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Research Doctorate in Agricultural Science and Biotechnology

In agreement with Fondazione Edmund Mach, San Michele all'Adige

Cycle XXX

PhD Thesis

USE OF NATURAL PRODUCTS AND CULTURAL PRACTICES IN THE SUSTAINABLE MANAGEMENT OF GRAPEVINE PESTS

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Academic year 2016/2017

Index

Chapter 1 - Grapevine arthropod pests in north-eastern Italy

1. Most important pests

In north-eastern Italy, vineyards are threatened by a wide array of arthropod pests. The main carpophagous are *Lobesia botrana* (Denis and Schiffermüller) and *Eupoecilia ambiguella* (Hübner) (Lepidoptera: Tortricidae). Grapevines are also interested by sap feeders such as *Empoasca vitis* (Göethe), *Zygina rhamni* Ferrari and *Schapoideus titanus* Ball (Hemiptera: Cicadellidae). Other sap feeders belong to the Coccoidea superfamily such as *Planococcus ficus* (Signoret), *Parthenolecanium corni* (Bouché), *Parthenolecanium persicae* (Fabricius), *Neopulvinaria innumerabilis* (Rathvon), *Pulvinaria vitis* (L.) and *Heliococcus bohemicus* Sulc. Furthermore, grapevines are also damaged by mites, such as *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans) (Acari: Tetranychidae), and *Calepitrimerus vitis* Nalepa and *Colomerus vitis* (Pagenstecher) (Acari: Eriophyidae), and thrips, such as *Drepanothrips reuteri* (Uzel) (Thysanoptera: Thripidae).

The following are abstracts about biology and damage of the insect pests that will be studied in the present PhD thesis.

1.1. *Lobesia botrana*

The European grapevine moth, *Lobesia botrana* (Den. and Schiff.) (Lepidoptera: Tortricidae) is the most important carpophagous pest in European vineyards and is recently spreading to the Nearctic region (Ioriatti *et al.*, 2012). In relation to the different grape-growing areas and years, the moth can complete 2 to 4 generations per year (Martín-Vertedor *et al.*, 2010; Pavan *et al.*, 2013). The larvae from second to fourth generations are carpophagous and can cause yield losses and promote the spread of the botrytis bunch rot (*Botrytis cinerea* Pers. Fr.) (Fermaud and Giboulot, 1992; Pavan *et al.*, 1998, 2014; Moschos, 2006) as well as, after veraison, the grape sour rot associated with *Acetobacter* spp. and some yeasts (Bisiach *et al.*, 1986; Barata, 2011).

1.2. *Empoasca vitis* and *Zygina rhamni*

Empoasca vitis (Göthe) and *Zygina rhamni* Ferrari (Hemiptera: Cicadellidae) are the most common and widespread leafhoppers in European vineyards (Vidano and Arzone, 1983). *Empoasca vitis* is a polyphagous species that overwinters as adults on evergreen plants and can complete from one to four generations a year in different European grape-growing areas (Schvester *et al.*, 1962; Vidano 1963; Baggiolini *et al.*, 1968; Cerutti *et al.*, 1988). This leafhopper is a phloem feeder of grapevine leaf veins, and the leaf margins can become reddish or yellowish and then desiccate (Vidano, 1963; Carle and Moutous, 1965). Leaf symptoms are associated with physiological damage such as reductions in photosynthesis, mesophyll conductance and transpiration rate (Candolfi *et al*., 1993), which can lead to economic damage (i.e., yield losses and sugar content reduction of berries) when the infestation exceeds one-two nymphs per leaf (Moutous and Fos, 1971; Baillod *et al*., 1993; Pavan *et al*., 2000; Lehmann *et al*., 2001).

The leafhopper *Z. rhamni* is an oligophagous species that in northern Italy overwinters mostly on *Rubus* spp., on which it can complete a generation in early spring before migrating towards vineyards where it has three generations per year (Vidano, 1963; Pavan, 2001; Mazzoni *et al*., 2008). It is a mesophyll feeder leaving white dots on the upper surface of leaves where cell contents were extracted. Relatively to mesophyll-feeder grapevine leafhoppers, economic damage is reported for American species (Jubb *et al.,* 1983; Martinson *et al*., 1997). For *Z. rhamni* strong symptoms are described (Vidano, 1963) and an economic threshold of two-three nymphs per leaf was suggested for northern Italy (Girolami *et al*., 1989).

