



# Vine and citrus mealybug pest control based on synthetic chemicals. A review

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## Abstract

Synthetic chemicals are extensively used to limit the substantial crop damage induced by two closely related scale insects, the vine mealybug *Planococcus ficus* (Signoret) and the citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae). Both organisms are economically important pests occurring in vineyards and/or in citrus orchards worldwide. Synthetic chemicals can be either incorporated in pesticides aimed at directly controlling these pests or used as semiochemicals (i.e., sex pheromones) for monitoring, mass trapping, mating disruption, and/or for kairomonal attraction to enhance parasitoid performances. Growing evidence of both an alarming bee decline and destruction of auxiliary fauna driven by pesticides have stimulated an urgent need for in-depth research clarifying the adverse side effects of pesticides on beneficial arthropods. We have reviewed the current knowledge on mealybug pest control based on insecticides and semiochemicals. We highlight the following major advances: (1) How the active substances of insecticides (four organophosphates, imidacloprid, buprofezin, and spirotetramat) affect target and non-target organisms, (2) in which contexts and how a semiochemical-based strategy could be applied to deal with serious mealybug infestations, and (3) the implications of the appropriate exploitation of these synthetic chemicals for sustainable development. Using selective insecticides with novel modes of action and long-lasting efficacy in combination with eco-friendly semiochemical-based tools is a promising strategy for developing sustainable integrated pest management programs. This would help to maintain biodiversity dynamics and vital ecosystem services, thereby sustaining crop yields.

**Keywords** *Planococcus ficus* · *Planococcus citri* · Pesticides · Semiochemicals · Biocontrol · Bees · Ecotoxicology · Integrated pest management · Citrus · Grapevine

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## Contents

1. Introduction
2. Pesticides: field performance, limitations, and side effects on beneficial arthropods
  - 2.1 Acetylcholinesterase inhibitors (nerve action): organophosphate insecticides
    - 2.1.1 Efficacy against the vine mealybug
    - 2.1.2 Efficacy against the citrus mealybug
    - 2.1.3 Side effects on natural enemies
    - 2.1.4 Side effects on pollinators
  - 2.2 Nicotinic acetylcholine receptor competitive modulators (nerve action): the neonicotinoid imidacloprid
    - 2.2.1 Efficacy against vine and citrus mealybugs
    - 2.2.2 Side effects on natural enemies
    - 2.2.3 Side effects on pollinators

- 2.3 Inhibitors of chitin biosynthesis, type 1 (growth regulation): buprofezin
    - 2.3.1 Efficacy against vine and citrus mealybugs
    - 2.3.2 Side effects on natural enemies and pollinators
  - 2.4 Inhibitors of acetyl-coA carboxylase (lipid synthesis, growth regulation): spirotetramat
    - 2.4.1 Efficacy against vine and citrus mealybugs
    - 2.4.2 Side effects on natural enemies and pollinators
  - 3. Semiochemicals exploited for vine and citrus mealybug pest management
    - 3.1 Monitoring and mass trapping
    - 3.2 Mating disruption
    - 3.3 Kairomone-based control systems
  - 4. Alternative tools for sustainable control
  - 5. Conclusion
- Acknowledgements  
References

## 1 Introduction

Scale insects (Hemiptera: Coccoidea) are considered to be among the most important pests of cultivated crops worldwide (Daane et al. 2012; Franco et al. 2009; Mansour et al. 2017a; Miller et al. 2002; Sforza 2008). Within this large group of insects, mealybugs (Pseudococcidae) constitute the second most species-rich family, following armored scales (Diaspididae), exceeding 2000 described species to date (García Morales et al. 2016). Mealybugs are small, soft-bodied insects, usually covered by a white mealy wax, which feed on plant phloem and excrete honeydew (Williams and Watson 1988). About 160 species of mealybugs are recognized as plant (crop and non-crop) pests worldwide (Franco et al. 2009; Miller et al. 2002).

The vine mealybug, *Planococcus ficus* (Signoret) (Fig. 1), is the key economic scale insect pest species occurring in vineyards worldwide, including the Mediterranean basin, which represents its native range (Daane et al. 2012; Dalla Montà et al. 2001; Güleç et al. 2007; Mansour et al. 2017a; Pacheco-da-Silva et al. 2014; Reineke and Thiéry 2016; Sforza

et al. 2005; Walton et al. 2009). The citrus mealybug, *Planococcus citri* Risso (Fig. 2), is an economically important insect pest in Mediterranean citrus orchards (Franco et al. 2004; Jacas et al. 2010; Karamaouna et al. 2010; Küçük et al. 2014; Mansour et al. 2017a; Zappalà 2010) and in other citrus-growing areas of the world (Gill et al. 2013; Kerns et al. 2001; Rao et al. 2006; Wakgari and Giliomee 2003). It has also been reported to attack and damage grapevines in Italy (Bertin et al. 2016; Dalla Montà et al. 2001), France, Portugal, Greece (Sforza 2008), Spain (Cabaleiro and Segura 1997; Cid et al. 2010), Tunisia (Mansour et al. 2017a), and Brazil (Morandi Filho et al. 2015; Pacheco-da-Silva et al. 2014). The number and distribution of the multilocular disc pores and tubular ducts in the adult females can be used for morphological discrimination between *P. ficus* and *P. citri*, although genetic analysis is probably easier to apply (Cavaliere et al. 2008; Daane et al. 2011; Mansour et al. 2011a, 2012b).

In vineyards, *P. ficus* and *P. citri* excrete honeydew that supports the growth of sooty mold fungi, lowering the quality and the market value of grape clusters. Vine mealybug feeding on grapevine leaves can inhibit photosynthesis and provoke defoliation (Reineke and Thiéry 2016). Vine and citrus mealybugs have been proven to be prominent vectors of grapevine viruses, such as Leafroll Associated Virus III (GLRaV-III) (Almeida et al. 2013; Cabaleiro and Segura 1997; Golino et al. 2002; Tsai et al. 2010). In citrus orchards, the citrus mealybug occurs primarily in older, well-shaded groves planted in heavy soils and will feed on the roots, bark, foliage, and fruit (Kerns et al. 2001)

Infestations of citrus by citrus mealybugs can reduce plant growth and fruit size and lead to fruit downgrading; high infestations can cause defoliation (up to 80%), fruit splitting, and fruit drop (up to 100%) (Kerns et al. 2001; Zappalà 2010). Development of sooty mold fungi growing on honeydew can degrade citrus fruit quality by reducing the photosynthetic capacity of leaves and lead to a commercially unacceptable appearance of fruits, requiring vigorous scrubbing before packing (Gill et al. 2013)

To prevent and/or limit major economic losses due to severe attacks on grapevines by vine mealybugs and on citrus by

**Fig. 1** Immature and mature vine mealybugs residing under the grapevine trunk bark (on the left) and severely infesting a ripe cluster (on the right)



**Fig. 2** Mature and young instar nymphs of citrus mealybug infesting a cluster of lemons (on the left); honeybee visiting mandarin flowers as a key non-target beneficial arthropod (pollinator) that could be adversely affected by the insecticides used to control citrus mealybugs (on the right)



citrus mealybugs, a range of pest management control strategies have been developed and implemented. Integrated pest management programs against both mealybug species encompass a range of approaches including prophylaxis and cultural practice tools, biological control using specialized encyrtid parasitoids and/or efficient coccinellid predators, the application of insecticide treatments, and the exploitation of semiochemicals in various pheromone-mediated pest management tactics.

Chemical control is considered as the most common control tactic used against mealybug pests (Franco et al. 2009). However, repeated applications of non-selective pesticide treatments can seriously compromise the efficacy of integrated pest management programs owing to the non-target effects on beneficial arthropods, i.e., parasitoids, predators and bees (Fig. 2) (Belzunces et al. 2012; Biondi et al. 2012, 2015; Desneux et al. 2007; Gill and Garg 2014; Pisa et al. 2015), including those applied against vine and citrus mealybugs (Campos et al. 2008; Mansour et al. 2011b; Mgocheki and Addison 2009; Planes et al. 2013; Suma et al. 2009; Walton and Pringle 1999).

To promote the integration of biocontrol agents in integrated pest management strategies, the compatibility of insecticides and biocontrol candidates is essential (Johnson and Tabashnik 1999; Stark et al. 2007). Decisions regarding the timing and modes of action of the selected insecticide should be based on physiological and bio-ecological characteristics of the target pest. For instance, new synthetic pesticides that penetrate into the protected locations of mealybugs (e.g., under the vine bark) are fundamental for the successful management of mealybugs in vineyards.

