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Insecticidal activity of natural products against vineyard mealybugs (Hemiptera: Pseudococcidae)

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ABSTRACT

Mealybugs are worldwide pests in vineyards due to direct damage and spread of grapevine leafroll disease. The objective of this study was to find natural products valuable as alternatives to synthetic insecticides for their control. Laboratory experiments and field trials were conducted in New Zealand in 2015–16 on *Pseudococcus calceolariae* (Maskell) and *Pseudococcus longispinus* (Targioni Tozzetti), and in Italy in 2016–17 on *Planococcus ficus* (Signoret) to assess the insecticidal activity of kaolin and citrus essential oil. Although kaolin increased *Ps. calceolariae* mortality in the laboratory, it was ineffective at controlling the three mealybug species in the vineyards. In contrast, citrus essential oil increased mortality of *Ps. calceolariae* and *Pl. ficus* in the laboratory and reduced leaf infestations of the latter in the vineyards. Citrus essential oil could be a good alternative to synthetic insecticides against vineyard mealybugs.

1. Introduction

Vineyard mealybugs (Hemiptera: Pseudococcidae) are among the most important pests in many grape-growing regions of the world and economic losses due to infestations have increased substantially over the past decade (Daane et al., 2012).

In New Zealand vineyards, *Pseudococcus longispinus* (Targioni Tozzetti), *Ps. calceolariae* (Maskell) and *Ps. viburni* (Signoret) are the most widespread mealybugs (Charles, 1993), with the first two being the key pests on grapevines (Charles et al., 2006, 2010). *Pseudococcus calceolariae* overwinters largely in the juvenile stages under the grapevine bark and on the roots of grapevines and ground cover plants, and completes two and possibly a partial third generation per year (V.A. Bell, unpublished data). *Pseudococcus longispinus* overwinters under the bark of grapevines as adult females and crawlers (first instar nymphs), and in the north of New Zealand completes three generations per year (Charles, 1981).

In European vineyards, four species of mealybugs are present: *Planococcus ficus* (Signoret), *Pl. citri* (Risso), *Heliococcus bohemicus* Sulc and *Phenacoccus aceris* (Signoret) (Sforza et al., 2003; Bertin et al., 2010; Cid et al., 2010; Mansour et al., 2017; Cocco et al., 2018). *Planococcus ficus* is the most important mealybug species in Italian vineyards (Duso, 1989) and is also a significant pest in South African and North American vineyards (Walton and Pringle, 2004; Prabhaker et al., 2012). In Italian vineyards, *Pl. ficus* overwinters under grapevine bark and on roots, mostly as fertilized females, and completes at least three generations per year (Duso, 1989; Lentini et al., 2008).

Mealybugs can adversely influence grape yield and fruit quality due to infestation of grapevine woody parts, and by fouling leaves and bunches through the excretion of honeydew upon which sooty mould subsequently develops (Charles, 1982; Cocco et al., 2014; Beltrà et al., 2017). However, in many grape-growing areas the most serious issue of mealybugs is their status as vectors of Grapevine leafroll-associated virus 3 (GLRaV-3), the predominant type species causing the grapevine leafroll disease (GLD) (Cabaleiro and Segura, 2006; Bell et al., 2009; Tsai et al., 2010). It reduces crop yield and must quality to such an extent that heavy infected vineyards have to be removed (Pietersen et al., 2013). To prevent GLRaV-3 spreading, it is necessary to identify and quickly remove virus-infected vines, and to control the insect vectors (Pietersen et al., 2013).

The control of mealybug infestations commonly relies on multiple applications of synthetic insecticides such as organophosphates, neonicotinoids and chitin-biosynthesis inhibitors (Daane et al., 2008; Cocco et al., 2014; Wallingford et al., 2015). However, insecticide applications can be of limited effectiveness because of the cryptic nature of mealybugs, which live in sheltered parts of the grapevines (i.e., under bark, on roots, in cracks and crevices on old wood, inside bunches, underside of leaves) (Lo and Walker, 2011), and resistance issues (Prabhaker et al., 2012). Therefore, effective control strategies should rely on integrated pest management (i.e., multi-tactic) programs based on

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environmentally sustainable tools that, wherever possible, limit the use of synthetic insecticides. This approach is important for sustainable wine production and vital for organic vineyards where relatively few products are available for use against mealybugs (e.g., mineral oil or insecticidal soap) (Regulation 2008/889/EC). Biological control by releasing of natural enemies (Daane et al., 2008; Marras et al., 2016; Pozzebon et al., in press) and mating disruption (Walton et al., 2006; Cocco et al., 2014; Sharon et al., 2016; Lentini et al., 2018) can be valid alternatives to chemical control. Cultural practices aimed to reduce plant vigour or change canopy-microclimate conditions can also reduce mealybug infestations (Duso et al., 1985; Cocco et al., 2015; Muscas et al., 2017).

As a substitute for chemical inputs, it is our view that there should be some effort made to identify natural products able to exert some influence in controlling numbers of mealybugs on grapevines. In this respect, plant essential oils have been found to exhibit some biological activity against mealybugs (Karamaouna et al., 2013). In particular, citrus essential oil (CEO), with limonene as the main component, was shown to be lethal against *Pl. ficus* and *Ps. longispinus* under laboratory conditions (Hollingsworth, 2005; Karamaouna et al., 2013). Similarly, kaolin, an inert white clay, is known to be harmful to insects (Glenn et al., 1999), including some grapevine pests (Puterka et al., 2003; Tacoli et al., 2017a, 2017b). Laboratory studies also suggest its effectiveness against *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae), the key insect pest in European vineyards (Pease et al., 2016). Against mealybugs, kaolin has only been tested in mango orchards where it was ineffective (Joubert et al., 2004).