1.3. *Scaphoideus titanus*

The leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) is the vector of '*Candidatus* Phytoplasma vitis', the causal agent of flavescence dorée (FD), which is a Grapevine Yellows Disease (GYD) causing severe damage in European vineyards (Schvester *et al*., 1963; Carraro *et al*., 1994; Bianco *et al*., 2001; Mori *et al*., 2002). *Scaphoideus titanus* is a monophagous species on plants of *Vitis* sp. on which completes one generation a year after overwintering as an egg (Schvester *et al*., 1962; Vidano, 1964; Chuche and Thiéry, 2014).

1.4. *Planococcus ficus*

Planococcus ficus (Signoret) (Hemiptera: Pseudococcidae) is the most important mealybug pest for Italian vineyards (Duso, 1989) and is of great importance in other relevant grape-growing regions such as South Africa and North America (Walton and Pringle, 2004; Prabhaker *et al*., 2012). In Italian vineyards, *P. ficus* overwinters under grapevine bark and on roots, mostly as fertilized females, and completes three generations a year (Duso, 1989; Lentini *et al*., 2008).

The mealybug can affect grape yield and quality due to infestation of grapevine woody parts and fouling of leaves and bunches by honeydew excretion on which sooty mould develops (Charles, 1982; Cocco *et al*., 2014). Moreover, *P. ficus* is an important vector of grapevine leafroll-associated virus 3, the predominant type species causing the grapevine leafroll disease (GLD) (Tsai *et al*., 2010). It reduces crop yield and must quality to such an extent that heavy infected vineyards have to be removed (Lo and Walker, 2011).

2. Integrated pest management

Insects are traditionally controlled using synthetic insecticides. Their use provided benefits during human history such as increasing the yield of crops and making food available to a wider amount of people, but many risks are tied to their usage. The reported undesirable effects of pesticides on non-target species, including humans, such as acute and chronic health effects (Hayes and Vaughn, 1977), domestic-animal poisoning (Caldwell *et al*., 1977), effects on wild birds and mammals, fish, bees, beneficial organisms, and small organisms in the soil (Van Steenwyk *et al*., 1975; Brown, 1978; Bairlein, 1990; Pimentel *et al*., 1993; Johnson *et al*., 2010) led to a greater focus on Integrated Pest Management (IPM) programs in order to control pests in a safer way. This approach to pest management consists in the definition of economic thresholds, and in the use of a multi-tool strategy to keep pest populations below the economic injury level minimizing synthetic-insecticide applications with the aim to reduce risks for human health and the environment.

In the context of IPM, the substitution of synthetic insecticides with natural products and the adoption of alternatives to chemical control (i.e., biological control, cultural control and semiochemical-based control) must be favoured. In the present PhD thesis, a particular interest was directed to natural products and to cultural and biological control.

2.1. Natural products

Natural insecticides are part of IPM approach since they have reduced toxicity to non-target animals making them not dangerous for users, consumers and the environment (Sarwar and Salman, 2016). Natural insecticides can have different origin and can be categorized as botanical insecticides, microbial-origin insecticides and mineral insecticides.

2.1.1. Botanical insecticides

Botanical insecticides are obtained from plants, usually by macerating plants tissues with a high percentage of active constituent that is then distilled. The advantage of plant botanical insecticides is their low persistence in the environment. However, this low persistence is also a disadvantage since they may require multiple applications to achieve a good pest control (Isman, 2006). The most important botanical insecticide is pyrethrum, a neurotoxic product extracted from the seeds of *Chrysanthemum cinerariaefolium* L., which is effective against a very wide array of pests. In vineyards, it is used against *S. titanus* and *E. vitis*, mostly in the context of organic viticulture (e.g., Jermini *et al*., 2014; Mori *et al*., 2014).

Another important plant extract is azadirachtin, an insect repellent, antifeedant and growth regulator obtained from *Azadirachta indica* Juss, which is effective against caterpillars, flies, whiteflies, scales and aphids (Schmutterer, 1990). Other essential oils from various plants also exhibit insecticides activity (Zoubiri and Baaliouamer, 2014), but their role in pest management needs to be further studied.

Horticultural oils are usually obtained from petrol, but plant-based oils are also offered. They derive from oilseed crops (e.g., soybean) and are available as dormant and narrow-range (i.e., summer) oils. Dormant oils are effective in the control of overwintering eggs and soft bodied insects and are used in spring before plant budburst begins. Summer oils, instead, are used on foliage and can control scales, whiteflies, aphids and mites (Nielsen, 1990).