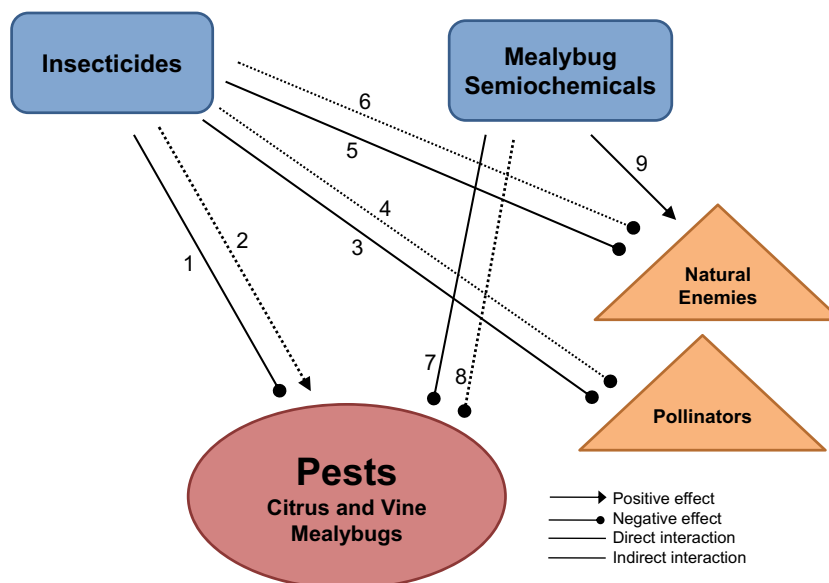
In addition to pesticide-induced reduction/extinction of mealybugs' natural enemies, resistance to the active substances of insecticides has developed as a consequence of the intensive and repeated use of insecticides (Flaherty et al. 1982; Franco et al. 2004, 2009; Venkatesan et al. 2016). Researchers are thus investigating alternative innovative, environmentally sound, and effective pest management strategies to control mealybugs. From a sustainability perspective, the development of naturally derived compounds with a potential insecticidal activity should be pursued (Campolo et al. 2014; Karamaouna et al. 2013).

Reduced pesticide use could also be combined with other biorational tactics (using pheromones or natural enemies), which individually may be less efficient than pesticides (Barzman et al. 2015). In this regard, semiochemicals can be used in different strategies and are currently important for growers and pest management specialists. Using semiochemicals for controlling vine and citrus mealybugs includes pheromone-based monitoring and/or mating disruption options (semiochemical-based intraspecific communication) (Cocco et al. 2014a; Daane et al. 2006; Mansour et al. 2017a; Walton et al. 2004, 2006), and the use of a kairomone-mediated system to enhance the mealybug's parasitoid performance (semiochemical-based interspecific communication) in vineyards or citrus orchards (Franco et al. 2008, 2011; Mansour et al. 2010b).

In this review article, we summarize the most important aspects linked to the most commonly used synthetic chemicals for vine and citrus mealybug management throughout the largest global grape-growing and citrus-producing areas. We focus on the direct and indirect (i.e., mediated by other organisms) interactions (either positive or negative) of the reviewed synthetic chemicals (insecticides and semiochemicals) with both mealybug pests, natural enemies, and pollinators (see the outline in Fig. 3).

We reviewed the current bibliography on these topics and tried to answer the following key questions: (1) which active substances in pesticides have commonly been used against the vine and citrus pest mealybugs? (2) How do these insecticides affect target and non-target organisms? (3) In which context and how could a semiochemical-based tactic deal with serious mealybug infestations? (4) What are the implications of the appropriate exploitation of these synthetic chemicals for sustainable pest management?

All the scientific literature used in this review was retrieved from the following online databases: Google Scholar, Web of Science, Scopus, PubMed, and ScaleNet (<http://scalenet.info/>). The last reference update was carried out on 30 April 2018. The keywords used for the searches alone or in various combinations were bees, buprofezin, chlorpyrifos, citrus orchards, citrus mealybug, imidacloprid, insecticides,



**Fig. 3** The main direct and indirect (i.e., mediated by other organisms) effects of insecticides and semiochemicals used in citrus and grape integrated mealybug management programs. (1) Acute and chronic insecticide toxicity to pests. (2) Positive effects due to insecticide side effects on natural enemies of the pests. (3) Direct side effects of insecticides on pollinators exposed to direct sprays or to residues on citrus or non-crop plants in vineyards. (4) Indirect side effects of systemic insecticides mediated by the plants on pollinators feeding on pollen, floral, and extrafloral nectars. (5) Direct side effects of

insecticides on mealybug natural enemies exposed to direct sprays or to residues on plants. (6) Indirect side effects of insecticides mediated by the plants on mealybugs natural enemies (predators and parasitoids) feeding on floral and extrafloral nectars or mediated by contaminated hosts/preys. (7) Direct negative effects on pests due to monitoring, mass trapping, and mating disruption. (8) Indirect negative effects due to the kairomonal activity on mealybug natural enemies. (9) Direct positive kairomonal effects on mealybug natural enemies in terms of host/prey location

organophosphates, parasitoid, predator, *Planococcus ficus*, *Planococcus citri*, pollinator, scale insects, side effect, spirotetramat, sublethal effect, vine mealybug, and vineyards.

## 2 Pesticides: field performance, limitations, and side effects on beneficial arthropods

This section highlights the target effects of the insecticides currently authorized and thus used for controlling *P. ficus* in vineyards and *P. citri* in citrus orchards (and, to a lesser extent, in vineyards) worldwide. We also focus on the non-target effects of these chemicals on beneficial arthropods (predatory insects and mites, parasitoids, and pollinators) occurring in both agroecosystems. The synthetic pesticides that have been occasionally or rarely used or experimented were not included due to the absence of relevant scientific literature. The most common pesticides used against *P. ficus* and/or *P. citri* and their main physiological and biochemical characteristics are listed in Table 1.

### 2.1 Acetylcholinesterase inhibitors (nerve action): organophosphate insecticides

Organophosphate insecticides inhibit acetylcholinesterase, i.e., the enzyme that terminates the action of the excitatory neurotransmitter acetylcholine at nerve synapses, causing hyperexcitation in target organisms (IRAC 2016).

Acetylcholinesterase inhibitors that have traditionally been involved in both *P. ficus* and *P. citri* management above all include four organophosphate contact insecticides. These four insecticides are chlorpyrifos (also known as chlorpyrifos-ethyl), chlorpyrifos-methyl, and, to a lesser extent, methidathion and malathion. Despite their extensive use, these insecticides have shown relatively limited field effectiveness against both mealybug species compared to next-generation active substances with new modes of action.

#### 2.1.1 Efficacy against the vine mealybug

The organophosphate chlorpyrifos applied at 960 g of active ingredient per hectare and methidathion at 480 g of active ingredient per hectare against the vine mealybug in Mediterranean vineyards located in Spain, Greece, and Portugal have shown a mean efficacy level of 70% (Brück et al. 2009). Methidathion-based treatments against vine mealybug populations in northeastern vineyards in Tunisia (Cap-Bon) exhibited a limited effectiveness in reducing densities of eggs, nymphs, and adult females of this insect on grapevines (Mansour et al. 2010a). This could be explained by the cryptic behavior of the vine mealybug (residing under the trunk bark) and the waxy excretions covering its body, which could hamper the ability of this insecticide to achieve full contact with the pest. In addition, the traditional repetitive use of this insecticide against vine mealybugs in vineyards in Tunisia could have



**Table 1** Most common pesticide active substances tested against the vine and/or citrus mealybug in vineyards and/or citrus orchards worldwide

