following the announcement of EGVM detection in Chile, Argentina established a phytosanitary alert to guard against entry of the pest (SENASA 2009). However, despite these measures, the first detection occurred in 2010 in Maipú, in the Province of Mendoza (Ioriatti et al. 2012, Dagatti & Becerra 2015). In response, a national official control program for the EGVM prevention and eradication and a surveillance trapping network was established (SENASA 2010). In Mendoza, program treatment zones were set up based on the degree of infestation, and protocols were established for the

transport of grapes within each zone. The program provided funds to pay for pesticides and MD for small and medium-sized grape growers. MD applications were applied using hand-tied dispensers, mechanised aerosol release devices and aerial spray applications. Quarantine measures included restriction of movement of grapes and other host materials, tarping of bulk shipments of grapes to prevent larvae leaving infested shipments, destruction of wine making wastes, and removal of remaining fruit after harvest. After 2010, EGVM was detected in the provinces of San Juan, Entre Rios and Salta and control programs were initiated in those locations. Additional control measures included a pilot project to develop and evaluate the use of the SIT and releases of the classical biological control agent *C. capitator*.

Since program initiation, by 2021 the provinces of Entre Rios and Salta have achieved pest free area status after two years without detections and reductions in moth captures of about 50% have been achieved in the northern, eastern, and central grape growing regions of Mendoza with very low populations in the southern grape growing regions of the province. EGVM is still present in southern grape growing areas in the province of San Juan though population levels are low (W. Ramirez, SENASA, pers. comm.).

## 7 The future: challenges for prevention and management strategies

Overall, IPM offers several possible solutions for EGVM control. However, several key challenges in this field still need to be addressed. Below, we propose an agenda formulating major aims for future research.

To date, as the problems induced by synthetic pesticides worsened, applied entomologies started to realise that CBC, in the frame of IPM, could represent a pivotal solution for the timely and sustainable management of this pest. It is of strategic relevance to focus on prevention rather than on post detection responses, which are usually much more costly. Molecular analyses represent a useful and reliable tool for improved insect identification and, in case of new detections, for characterising populations and determining the origin of the seeded populations (Daane et al. 2018a). Molecular tools could also be successfully used to distinguish among larval stages of parasitoids attacking EGVM (Papura et al. 2016).

More studies on BCAs are needed. For example, research on the biology and mass production of the two very efficient parasitoids in Europe (*C. capitator* and *P. nigrina*) has to be intensified. Interesting laboratory results were also obtained with *D. microgastri* (Chuche et al. 2006) and the technique based on *Trichogramma* spp. has to be developed as well. Focusing deeper on the egg/larval immune system quality of EGVM will provide interesting perspectives. The analysis of the prophenol oxidase (Vogelweith et al. 2014) suggests that encapsulation may occur after *C. capitator* egg laying in

its larval host. The immune function varies with larval food and grape cultivar (Vogelweith et al. 2015); this could help in the future to adapt the biological control procedures to the immune efficiency (e.g., ratio parasitoids/hosts).

The extension of areas under MD worldwide is a very efficient current option for EGVM control, and the promising rapid results obtained in California (Simmons et al. 2021a) suggest that a large-scale project can be very effective. Progress in sensing such concentrations are still needed to efficiently manage MD vineyards (Harari et al. 2011; Ivaskovic et al. 2021).

Gene editing tools have been proposed for manipulating natural enemy biology or plant defences (Gurr & You 2016). Recent research opens promising new prospects for controlling EGVM through interference with its interspecific communication (Salvagnin et al. 2018).

Some of the control methods already available or under development against EGVM, if applied on a large scale and in the frame of IPM programs, can provide efficient, sustainable, and lasting control. However, the reduction of chemical input in the vineyard that these strategies can ensure may open the way for resurgence of secondary pests or invasion of alien species (Vezzulli et al. 2022). These phenomena are exacerbated by the mechanisms of globalisation and, in some contexts, by the ongoing climate change. The challenge for those involved on EGVM research and management is therefore to quickly find effective and compatible solutions against these new species to safeguard hitherto developed IPM and sustainable control of EGVM (Nieri et al. 2022).

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