Natural soaps can derive from plants or animal fat and are used to control soft-bodied insects like aphids, scales, whiteflies, mealybugs, thrips, and spider mites because they dissolve the protective layers of the cuticle causing the pest to desiccate. In vineyards, a formulation whose the active constituent is obtained from olive oil is registered against cicadellids.

2.1.2. Microbial-origin insecticides

Other products can be obtained from the fermentation of microorganisms such as avermectins obtained from *Streptomyces avermitilis* Kim and Goodfellow, which is used in baits for household insect pests (Dybas, 1989) and spinosad obtained from *Saccharopolyspora spinosa* Mertz and Yao used in the control of caterpillars, fruit flies, thrips and beetles (Salgado *et al*., 1998). In vineyards, spinosad is used against thrips such as *D. reuteri* (e.g., Posenato *et al*., 2004) and it is a valid alternative to *Bacillus thuringiensis* Berliner in the control of *L. botrana* in organic viticulture (e.g., Vassiliou, 2011).

2.1.3. Mineral insecticides

Petroleum-derived horticultural oils (i.e., mineral oils) are the most frequently used oils against pests (see 2.1.1.). In vineyards, mineral oils are used at the highest rates against overwintering stages of some scales, such as *P. corni* (Pavan, personal communication), and at the lowest rates against nymphs of vineyard mealybugs (e.g., D'Arcangelo, 2012 for *P. ficus* control) and thrips such as *D. reuteri*.

Other organic insecticides are obtained from mineral sources mined from earth. To this category belong: elemental sulphur, iron phosphate, diatomaceous earth and kaolin. Elemental sulfur is a powder that can be applied as dust or spray depending on the formulation and acts as a metabolic disruptor to insects such as aphids and thrips, and spider mites (Bloem *et al*., 2005). Iron phosphate is used in slug and snail management, usually in combination with baits. After eating the bait, the pest stops feeding and dies due to starvation (Edwards *et al*., 2009). Diatomaceous earth is a fine dust comprised of fossilized diatoms; the dust is abrasive and disrupts the cuticle of insects causing death due to desiccation. It is effective in the control of slugs, soil-dwelling insects and stored-product pests (Doumbia *et al*., 2014). Kaolin is a fine-grained clay used as a crop-protectant, which reduces sunburn damage and affects insects in various ways such as deterring feeding and egg laying (Glenn *et al*., 1999). An exhaustive bibliography on kaolin can be found in various chapters of this thesis.

2.2. Biological control

Biological control consists of the management of pests by other living organisms released in crops or favoring and preserving populations of naturally occurring ones. In the first category there are two cases, a natural enemy can be released once in relatively low amounts and then permanently find a place in the vineyard ecosystem (permanent release) or a natural enemy can be released in big amounts for the management of an occurring pest infestation without being able to establish permanently in the crop (periodical release). The last category (conservation biological control) consists in adopting practices that improve the abundance and species richness of natural enemies already living in vineyards and in avoiding the ones that disrupt their populations. This action can have an important impact on pest management, since pest outbreaks are much less likely to occur in a balanced, biodiverse and species-rich ecosystem. At this purpose, diversification of vineyards ground cover flora, presence of shrubs, vegetation corridors, and other ecological structures can supply natural enemies with refuges, preys and alternative host and food. Not less important, avoiding the use of insecticides harmful to non-target arthropods is vital in the maintenance of a healthy and resilient ecosystem (Altieri *et al*., 2005).

Among natural enemies, also pathogens have to be considered. To this category belongs *B. thuringiensis* Berliner, a naturally-occurring bacteria which toxins released in insect gut determine degradation of the mesentery lining causing death. It is mainly used in the control of caterpillars and mosquitoes (Jurat-Fuentes and Jackson, 2012). In viticulture, it is largely used against *L. botrana* (e.g., Ifoulis and Savopoulou-Soultani, 2004). Another pathogen is the fungus *Beauveria bassiana* (Bals.-Criv.) that develops a mycelium inside the body of the insect causing death. It can be used for the management of thrips, aphids, whitefly, caterpillars, beetles and subterranean insects like ants and termites (Vega *et al*., 2012). The main advantage of using insecticides based on microorganisms is given by the selectivity for non-target organisms while the main disadvantages come from low persistence and so the need of increasing the number of applications and the usually complex mode of use they require due to the living nature of their active constituent (Leacy *et al*., 2001).