Insecticide main group/ primary site of action	Chemical subgroup or exemplifying active ingredient	Insecticide active ingredient	Target mealybug species	References
Acetylcholinesterase inhibitors/nerve action	Organophosphates	Chlorpyrifos (chlorpyrifos-ethyl) or chlorpyrifos-methyl	<i>P. ficus</i> <i>P. citri</i>	Brück et al. 2009; Mansour et al. 2011b Campos et al. (2008); Jacas et al. (2010); Karamaouna et al. (2010); Kerns et al. (2001); Küçük et al. (2014); Satar et al. (2013); Suma et al. (2009); Zappalà (2010)
		Methodathion	<i>P. ficus</i> <i>P. citri</i>	Brück et al. (2009); Mansour et al. (2010a, 2010c) Kerns et al. (2001); Michelakis and Hamid (1995); Wakgari and Giliomee (2003)
		Malathion	<i>P. ficus</i> <i>P. citri</i>	Mohamed et al. (2012) Jacas et al. (2010); Kerns et al. (2001)
Nicotinic acetylcholine receptor competitive modulators/nerve action	Neonicotinoids	Imidacloprid	<i>P. ficus</i> <i>P. citri</i>	Daane et al. (2006); Mansour et al. (2010a, 2010c) Satar et al. (2013)
Inhibitors of chitin biosynthesis, type I/growth regulation	Buprofezin	Buprofezin	<i>P. ficus</i> <i>P. citri</i>	Daane et al. (2006); Mansour et al. (2010b) Jacas et al. (2010); Karamaouna et al. (2010); Kerns et al. (2001); Satar et al. (2013); Suma et al. (2009); Zappalà (2010)
Inhibitors of acetyl CoA carboxylase/lipid synthesis, growth regulation	Tetronic and Tetramic acid derivatives	Spirotetramat	<i>P. ficus</i>	Brück et al. (2009); Haviland et al. (2010); Mansour et al. (2010a, 2011b)
			<i>P. citri</i>	Kütük et al. (2014); Planes et al. (2013); Satar et al. (2013)

The classification of pesticide modes of action is presented according to the standards of the Insecticide Resistance Action Committee (IRAC 2016)

promoted resistance in this insect. In California vineyards, which are characterized by similar climatic conditions to those of the Mediterranean basin, applying chlorpyrifos at delayed dormant or post-harvest periods is a common component of most insecticide programs against *P. ficus* (Daane et al. 2012).

### 2.1.2 Efficacy against the citrus mealybug

Chemical control using organophosphate insecticides such as chlorpyrifos-methyl can be applied in the fall in the case of high infestations by citrus mealybug in citrus orchards in Italy (Zappalà 2010). As such, chlorpyrifos has been registered for use against the citrus mealybug in Spanish and Greek citrus orchards (Jacas et al. 2010), and is the most commonly used pesticide for controlling mealybugs and armored scales in Spanish citrus orchards (Campos et al. 2008). In Turkish citrus orchards (Antalya area), a single chlorpyrifos insecticide treatment was shown not to reduce the citrus mealybug infestation rate to less than 10% (Kütük et al. 2014). However, under controlled climatic conditions ( $25 \pm 1$  °C temperature;  $60 \pm 10\%$  humidity), chlorpyrifos can cause 100% mortality of both eggs and nymphs of citrus mealybugs on sour orange plants (Satar et al. 2013). In Greece, chlorpyrifos is commonly used against citrus mealybugs occurring on lemon, grapefruit,

mandarin, orange, and pomelo (Karamaouna et al. 2010). In mandarin orchards in Greece, methodathion treatments applied in late August led to a satisfactory control of immature citrus mealybugs, as this is the most susceptible life stage to this insecticide (Michelakis and Hamid 1995).

Malathion, another organophosphate, has been registered for use against *P. citri* in citrus orchards in Spain and Italy (Jacas et al. 2010). Nevertheless, this insecticide has been excluded from annex one of the Directive 91/414 EEC, which lists the active substances authorized for pest control in the European Union (Urbaneja et al. 2009). Accordingly, malathion is no longer used in Mediterranean citrus orchards belonging to the European Union, but it is still applied in the southern Mediterranean, the Middle East, and in other areas around the world, above all to control the key fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae).

### 2.1.3 Side effects on natural enemies

Organophosphate-based treatments may not be safe for beneficial arthropods occurring in vineyards and citrus orchards (Table 2). In southern Italian (Sicily) vineyards, treatments with chlorpyrifos-methyl significantly affected

**Table 2** Overview of the scientific literature showing the adverse side effects of pesticides used to control the vine and citrus mealybugs on non-target natural enemies present in vineyards and/or citrus orchards worldwide

Pesticide active substances	Non-target natural enemies	References
Chlorpyrifos	<i>Coccidoxenoides peregrinus</i> (pa)	Walton and Pringle (1999)
	<i>Aphytis melinus</i> (pa)	Gonzalez-Zamora et al. (2013); Vanaclocha et al. (2013)
	<i>Ageniaspis citricola</i> (pa)	De Morais et al. (2016)
	<i>Leptomastix dactylopii</i> (pa)	Jacas Miret and Garcia-Marí (2001)
	<i>Cryptolaemus montrouzieri</i> (pr)	Jacas Miret and Garcia-Marí (2001); Kütük et al. (2014); Planes et al. (2013)
	<i>Chrysoperla carnea</i> (pr)	Kütük et al. (2014)
	<i>Typhlodromus pyri</i> (pr)	Pozzebon et al. (2015)
	<i>Amblyseius andersoni</i> (pr)	Pozzebon et al. (2015)
	<i>Kampimodromus aberrans</i> (pr)	Tirello et al. (2013)
	Chlorpyriphos-methyl	<i>Anagyrus</i> sp. near <i>pseudococci</i> (pa)
<i>L. dactylopii</i> (pa)		Suma et al. (2009)
<i>A. melinus</i> (pa)		Suma et al. (2009)
<i>Coccophagus lycimnia</i> (pa)		Suma et al. (2009); Vanaclocha et al. (2013)
Methidathion	<i>C. montrouzieri</i> (pr)	Jacas Miret and Garcia-Marí (2001); Rahmouni et al. (2015)
	<i>L. dactylopii</i> (pa)	Jacas Miret and Garcia-Marí (2001)
	<i>C. peregrinus</i> (pa)	Wakgari and Giliomee (2003)
Malathion	<i>C. montrouzieri</i> (pr)	Jacas Miret and Garcia-Marí (2001); Rahmouni et al. (2015)
	<i>L. dactylopii</i> (pa)	Jacas Miret and Garcia-Marí (2001)
Imidacloprid	<i>A. sp.</i> near <i>pseudococci</i> (pa)	Mgocheki and Addison (2015)
	<i>Coccidoxenoides perminutus</i> (pa)	Mgocheki and Addison (2015)
	<i>Rodolia cardinalis</i> (pr)	Grafton-Cardwell et al. (2008); Jacas Miret and Garcia-Marí (2001)
	<i>Neoseiulus californicus</i> (pr)	Sá Argolo et al. (2013)
	<i>A. melinus</i> (pa)	Grafton-Cardwell et al. (2008); Prabhaker et al. (2011)
	<i>Comperiella bifasciata</i> (pa)	Grafton-Cardwell et al. (2008)
	<i>Euseius tularensis</i> (pr)	Grafton-Cardwell et al. (2008)
	<i>A. citricola</i> (pa)	De Morais et al. (2016)
Buprofezin	<i>A. sp.</i> near <i>pseudococci</i> (pa)	Mansour et al. (2010b); Suma and Mazzeo (2008)
	<i>Anagyrus pseudococci</i> (pa)	Campos et al. (2008)
	<i>C. lycimnia</i> (pa)	Suma et al. (2009)
	<i>A. melinus</i> (pa)	Suma et al. (2009)
	<i>Harmonia axyridis</i> (pr)	James (2004)
	<i>Stethorus punctum picipes</i> (pr)	James (2004)
	<i>Chilochorus nigritus</i> (pr)	Magagula and Samways (2000)
	<i>C. montrouzieri</i> (pr)	Cloyd and Dickinson (2006); Jacas Miret and Garcia-Marí (2001)
	Spirotetramat	–

pa, parasitoid; pr, predator

the parasitization potential of *Anagyrus* sp. near *pseudococci* (Girault) (Mansour et al. 2010b), one of the most common parasitoids of the vine mealybug in the Mediterranean basin (Franco et al. 2009; Mansour et al. 2017a; Suma et al. 2012a, b). Twenty-four hours after

tarsal contact, chlorpyrifos-methyl was shown to be harmful (toxicity category 4 of the International Organization for Biological Control), causing 100% mortality to adult *A. sp.* near *pseudococci* (Mansour et al. 2011b). Similarly, chlorpyrifos proved to be highly toxic to the parasitoid

*Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae) in laboratory conditions (Walton and Pringle 1999). In northeastern Italian vineyards (Veneto), chlorpyrifos applied at 70 g of active ingredient  $\text{hL}^{-1}$  significantly reduced densities of the generalist predatory mites *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae), i.e., key biocontrol agents of herbivorous mites on grapevine (Pozzebon et al. 2015). Similarly, Tirello et al. (2013) found that chlorpyrifos reduced the fecundity of another phytoseid mite, *Kampimodromus aberrans* (Oudemans), the most important predator of herbivorous mites in vineyards treated with selective pesticides in northern Italy. This organophosphate has been classified as moderately harmful to this predatory mite (Tirello et al. 2013).