The aim of this study is to evaluate in laboratory experiments and in field trials, the effects of CEO, terpenes, kaolin and insecticidal soap against mealybugs infesting grapevines in New Zealand and Italian vineyards. For products containing essential oils (i.e., CEO and terpenes), it was expected that contact toxicity (Isman, 2000), which had already been observed in the laboratory against mealybugs (Hollingsworth, 2005; Karamaouna et al., 2013), would also be detectable against this pest group under field conditions. For kaolin, it was predicted evidence of some feeding deterrence among mealybugs, analogous to that which was observed among other sap feeders (Tacoli et al., 2017a, 2017b), together with an inhibition of mealybug crawler migration from bark to leaves or from leaves to bunches (Glenn et al., 1999). For insecticidal soap, it was predicted that could be toxic to *Pl. ficus* crawlers, analogous to that which was observed for other mealybug species on potted plants (Hollingsworth, 2005).

2. Materials and methods

Natural products used in the laboratory experiments and field trials carried out against vineyard mealybugs in New Zealand and in Italy are reported in Table 1.

2.1. Insecticidal effect of kaolin and CEO on *Ps. calceolariae* and *Ps. longispinus*

2.1.1. Laboratory experiments

Two laboratory experiments on the mortality of *Ps. calceolariae* first-instar nymphs were carried out in the Southern Hemisphere early-summer in 2015 (Table 2).

For the experiments, new grapevine leaves were randomly collected from a block planted in mature Viognier vines grown in an organic vineyard where synthetic pesticides were not used (Hastings, 39°37′15″S, 176°45′36″E, 20 m a.s.l.). Mealybug nymphs of first and second instars were taken from a *Ps. calceolariae* mass rearing on seed potatoes and were allowed to naturally disperse onto the grapevine leaves so as to not damage the delicate feeding apparatus. The products were applied with a Potter spray tower to each leaf separately (Burkard Scientific Ltd, Uxbridge, UK) spraying 2 mL of solution per leaf at the pressure of 15 psi (103 kPa). Fifty grapevine leaves per treatment were individually placed together with one first instar *Ps. calceolariae* into transparent self-sealing plastic bags (20 × 35 cm). Laboratory conditions were controlled (23 ± 2 °C and a 16:8 L:D daily cycle). After 8, 20 and 44 h for the first experiment and after 8, 20, 44, 68 and 92 h for the second experiment, bags were checked to record nymph mortality and position (on leaf or on bag surfaces).

2.1.2. Field trials

During the 2015–2016 Southern Hemisphere growing season, two field trials were carried out in two vineyards (A and B) in New Zealand (Table 2). Vineyard A (Hastings Metropolitan Area, 39°36′40″S, 176°45′18″E, 28 m a.s.l., cultivar Cabernet Sauvignon) was a 9-year-old conventionally managed vineyard growing using double Guyot training system. Distances between and along rows were 2.7 m and 1.6 m, respectively. Buprofezin (Ovation 50 WDG, Etec crop solutions, Auckland, New Zealand, 0.25% solution) was applied against mealybugs in late October (i.e., a month earlier than flowering) before the trial commenced. The application of the insecticide could not be avoided due to the risks associated with vector-mediated GLRaV-3 transmission to healthy vines (Charles et al., 2006).

Vineyard B (Hastings Metropolitan Area, 39°37′16″S, 176°46′00″E, 21 m a.s.l., cultivar Gewurztraminer) was a 16-year-old organic vineyard with vines grown on double cordon training system with distances between and along rows of 2.1 m and 2.0 m, respectively. No farm application of insecticides occurred before the trial commenced.

In both vineyards, kaolin was sprayed three times (Table 3) with the first two applications timed before the adult emergence of the second generations of *Ps. calceolariae* and *Ps. longispinus*. Kaolin was sprayed using a two rows recycling sprayer (FMR, 2300R, FMR GROUP LTD, Blenheim, New Zealand) at a rate of 1000 L/ha and an alternate block design with 20 replicates was adopted. Each replicate consisted of 30 (Vineyard A) or 32 (Vineyard B) grapevines equally distributed on two rows, as a result of the use of the two-row recycling sprayer.

Adult males of *Ps. calceolariae* and *Ps. longispinus* were monitored

Table 1
Natural products tested in the laboratory and in the field in New Zealand and Italy.

Active ingredient	Commercial product	
	Name	Formulation
Kaolin	Surround WP (Tessenderlo Kerley Inc., Phoenix, Arizona, USA)	WP, 95% kaolin
Citrus essential oil (CEO)	Orange oil emulsion (Hawkins Watts, Auckland, New Zealand) ([a])	SL, 10% orange oil Brazilian IN105118 (Lionel Hitchen Ltd, Winchester, UK) containing at least 90% of D-limonene
	Prev-Am Plus (Nufarm Italia, Milano, Italy)	SL, 5.88% orange oil containing at least 90% of D-limonene
Terpenes	3logy (SIPCAM, Milano, Italy)	CS, 3.2% eugenol, 6.4% geraniol, 6.4% thymol
Insecticidal soap	Flipper (Dow Agrosciences Italia, Milano, Italy)	SL, 47.8% potassium fatty acids (unsaturated carboxylic acids C14–20 from olive oil)

[a] The product was added with 4% citric acid and 1% sodium laureth sulphate according to Hollingsworth and Hamnett (2010).