2.3. Cultural control

In IPM, a very important role is given to the impact of cultural practices on pests. These practices can affect pest population indirectly, through the modification that they induce on plants, or directly by increasing pest mortality. In vineyards, some cultural practices (e.g., irrigation, fertilization, weed control, training system and pruning) can make grapevines less attractive or less favourable to the development of certain pests. For example, nitrogen fertilization affects *L. botrana* (Vartholomaiou *et al*., 2008), *E. vitis* (Decante and van Helden, 2001; Decante *et al*., 2009) infestation and *P. ficus* (Cocco *et al.,* 2015) and bunch-zone leaf removal is effective in reducing *L. botrana* and *B. cinerea* Pers. Fr. (Pavan *et al*., 2016).

3. General aim of PhD thesis

In the context of IPM strategies, the presented PhD thesis aims to investigate the possibility to control grapevine pests through environmental-friendly strategies, such as the use of natural products (i.e., kaolin, sulfur dust, essential oils) and cultural practices (i.e., bunch-zone leaf removal). The interaction between kaolin and bunch-zone leaf removal was also considered. In the context of conservation biological control, the side effects of kaolin and bunch-zone leaf removal on natural enemies were also studied.

4. References

Altieri, M. A., L. Ponti, and C. I. Nicholls. 2005. Manipulating vineyard biodiversity for improved insect pest management: case studies from northern California. International Journal of Biodiversity Science and Management. 1: 191–203.

Baggiolini, M., V. Canevascini, Y. Tencalla, R. Caccia, G. Sobrio, and S. Cavalli. 1968. La cicadelle verte, Empoasca flavescens F. (Homopt., Typhlocybidae), agent d'altérations foliaires sur vigne. Recherche Agronomique en Suisse 7 (1): 43–69.

Bairlein, F. 1990. Estimating density and reproductive parameters for terrestrial field testing with birds. *In* Somerville, L., and Walker C. H. (eds), pp. 113-128, *Pesticide Effects On Terrestrial Wildlife*, Taylor and Francis, London.

Barata, A. 2011. PhD Thesis: Microbial ecology of sour rotten grapes and their influence on chemical and sensorial wine quality. Instituto Superior de Agronomia. Universidade Técnica de Lisboa, 232 pp.

Bianco, P. A., A. Alma, P. Casati, G. Scattini, and A. Arzone. 2001. Transmission of 16SrV phytoplasmas by *Scaphoideus titanus* Ball in Northern Italy. Plant Prot. Sci. 32: 49–56.

Bisiach, M., G. Minervini, and F. Zerbetto. 1986. Possible integrated control of grapevine sour rot. Vitis 25: 118–128.

Bloem, E., S. Haneklaus, and E. Schung. 2005. Significance of sulfur compounds in the protection of plants against pests and diseases. J. Plant Nutr. 28: 763–784.

Brown, A.W.A. 1978. Ecology of pesticides. *John Wiley & Sons,* New York, 525 pp.

Caldwell, S. T., J. E. Keil, and S. H. Sandifer. 1977. Survey of animal pesticides poisoning in South Carolina, 1976. Vet. Hum. Toxicol. 19: 166–168.

Candolfi, M. P., M. Jermini, E. Carrera, and M. C. Candolfi-Vasconcelos. 1993. Grapevine leaf exchange, plant growth, yield, fruit quality and carbohydrate reserves influenced by the grape leafhopper, *Empoasca vitis*. Entomol. Exp. Appl. 69: 289–296.

Carle, P., and G. Moutous. 1965. Observation sur le mode de nutrition sur vigne de quatre espèces de cicadelles. Annales des Epiphyties 16: 333–354.

Carraro, L., N. Loi, C. Kuszala, D. Clair, E. Boudon-Padieu, and E. Refatti. 1994. On the ability-inability of *Scaphoideus titanus* Ball to transmit different grapevine yellow agents. Vitis 33: 231–234.

Cerutti, F., J. Baumgärtner, and V. Delucchi. 1988. Ricerche sull'ecosistema "vigneto" nel Ticino: I. Campionamento delle popolazioni di *Empoasca vitis* Goethe (Hom., Cicadellidae, Typhlocybinae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 61: 29–41.

Charles, J. G. 1982. Economic damage and preliminary economic thresholds for mealybugs (*Pseudococcus longispinus* T-T.) in Auckland vineyards. N. Z. J. Agric. Res. 25: 415–420.

Chuche, J., and D. Thiéry. 2014. Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: a review. Agron. Sustain. Dev. 34: 381–403, http://doi.org/10.1007/s13593-014-0208-7

Cocco, A., A. Lentini, and G. Serra. 2014. Mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in vineyards using reservoir pheromone dispensers. J. Insect Sci. 14: 1–8.