In laboratory trials, Suma et al. (2009) demonstrated that 24 h after treatment, chlorpyrifos-methyl caused 100% mortality of scale insect parasitoids occurring in Italian (Mediterranean) citrus orchards, namely *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), *Aphytis melinus* DeBach, and *Coccophagus lycimnia* Walker (Hymenoptera: Aphelinidae). Under semi-field conditions, chlorpyrifos-methyl was less harmful to *L. dactylopii* adults, but negatively affected the longevity of the surviving females (Suma et al. 2009). In Spanish citrus orchards, chlorpyrifos caused 100% mortality of adult females of *A. melinus*, a key parasitoid of the California red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) (Gonzalez-Zamora et al. 2013).

Based on acute toxicity tests in laboratory conditions, chlorpyrifos was considered harmful and persistent, whereas chlorpyrifos-methyl was slightly harmful and moderately persistent on the parasitoid *A. melinus* (Vanaclocha et al. 2013). Chlorpyrifos-based treatments in citrus orchards in Turkey caused high mortality of the predators *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Kütük et al. 2014). Additional studies demonstrated that chlorpyrifos was harmful, causing 89% of mortality in the adults of the parasitoid *Ageniaspis citricola* Longvovskaya (Hymenoptera: Encyrtidae), a principal biological control agent of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in citrus orchards worldwide (De Morais et al. 2016).

In Tunisia, both active substances, methidathion and malathion, applied in citrus orchards in Cap-Bon were highly toxic to larvae and adults of the predatory beetle *C. montrouzieri*, thus implying that the use of both insecticides would not be compatible with releases of *C. montrouzieri* for controlling *P. citri* (Rahmouni et al. 2015). Based on contact toxicity trials, both organophosphates chlorpyrifos and malathion were classified as harmful to both *C. montrouzieri* pupae and *L. dactylopii* adults,

two common natural enemies of pests occurring in Spanish citrus orchards. Besides, methidathion was categorized as moderately harmful for pupae of *C. montrouzieri* and moderately harmful to *L. dactylopii* adults (Jacas Miret and Garcia-Mari 2001). Based on its side effects on fecundity, egg hatching, and offspring survival, chlorpyrifos was classified as moderately toxic to adults of the predator *C. montrouzieri* (Planes et al. 2013). Moreover, laboratory bioassays demonstrated that the contact insecticide methidathion was highly toxic, causing 98–100% mortality to the citrus mealybug parasitoid *C. peregrinus* within less than 6 h of treatment (Wakgari and Giliomee 2003).

#### 2.1.4 Side effects on pollinators

Organophosphates are commonly used in vineyards and citrus orchards and can be categorized as bee-toxic pesticides. Their application is therefore restricted in order to preserve bee safety. On flowers, honeybees use the proteins contained in pollen for their development and growth, and require the sugar contained in nectar to cover their energetic expenses (Rortais et al. 2005). The active substance, chlorpyrifos, can be recovered in wax and pollen matrices at concentrations of up to  $890 \mu\text{g kg}^{-1}$  (medians  $4.3$  and  $4.4 \mu\text{g kg}^{-1}$ , respectively) (Mullin et al. 2010) and in honey ( $2.2 \mu\text{g kg}^{-1}$ ) (Rodríguez López et al. 2014). Chlorpyrifos has been shown to acutely affect bees, and mostly the honeybees *Apis mellifera* L. (Hymenoptera: Apidae), but also other pollinators, and plays an important role in bee decline (Tirado et al. 2013). In vitro experiments have shown that chlorpyrifos via larval food causes a high larval mortality in the neotropical social bee (*Plebeia droryana* (Friese)), and the surviving larvae develop into workers although destined to become queens (Dos Santos et al. 2016). Following contact and oral exposure experiments, chlorpyrifos proved to be highly toxic to honeybee, while the bumblebee *Bombus terrestris* (L.) (Hymenoptera: Apidae) was 10 times more sensitive than *A. mellifera* to the active substance, chlorpyrifos-methyl (Mommaerts and Smaghe 2011).

Besides its acute lethal effects, chlorpyrifos severely affected the formation and retrieval of appetitive memory in the honeybee, which could have an impact on the foraging behavior and honey production (Urlacher et al. 2016). Eighty percent of dead honeybee samples collected periodically from four different locations during the citrus and stone fruit tree blooming season in the Valencian Community (Spain) had chlorpyrifos (Calatayud-Vernich et al. 2016). Moreover, chlorpyrifos can contaminate honey. In fact, 29% of the sampled organic citrus-monofloral honey produced in an intensive citrus growing area in Calabria (southern Italy) is contaminated (Chiesa et al. 2016).

## 2.2 Nicotinic acetylcholine receptor competitive modulators (nerve action): the neonicotinoid imidacloprid

Neonicotinoid insecticides interact with the acetylcholine (the major excitatory neurotransmitter in the insect central nervous system) binding site of nicotinic acetylcholine receptors, causing a range of symptoms from hyperexcitation to lethargy and paralysis (IRAC 2016). Neonicotinoids are the most important chemical family of insecticides, which have been introduced to the global market in the last few decades (Jeschke et al. 2011). They exhibit long-lasting residual effects, especially in seed treatment and soil application (Elbert et al. 2008).

Imidacloprid is the most commonly used neonicotinoid insecticide against both vine and citrus mealybugs. This systemic active substance was the first neonicotinoid insecticide introduced to the pesticide market in 1991 (Nauen and Denholm 2005) and is regarded as the most successful molecule from this chemical family and the largest-selling insecticide in the world (Elbert et al. 2008). As a systemic active substance, imidacloprid is absorbed by the roots or leaves and then it is transported via the xylem and phloem through the whole plant, where it may persist for weeks or months depending on the application rate and abiotic conditions (Dively et al. 2015).

### 2.2.1 Efficacy against vine and citrus mealybugs

The performance of imidacloprid in controlling mealybugs in vineyards mainly depends on its application mode: either as foliar sprays, through a furrow irrigated system, or in drip irrigation. Imidacloprid applied through a furrow irrigated system did not exhibit a promising control of vine mealybugs on grapevines in northeastern Tunisia, compared to results obtained with both the lipid biosynthesis inhibitor insecticide spirotetramat and a novel biopesticide containing sweet orange essential oil, borax, and organic surfactants (Mansour et al. 2010a). Indeed, furrow irrigated vines have a wider root zone, which makes delivery of imidacloprid to the entire root zone difficult and results in a more diluted application and poorer uptake of the applied product (Daane et al. 2006). In contrast, when this neonicotinoid was applied through a drip-irrigation system, it significantly reduced vine mealybug densities in grape-growing areas in northeastern Tunisia. In this region, imidacloprid provided a long-lasting control of this pest with an outstanding efficiency, reaching 100% mortality on all mealybug life stages, 40 days after treatment (Mansour et al. 2010c).

Imidacloprid has been commonly used near bloom time to control the vine mealybug in North American (California and Mexico) vineyards (Daane et al. 2012). Similarly to Mediterranean (Tunisian) vineyards, in California vineyards, imidacloprid provided the greatest reduction in cluster damage

caused by *P. ficus* when applied in April or May through a drip-irrigation system, and was less effective when delivered through the furrow irrigation system (Daane et al. 2006). More recently, imidacloprid applied through drip irrigation in combination with pheromone-based mating disruption significantly decreased male, adult female, and nymph numbers of vine mealybugs in Tunisian vineyards (Mansour et al. 2017b).

Imidacloprid could also be used to control the citrus mealybug in citrus orchards. For example, sprays with imidacloprid, which is commonly used by farmers in Turkey for controlling *P. citri*, caused 100% mortality on eggs and nymphs of this insect on sour orange (Satar et al. 2013). In Tunisia, imidacloprid is also registered for use against citrus mealybug in citrus orchards (Mansour et al. 2010c) and currently is used by farmers in chemical control programs of this pest, in alternation with other chemicals (see Section 2.4).

### 2.2.2 Side effects on natural enemies

Although imidacloprid effectively controls both vine and citrus mealybugs, its applications may adversely affect non-target beneficial arthropods, i.e., auxiliary fauna (Table 2) and pollinators. Laboratory studies have shown that both parasitic wasps *A. sp.* near *pseudococci* and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) failed to emerge from vine mealybugs contaminated with high doses of imidacloprid. The same experiments also showed that survival of *C. perminutus* was significantly affected when it was allowed to feed on imidacloprid-contaminated vine mealybugs (Mgocheki and Addison 2015).

Direct sprays of imidacloprid on citrus leaves were shown to be harmful to pupae of *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), a common predator of the cottony cushion scale *Icerya purchasi* Maskell (Hemiptera: Margarodidae) in citrus orchards in Spain (Jacas Miret and Garcia-Marí 2001). Imidacloprid applied as a drench negatively affected the demographic parameters of the predatory phytoseiid mite *Neoseiulus californicus* (McGregor) under laboratory conditions, whereas a combination of drench imidacloprid applications with field releases of another predatory phytoseiid mite, *Phytoseiulus persimilis* Athias-Henriot, proved to be highly effective (compatible) for the management of the key pests of young clementine plants in citrus nurseries in Spain (Sá Argolo et al. 2013).

Foliar applications of imidacloprid in citrus orchards in California caused short-term suppression of the parasitoids *A. melinus* and *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), the predator *R. cardinalis*, and the predacious phytoseiid mite *Euseius tularensis* (Congdon), disrupting biological control and allowing scales and mites to maintain higher populations in the treated areas (Grafton-Cardwell et al. 2008). The same authors suggested the use of insecticides with a greater compatibility with biological control than



imidacloprid for ensuring a sustainable integrated pest management approach in California citrus. Similarly, negative effects on the adult survival of the parasitoid *A. melinus* were observed after exposure to imidacloprid systemically treated citrus leaves under laboratory conditions (Prabhaker et al. 2011). De Morais et al. (2016) also found imidacloprid to be harmful (causing 90% mortality) for adults of the citrus leafminer parasitoid *A. citricola* on contaminated leaf discs of Valencia sweet orange under laboratory conditions.

### 2.2.3 Side effects on pollinators

In addition to its negative side effects on auxiliary fauna in vineyards and citrus orchards, imidacloprid is a clear threat to *A. mellifera*, which provides vital ecosystem services as a pollinator and produces honey (Blacqui re et al. 2012; Desneux et al. 2007; Goulson et al. 2015; Rortais et al. 2005). Phloemic and xylemic transport of imidacloprid inside plants results in translocation toward pollen and nectar (Van der Sluijs et al. 2013), which can be consumed by honeybees. Imidacloprid is highly toxic to honeybees, with acute oral LD<sub>50</sub> for *A. mellifera* equal to 0.004–0.005 µg per bee and acute contact LD<sub>50</sub> equal to 0.02–0.08 µg per bee (EFSA 2012). It also exhibits a high chronic oral toxicity at environmental contamination levels observed in citrus nectar and pollen (Byrne et al. 2014; Suchail et al. 2001), which could explain the impairment of overwintering, before colony collapse disorder (defined as a rapid loss of adult worker honeybees), in colonies exposed to imidacloprid (Lu et al. 2014). As such, contact or oral exposure to this neonicotinoid led to acute worker bumblebee mortality (Mommaerts et al. 2010).

At sublethal levels, imidacloprid can cause a decrease in both the learning performance in an age-dependent mode, although the exposure occurred at honeybee larval stages (Decourtye et al. 2003; Desneux et al. 2007; Guez et al. 2001; Tan et al. 2015; Wright et al. 2015; Yang et al. 2012; Zhang and Nieh 2015), an impairment in odor coding (Andrione et al. 2016), an alteration in the viability of sperm stored in the honeybee queens' spermatheca (Chaimanee et al. 2016), a decrease in queen fecundity (Wu-Smart and Spivak 2016), and alterations in foraging activity (behavior) or flight capacity and motor functions in honeybees (Belzunces et al. 2012; Blanken et al. 2015; Desneux et al. 2007; Eiri and Nieh 2012; Fischer et al. 2014; Karahan et al. 2015; Schneider et al. 2012; Tan et al. 2014; Williamson et al. 2014; Yang et al. 2008). Imidacloprid also caused alterations in the foraging activity or the flight capacity and motor functions in bumblebee workers when collecting pollen (Feltham et al. 2014; Gill and Raine 2014; Switzer and Combes 2016).

The exposure of colonies of the bumblebee *B. terrestris* to field-realistic doses of imidacloprid also significantly reduced the growth rate and led to an 85% reduction in the production of new bumblebee queens (Whitehorn et al. 2012).

Imidacloprid damages the development of the stingless bee and honeybee brain (Palmer et al. 2013; Tom e et al. 2012; Wu et al. 2015), and especially in regions responsible for both olfaction and vision in adult honeybees exposed during the larval stage (Peng and Yang 2016). Imidacloprid also affects physiological processes linked with feeding activities of the honeybee, such as the production of midgut proteolytic enzymes and the development of the hypopharyngeal glands (Han et al. 2012). Furthermore, imidacloprid affects the health of the honeybee colony, cleaning behavior (Dively et al. 2015; Naranjo et al. 2015), and individual immunocompetence, thus reducing hemocyte density, encapsulation response, and antimicrobial activity also at field realistic concentrations in the immune system of worker honeybees, possibly leading to an impaired disease resistance capacity (Brandt et al. 2016; Pettis et al. 2012; Sanchez-Bayo et al. 2016).

Overall, at field realistic doses, neonicotinoids including imidacloprid cause a wide range of sublethal effects in honeybee and bumblebee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, and susceptibility to diseases (Van der Sluijs et al. 2013). All of these effects should be considered of high concern because bees have a tendency to prefer food containing neonicotinoids, which could increase the exposure to imidacloprid and, in turn, the effects induced (Kessler et al. 2015). Thus, for all of these reasons, it is highly recommended that the use of imidacloprid for mealybug pest control in the vicinity of blooming (either in vineyards or in citrus orchards) should be limited, considering that honeybees and bumblebees frequently visit host flowers. Using pollinator-friendly pesticides or other environmentally friendly control alternatives would thus be a suitable option for ensuring sustainable pollination services from bees.

## 2.3 Inhibitors of chitin biosynthesis, type 1 (growth regulation): buprofezin

Type 1 growth regulator insecticides have an incompletely defined mode of action leading to inhibition of chitin biosynthesis in various insect species (IRAC 2016), including scale insects, plant hoppers, and whiteflies (De Cock and Degheele 1998). In this group of pesticides, buprofezin is the primary active substance used against both *P. ficus* and *P. citri*. Buprofezin is a thiadiazine-like compound that has both contact and fumigant activities and affects the nymph stages of the target pest without altering the adult stage (Ghanim and Ishaaya 2011).

### 2.3.1 Efficacy against vine and citrus mealybugs

Buprofezin applied at 1 L ha<sup>-1</sup> is one of the most commonly used insecticides for controlling the vine mealybug in

vineyards in Italy (Mansour et al. 2010b) similarly to North American (California and Mexico) vineyards, where a foliar application in late spring or early summer is incorporated in most insecticide programs (Daane et al. 2012). In California vineyards, buprofezin-based treatments have been shown to be the most effective in reducing cluster damage caused by *P. ficus* infestations (Daane et al. 2006). Daane et al. (2006) suggested that because buprofezin is most effective on smaller vine mealybugs undergoing insect molts, it would thus have a greater impact when applied earlier in the season, before mealybug populations have overlapping generations.

In the case of high infestations by *P. citri* in citrus orchards in Italy, insect growth regulators such as buprofezin should be applied in the summer–fall (Zappalà 2010). This insecticide has also been registered for use against *P. citri* on citrus in Spain (Jacas et al. 2010). Buprofezin is also one of the active substances authorized for use as an insecticide against *P. citri* on citron, lemon, grapefruit, mandarin, and orange orchards in Greece (Karamaouna et al. 2010). Within 3 days of its application, buprofezin, which is commonly used against citrus mealybugs in citrus orchards in Turkey, caused about 85% mortality of mealybug eggs and 100% mortality of nymphs exposed on sour orange plants (Satar et al. 2013).

### 2.3.2 Side effects on natural enemies and pollinators

Buprofezin applications may induce adverse side effects on non-target beneficial arthropods in vineyards and citrus orchards (Table 2). Buprofezin-based insecticide treatments were found to significantly affect the parasitization performance of the encyrtid parasitoid *A. sp. near pseudococci* on host mealybugs in vineyards in southern Italy (Mansour et al. 2010b). Side effects of buprofezin on immature stages of the generalist coccinellid *Harmonia axyridis* (Pallas) and the mite-feeding coccinellid *Stethorus punctum picipes* Casey, two predatory insects present in vineyards in the state of Washington, were examined in laboratory bioassays. These assays showed that no early instar (larvae) and very few late instar larvae of *H. axyridis* completed development after exposure to buprofezin, and that ecdysis was prevented in about 70% of early instars of the coccinellid *S. punctum picipes* (James 2004). Laboratory experiments on oral exposure demonstrated that buprofezin significantly affected the longevity of the female parasitoid *A. sp. near pseudococci*, whereas contact toxicity tests did not show any negative effect of buprofezin on females of this common parasitoid of *P. citri* in citrus orchards in Sicily (Suma and Mazzeo 2008). Further laboratory studies revealed that buprofezin was harmful to the parasitoid *C. lycimnia* and slightly harmful to both parasitoids *L. dactylopii* and *A. melinus* (Suma et al. 2009). The same study showed that buprofezin did not affect the progeny production of *L. dactylopii* females, whereas it significantly reduced the progeny production of *A. melinus* females.

Similar laboratory studies testing the effect of exposure to pesticide residues on leaves of sprayed citrus showed that buprofezin did not alter the progeny production of the parasitoid *L. dactylopii*, but adversely affected *A. pseudococci* progeny (Campos et al. 2008). In addition, laboratory studies showed that direct sprays of buprofezin were moderately harmful to pupae of the predator *C. montrouzieri* (Jacas Miret and Garcia-Mari 2001). Laboratory studies performed by Cloyd and Dickinson (2006) provided evidence that buprofezin, sprayed at either the manufacturer's recommended rate or 4× that rate, was not toxic to exposed *L. dactylopii* adults; however, it showed minimal (10–20% mortality after 48 h) harmful effects on *C. montrouzieri*.

Other laboratory and field experiments demonstrated that buprofezin had a detrimental effect on immature stages of *Chilocorus nigritus* (Fabricius), one of the major coccinellid predators of the red scale *A. aurantii* in South African citrus orchards. In fact, all coccinellid first-instar larvae that had ingested buprofezin-treated scales (*A. aurantii*) died during their first molt, whereas the adult survival of this predator was not adversely affected by this insecticide (Magagula and Samways 2000).

Regarding the side effects of buprofezin on pollinators, it has been shown that this insecticide was less toxic by direct spraying on bees or by residual contact with a glass support (surface) than with treated citrus leaves (Machado Baptista et al. 2009). Health Canada/Santé Canada (2016) considered the risk induced by oral and contact exposure to buprofezin as negligible. However, concerning the effects on brood, it was concluded that the data were insufficient, especially data on brood exposure and brood toxicity (Health Canada/Santé Canada 2016).

### 2.4 Inhibitors of acetyl-coA carboxylase (lipid synthesis, growth regulation): spirotetramat

Insecticides belonging to the acetyl-coA carboxylase inhibitor group inhibit the first step of lipid biosynthesis, leading to insect death (IRAC 2016). Among the active substances belonging to this group, spirotetramat, a double systemic insecticide capable of moving up and down through the xylem and the phloem of the pest's host plant, has been proven significantly effective as an inhibitor of insect lipid biosynthesis and is now the most recommended insecticide for the vine or citrus mealybug control in vineyards and citrus orchards. After foliar application, spirotetramat penetrates through the leaf cuticle and is translocated as spirotetramat-enol via the xylem and phloem, up to the growing shoots and down to roots, and is metabolized in its active form a few days after the treatment (Brück et al. 2009). Spirotetramat exhibits a promising systemic and translaminar efficacy against target sucking pests and shows very good crop safety, excellent photostability, and is active over a broad temperature range (Nauen et al. 2008).

This active substance with its two-way systemicity is especially active against juvenile stages of sucking pests including mealybugs, soft and armored scales, aphids, psyllids, and whiteflies (Nauen et al. 2008) in vegetables, cotton, soybean, pome and stone fruit, grapes, hop, citrus, nut trees, and banana (Brück et al. 2009).

#### 2.4.1 Efficacy against vine and citrus mealybugs

Field experiments for controlling the vine mealybug in Mediterranean countries including Spain, Greece, and Portugal have demonstrated that a single application of spirotetramat at a rate of 72–88 g of active ingredient per hectare resulted in a mean efficacy exceeding 90%, which was sufficient to provide a very promising long-lasting protection of grapevine until harvest (Brück et al. 2009). Mansour et al. (2010a) demonstrated that 3 weeks after applying spirotetramat, vine mealybug eggs and adult females were absent from grapevines in northeastern Tunisia. This insecticide exhibited a long-residual activity against vine mealybug populations since it prevented further spread of first and second instar nymphs on grapevine leaves (> 90% efficacy) for up to 40 days after treatment (Mansour et al. 2010a).

In California vineyards, spirotetramat applications from late spring to early summer or in post-harvest are part of insecticide programs against the vine mealybug (Daane et al. 2012). In foliar applications in April, May, or June in table grape vineyards in California, spirotetramat was highly effective against the vine mealybug at all three timings. However, earlier applications in April were the most effective with reductions in damage ranging from about 50% to over 97%, with spirotetramat being the most effective post-harvest insecticide against vine mealybugs (Haviland et al. 2010). Spirotetramat may also be used to control citrus mealybug in citrus orchards. Seven days after its application, spirotetramat, which is registered in Turkey against *P. citri*, caused 100% mortality of nymphs of this pest on sour orange (Satar et al. 2013). In Tunisia, spirotetramat has successfully controlled *P. citri* in citrus orchards (Mansour et al. 2017a).

#### 2.4.2 Side effects on natural enemies and pollinators

Unlike all the pesticides previously described, i.e., organophosphates, imidacloprid, and buprofezin, key research studies performed have provided evidence that spirotetramat does not exhibit adverse side effects on non-target auxiliary fauna occurring in either vineyards or citrus orchards. Brück et al. (2009) classified spirotetramat as harmless to slightly harmful to the predatory mites *T. pyri* and *K. aberrans* in Italian vineyards. Toxicity trials in laboratory conditions demonstrated that spirotetramat treatments did not cause the mortality of the adult parasitoid *A. sp. near pseudococci* and did not adversely impact the development of its pupal stage inside vine

mealybug mummies or the survival of the emerged parasitoids (Mansour et al. 2011b). Additional studies have shown that spirotetramat did not affect the predator *C. montrouzieri*, which suggests that this insecticide may be compatible with augmentative releases of the coccinellid to control *P. citri* in Mediterranean citrus orchards (Planes et al. 2013) and also to control its other preferred host, the vine mealybug, occurring in Mediterranean vineyards (Mansour et al. 2012a). The absence of acute toxicity to *A. melinus* has been shown in citrus orchards in Spain (Vanaclocha et al. 2013); however, the assessment of the sublethal effects on surviving specimens is still warranted. Similarly, in citrus orchards in Turkey, this insecticide proved to be harmless to the predatory fauna of the citrus mealybug including the lady beetle *C. montrouzieri* and the green lacewing *C. carnea* (Kütük et al. 2014).

Interestingly, ecotoxicological studies have shown that spirotetramat is not acutely toxic to honeybees: the oral LD<sub>50</sub> is 107.3 µg of active ingredient per bee and 91.7 µg of active ingredient per bee for the OD 150 formulation, while the contact LD<sub>50</sub> is above 100.0 µg of active ingredient per bee and 162.0 µg of active ingredient per bee for the OD 150 formulation (Maus 2008). However, although spirotetramat was classified as non-toxic to honeybees on the basis of its acute oral and contact toxicity, brood feeding and tunnel studies revealed the potential of spirotetramat to elicit effects on brood at application rates lower than the maximum proposed rates. Among the detected effects, increased mortality in adults and pupae, massive perturbation of brood development, early brood termination, and decreased larval abundance appeared to be particularly significant (US-EPA 2008). These results have been supported by recent laboratory studies showing evidence that spirotetramat, applied at a field realistic dose, significantly shortened post-emergence longevity in larvae of the solitary bee, *Osmia cornuta* (Latreille) (Hymenoptera: Megachilidae). However, spirotetramat did not exhibit any adverse effects on survival, larval and spinning duration, emergence time, or food/body conversion rate in *O. cornuta* (Sgolastra et al. 2015). Further more accurate, long-term, and wider non-target effect surveys are still needed before drawing a final conclusion on the ecotoxicological profile of this systemic substance.

### 3 Semiochemicals exploited for vine and citrus mealybug pest management

Semiochemicals are defined as substances or mixtures of substances emitted by plants, animals, and other organisms that evoke a behavioral or physiological response in individuals of the same or other species (EU 2016). Insect sex pheromones, which are species-specific substances often emitted by virgin females to attract males for mating, are used in lures for pest monitoring by attracting males to traps and can also be

exploited as potential control tools including pest mass trapping and/or mating disruption. Semiochemicals are called kairomones when the response of the receiver (another species) is beneficial to the receiver but not the emitter. In this context, the pest's sex pheromones can be used as kairomones to attract parasitoids, thereby to enhance the host searching activity of the latter.

Natural sex pheromones of both mealybug species are monoterpenoid esters and have been isolated from virgin mealybug females, identified and then synthesized for application in open field conditions. The first isolation, identification, and synthesis of the citrus mealybug sex pheromone ((+)-(1R)-*cis*-2,2-dimethyl-3-isopropenylcyclobutanemethanol acetate) was achieved by Bierl-Leonhardt et al. (1981). Besides, the vine mealybug sex pheromone ((S)-(+)-lavandulyl senecioate) was first isolated, identified, and synthesized by Hinkens et al. (2001). Since then, both commercially available mealybug sex pheromones have been used for monitoring *P. ficus* and *P. citri* or for pest control purposes.

### 3.1 Monitoring and mass trapping

The monitoring of *P. ficus* or *P. citri* males using sex pheromone-baited traps is a crucial step for the early detection of both species in a cultivated environment, for a better understanding of season-long population dynamics in the field, and also for deciding the most suitable period to trigger insecticide treatments against the target mealybugs. In vineyards in southern France, the monitoring of vine mealybugs using sex pheromones helped confirm the presence of this insect and quantify infestation and damage on grapevine. Damage was observed in early August when the highest trap counts of vine mealybug males were reported (Maugin and Sforza 2006). In vineyards in Sardinia, both the vine and citrus mealybug sex pheromones were baited in bottle traps, which revealed the co-occurrence of both mealybug species in the same vineyard and demonstrated that the vine mealybug was more abundant than the citrus mealybug on grapevine (Ortu et al. 2006). Similarly, pheromone-based monitoring systems in vineyards in north-eastern Tunisia demonstrated that both mealybug species can coexist in the same vineyard (Mansour et al. 2009).

Franco et al. (2009) stated that monitoring systems using mealybug sex pheromones provide vital information for the timing of insecticide applications. Mansour et al. (2010a) exploited a sex pheromone-based monitoring in Tunisian vineyards in order to decide the most suitable timing of spirotetramat treatments against *P. ficus*. Based on male vine mealybug trap catches, insecticide treatments were applied 1 day after the first summer mealybug male flight peak was reported (when young instar nymphs were the most abundant life stages), and the results obtained were very promising in terms of pest control performance (Mansour et al. 2010a). In vineyards in South Africa, a

pheromone-based monitoring system was applied to monitor male vine mealybug flight activity. This showed that the pheromone lures were attractive to male vine mealybugs for 10 weeks or more, and that the numbers of male mealybugs captured in pheromone-baited traps were positively correlated to densities of mealybug life stages on grapevines (Walton et al. 2004). Overall, pheromone traps provide a sensitive and selective tool for monitoring the vine mealybug (needed for its early detection) and also provide a quantitative measurement of vine mealybug density and potential economic damage (Millar et al. 2002).

In addition to vineyards, pheromone-based monitoring systems of citrus mealybug populations have also been applied within citrus orchards in Italy, Portugal, Israel (Franco et al. 2001), Spain (Martinez-Ferrer et al. 2003, 2008), Greece (Karamaouna et al. 2010), and Tunisia (Mansour et al. 2017a). Pheromone-based monitoring of citrus mealybug in these Mediterranean areas provided useful data on male citrus mealybug flight activity (season-long peaks) and related fruit damage. In citrus orchards in Spain for example, the use of a pheromone trapping system revealed a positive relationship between male flights and the population of citrus mealybugs (nymphs and adult females) on the fruits (Martinez-Ferrer et al. 2008). According to the same authors, pheromone-baited traps detected the abundance of populations, both in terms of the number of insects per fruit and the percentage of attacked fruits, which led to the adoption of an insecticide treatment threshold for each male flight peak for each period of the growing season. The citrus mealybug sex pheromone can also be used in mass trapping systems, which means deploying them at higher densities in the field to capture larger numbers of male mealybugs in an attempt to limit crop damage. However, this control approach has not yet been incorporated into integrated pest management programs against citrus mealybug worldwide.

Mass trapping trials of male citrus mealybugs in Portuguese, Israeli, and Italian citrus plots using one pheromone-baited sticky plate trap per tree led to a significant reduction in male citrus mealybug numbers; however, this was not enough to significantly reduce the fruit infestation (Franco et al. 2003, 2004). These authors stated that the higher level of mating observed in mass-trapping plots early in the spring, when the mealybug density is usually very low, suggested that mass trapping led to a strong attractive effect in males from outside the subplots. In general, both the trap design and size are key elements in optimizing male citrus mealybug catches. In fact, based on pheromone-mediated mass-trapping field trials in citrus orchards in Israel, Zada et al. (2004) found that plate traps caught more male citrus mealybugs than delta traps, and large traps caught more than small ones. Delta traps are easier to operate under field conditions and, therefore, are recommended for monitoring, whereas the large plate traps are best for mass trapping.



Irrespectively of their use either in the monitoring or mass trapping of citrus mealybugs, sex pheromones are species specific. Consequently, they very rarely attract non-target beneficial arthropods to sticky traps. However, no study has provided clear evidence that monitoring or mass trapping of either *P. ficus* or *P. citri* adversely impacts on population dynamics of non-target beneficial arthropod fauna in both agroecosystems.

### 3.2 Mating disruption

Mating disruption consists in releasing large quantities (compared to quantities used for monitoring) of a synthetic sex pheromone to disrupt the mate location, thus reducing the number of the pest's offspring produced in the next generation (Suckling et al. 2014). Worldwide, mating disruption has been applied mainly against moths, which cause major crop losses and economic damage. In the case of the vine mealybug, mating disruption seems to be more successful at low insect population densities (Sharon et al. 2016; Suckling et al. 2014). However, at high mealybug population levels, mating disruption can be effective if applied for consecutive years (Sharon et al. 2016). Consequently, mating disruption needs to be deployed over several seasons to reach acceptable population levels.

Pheromone-mediated mating disruption has been tested against *P. ficus* in vineyards in both Italy and Tunisia. Cocco et al. (2014a) pointed out that this technique is the most suitable control strategy against vine mealybug infestations in organic viticulture, especially when integrated with pruning, nitrogen fertilization, and irrigation. In fact, mating disruption using reservoir pheromone dispensers significantly influenced vine mealybug male flight activity and reduced population densities on grapevine in Sardinia, Italy (Cocco et al. 2014a). Similarly, in vineyards in central-southern Tunisia, mating disruption, combined with imidacloprid-based treatments through drip irrigation, significantly disrupted male–female vine mealybug sexual communication. This disruption significantly reduced vine mealybug population densities on grapevines, with a cumulative effectiveness of mating disruption over time reaching 120 days (Mansour et al. 2017b). In addition to decreasing vine mealybug densities on grapevine, a potential consequence of applying mating disruption against this pest is the significant reduction in crop (cluster) damage, as demonstrated in California vineyards (Daane et al. 2006; Walton et al. 2006).

Millar et al. (2002) suggested that the vine mealybug system presents a number of features that may make it particularly amenable to mating disruption: (1) male flights are synchronized and occur over periods of only a few weeks, limiting the amount of time that pheromones need to be applied per generation; (2) adult males do not feed, live a few days at most, and rely on pheromones for mate location. Therefore, males

need to be disrupted for only short periods before they exhaust their energetic reserves and die, and (3) the replacement of insecticides with mating disruption would eliminate problems caused by insecticide-related outbreaks of secondary pests and would eliminate restrictions for workers being able to return to the work environment, environmental contamination, and other problems linked to pesticide application.

Unlike broad-spectrum insecticides, mating disruption does not negatively influence the movements of beneficial arthropods in vineyards or in their vicinity. In Sardinian vineyards (Italy), it has been shown that mating disruption did not negatively impact the parasitism rate of vine mealybug by its most common parasitoid, *A. sp. near pseudococci* (Cocco et al. 2014a). Similar results were obtained in California vineyards (Millar et al. 2002; Walton et al. 2006), which means that mating disruption is a perfectly safe pest control tactic for the most common non-target parasitoid, *A. sp. near pseudococci*.

### 3.3 Kairomone-based control systems

In addition to monitoring, mass trapping, and mating disruption, the vine mealybug sex pheromone has been exploited as a kairomonal cue to attract parasitoids to their host mealybugs in Mediterranean vineyards and citrus orchards. In the case of the vine mealybug, kairomonal attraction was first observed by Millar et al. (2002) in California vineyards where *A. sp. near pseudococci* was attracted to the sex pheromone of *P. ficus*. Similarly, Franco et al. (2008) demonstrated, in both field (Mediterranean vineyards and citrus orchards) and olfactometer experiments, that the females of *A. sp. near pseudococci* use the vine mealybug sex pheromone as a kairomonal cue in both *P. citri* and *P. ficus* host selection. They suggested that this was an innate behavior trait of this parasitoid, possibly due to evolutionary relationships with its host *P. ficus*. Additional experiments in Mediterranean (Portugal, Italy, and Israel) citrus orchards provided evidence that parasitism rates of citrus mealybug individuals by *A. sp. near pseudococci* were significantly increased by the vine mealybug sex pheromone acting as a kairomone (Franco et al. 2011). Further trials in vineyards in Sicily (southern Italy) showed that the application of the vine mealybug sex pheromone significantly increased parasitism rates of citrus mealybugs by *A. sp. near pseudococci*, implying that this sex pheromone is used by the parasitoid as a kairomone to ensure greater potential for host searching activity (Mansour et al. 2010b).

Franco et al. (2009) reported that because of the typical clumped spatial pattern of mealybugs, the sex pheromone is a convenient chemical cue by which the parasitoid can efficiently locate colonies of hosts, which are expected to emit a stronger pheromonal signal than single mealybug virgin females. Based on all of these aforementioned findings, we can

conclude that using the vine mealybug sex pheromone as a kairomonal cue could be a promising approach for improving biological control programs of *P. ficus* and/or *P. citri*, using their most common parasitoid *A. sp. near pseudococci*, and reducing pesticide inputs in Mediterranean vineyards and citrus orchards.

#### 4 Alternative tools for sustainable control

Although the use of synthetic chemicals (i.e., pesticides and pheromone lures) can be a crucial component of any sustainable control package against *P. ficus* and/or *P. citri*, more innovative biological and cultural, eco-friendly control tools should also be incorporated/combined as sustainable components of management programs of both mealybug species in vineyards and citrus orchards. Such non-chemical control tools include several aspects:

- (a) *Cultural practices*: the choice of appropriate grapevine cultivars could be useful in decreasing vine mealybug densities. Early-harvested cultivars often have lower infestation levels than late-harvested cultivars because the clusters are exposed for a shorter period (Daane et al. 2005). Hot-water immersion (during 5 min at 51°C) of dormant grape cuttings used for nursery stock can efficiently kill 99% of vine mealybug life stages (Haviland et al. 2005). In addition, adapted (balanced) nitrogen fertilization inputs can help reduce the vine mealybug population densities and prevent pest outbreaks (Cocco et al. 2014b). Besides, micropropagated citrus varieties, including Assam lemon (*Citrus limon*), Satkara (*C. macroptera*), and Pumelo (*C. grandis*), have proven to be highly resistant to *P. citri* attacks and are used as rootstocks in propagation programs (Rao et al. 2006).
- (b) *Bioinsecticides (botanical/microbial)*: a biopesticide containing sweet orange essential oil, borax, and organic surfactants has proven successful in controlling vine mealybug young instar nymphs when applied at the registered dose of 300 ml hL<sup>-1</sup> (Mansour et al. 2010a) and has not exhibited any negative side effects on the encyrtid parasitoid *A. sp. near pseudococci* (Mansour et al. 2011b). Microbial biopesticides composed of entomopathogenic fungi (*Lecanicillium longisporum* (Petch) or *Lecanicillium lecanii* (Zimmere)) are potentially useful biological control agents for citrus mealybug (Ghaffari et al. 2017).
- (c) *Optimized insecticide application timing*: to improve scouting and spray timing, grape growers could consider the fact that vine mealybug populations are more exposed on the grapevine at certain periods of the year (Wilson and Daane 2017).

- (d) *Natural enemies*: augmentative release of the parasitoid *A. pseudococci* can significantly decrease vine mealybug densities and reduce grapevine damages (Daane et al. 2006). Similarly, field releases of the parasitoid *L. dactylopii* could result in a significant reduction of *P. citri* densities in citrus orchards (Krishnamoorthy and Singh 1987; Smith et al. 1988; Zappalà 2010). In addition, augmentative releases of the predator *C. montrouzieri* have reduced *P. citri* populations in Mediterranean, Australian, and California citrus orchards (Franco et al. 2009).

#### 5 Conclusions

The exploitation of synthetic chemicals as pesticides and/or as semiochemical-mediated options (i.e., used as pheromones or kairomones) has consistently contributed to the successful management of *P. ficus* and *P. citri*, two economically important sap-sucking insect pests occurring in vineyards and/or citrus orchards. Most of the IPM programs applied against populations of both mealybug species have historically relied heavily on insecticide applications.

A number of insecticides for controlling both mealybug species are available on the market. Nevertheless, only very few insecticide active substances have shown promising results in decreasing outbreaks of mealybug populations and in limiting related crop damage and economic losses yet with no observed adverse side effects on beneficial arthropods.

To date, based on the current scientific literature, a modern insecticide, spirotetramat, has proven to be efficient in terms of control and safety in relation to beneficial arthropods including insect pollinators (bees) and the main natural enemies of the vine and citrus mealybugs. In contrast to spirotetramat, organophosphate insecticides, i.e., chlorpyrifos (chlorpyrifos-ethyl), chlorpyrifos-methyl, methidathion, and malathion, have been shown to be disruptive to key non-target auxiliary fauna in vineyards and citrus orchards and also to be highly toxic to pollinators such as honeybees and bumblebees. Furthermore, the neonicotinoid insecticide, imidacloprid, is harmful to non-target ecosystem service-providing insects, i.e., pollinator honeybees and bumblebees. Consequently, the use of imidacloprid should be avoided or minimized in vineyards and/or citrus orchards.

Based on these findings, incorporating spirotetramat into *P. ficus* and/or *P. citri* management programs could represent a valid option if used with caution and if integrated with other eco-friendly, sustainable control tools. A comprehensive understanding of the selectivity of the active substances of other pesticides to non-target beneficial arthropods is of utmost importance and is required for minimizing pesticide-induced disruption of vine and citrus mealybug natural enemies and

pollinators, and for strengthening pest management decision-making. Respecting and following pesticide label directions, which obviously mention restrictions aimed at protecting bees and spraying the infested host plants in periods when bees are not foraging, is a crucial step in minimizing the most hazardous pesticides for pollinators.

Frequent insecticide resistance and the detrimental side effects of pesticides on beneficial arthropods, which compromise the aims of biological control and ecosystems services, has led to the need for more environmentally friendly pest control tactics that also explore synthetic chemicals, but in a different way from how this is achieved with pesticides. Exploiting synthetic chemicals as bio-rational, behavior-modifying semiochemical-mediated tools, i.e., monitoring, mass trapping, mating disruption, and kairomonal attraction of parasitoids, has been suggested as an essential, sustainable alternative in integrated pest management programs against vine and citrus mealybugs.

Unlike broad-spectrum insecticides, semiochemicals do not adversely impact on survival, activities, and functionality of non-target beneficial arthropods. Monitoring vine and citrus mealybug populations using sex pheromone-baited traps is a powerful approach for the early detection of the right mealybug species in the field (avoiding misidentification), for studying season-long population dynamics (peaks and number of generations per year), and also for deciding the most suitable timing of insecticide application.

Mating disruption, a behavior-modifying, density-dependent control tactic, significantly disrupts male vine mealybug sexual behavior, inhibiting mating and thus decreasing outbreaks of mealybug populations on grapevines. Nevertheless, a vine mealybug pest management system based solely on mating disruption would not constitute a fully efficient sustainable control option, especially in the case of high mealybug infestations. Thus, using this approach in combination with an effective and safer insecticide, such as spirotetramat, could be the most appropriate way to ensure long-term field efficacy, thereby optimizing pest management programs for vine mealybugs in vineyards.

Mass trapping and kairomonal attraction are, however, semiochemical-based techniques that have been used for experimental purposes. Hence, further studies focusing on evaluating their field performance should be carried out in order to incorporate them as potential alternatives in integrated pest management programs of vine and citrus mealybugs.

New synthetic chemicals should continue to be exploited for vine and citrus mealybug pest management. It is also of great importance to select those substances that are not only sufficiently effective against target mealybugs but which are also perfectly safe for non-target beneficial arthropods, which are considered essential and stabilizing components of agroecosystem dynamics and sustainability. However, mealybug pest management should not rely solely on the use of synthetic

pesticides as the main control strategy. Alternative ecologically sound pest management options based on the use of pheromone-mediated tactics, cultural practices, and/or effective biocontrol agents are recommended in order to further decrease pest populations without hampering biodiversity dynamics and key ecosystem services.

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## Compliance with ethical standards

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