Cocco, A., M. P. Marras, E. Muscas, A. Mura, and A. Lentini. 2015. Variation of life-history parameters of *Planococcus ficus* (Hemiptera: Pseudococcidae) in response to grapevine nitrogen fertilization. J. App. Entomol. 139: 519–528.

D'Arcangelo, M. E. M. 2012. Prove di efficacia per il contenimento di *Planococcus ficus* (Homoptera Pseudococcidae) su vite in Toscana. Atti Giornate Fitopatologiche 2012, vol. 1: 2011–218.

Decante, D., and M. Van Helden. 2001. Intraplot distribution of leafhoppers damaging Bordelais vineyards. *IOBC/WPRS Bull.* 24 (7): 203–210.

Decante, D., C. Van Leeuwen, and M. Van Helden. 2009. Influence of plot characteristics and surrounding vegetation on the intra-plot spatial distribution of *Empoasca vitis*. Agric. For. Entomol. 11: 377–388.

Doumbia, M., B. G. Douan, K. Kwadjo, K. D. Kra, V. Martel, and M. Dagnogo. 2014. Effectiveness of diatomaceous earth for control of *Sitophilus zeamais* (Coleoptera: Curculionidae), *Tribolium castaneum* and *Palorus subdepressus* (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 57: 1–5.

Duso, C. 1989. Bioecological study on *Planococcus ficus* (Sign.) in Veneto. Boll. Lab. Entomol. Agrar. Filippo Silvestri 46: 3–20

Dybas, R. A. 1989. Abamection use in crop protection. *In* Campbell, W. C. (ed), pp. 287–310, *Ivermectin and abamectin*. Springer-Verlag. New York.

Edwards, C. A., N. Q. Arancon, M. Vasko-Bennet, B. Little, and A. Askar. 2009. The relative toxicity of metaldehyde and iron phosphate-based molluscicides to earthworms. Crop Prot. 28: 289–294.

Fermaud, M., and A. Giboulot. 1992. Influence of *Lobesia botrana* larvae on field severity of Botrytis rot of grape berries. Plant. Dis. 76: 404–409.

Girolami, V., C. Duso, E. Refatti, and R. Osler. 1989. Lotta integrata in viticoltura. Malattie della vite. IRIPA – COLDIRETTI, Treviso, Italy.

Glenn, D. M., G. Puterka, T. Vanderzwet, R. E. Byers and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. of Econ. Entomol. 92: 759–771.

Hayes, W. J., and W. K. Vaughn. 1977. Mortality from pesticides in the United States in 1973 and 1974. Toxicol. Appl. Pharmacol. 42: 235–252.

Ifoulis, A. A., and M. Savopoulou-Soultani. 2004. Biological control of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae by using different formulations of *Bacillus thuringiensis* in 11 vine cultivar under filed conditions. J. Econ. Entomol. 97: 340–343.

Ioriatti, C., A. Lucchi, and L. G. Varela. 2012. Grape berry moths in Western European vineyards and their recent movement into the New World. *In* Arthropod management in vineyards: pest, approaches, and future directions, ed by Bostanian NJ, Vincent C, and Isaacs R,. Springer Science+Business Media B.V., Dordrecht, The Netherlands, pp. 339–360.

Isman, M. B. 2006. Botanical Insecticides, deterrents, and repellents in modern agriculture and an increasingly regualated world. Annu. Rev. Entomol. 51: 45–66.

Jermini, M., M. Gusberti, L. Schaub, C. Linder, P. Gugerli, S. Schärer, and S. Emery. 2008. Flavescence dorée and *Scaphoideus titanus*: distribution and control in Switzerland. IOBC/WPRS Bull. 36: 107–111.

Jonsson, M., S. D. Wratten, D. Landis, J. M. L. Tompkins, and R. Cullen. 2010. Habitat manipulation to mitigate the impacts of invasive arthropod pests. Biol. Invasions. 12: 2933–2945.

Jubb, G. L., L. Danko, and C. W. Haeseler. 1983. Impact of *Erythroneura comes* Say (Homoptera: Cicadellidae) on caged 'Concord' grapevines. Environ. Entomol. 12: 1576–1580.

Jurat-Fuentes, J. L., and T. A. Jackson. 2012. Bacterial Entomopathogens. *In* Vega, F. E., and H. K. Kaya (eds), pp. 265–304, *Insect Pathology.* Academic Press, San Francisco.

Lacey, L. A., R. Furtos, H. K. Kayna, and P. Vail. 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control. 21: 230–248.

Lehmann, F., K.-J. Schirra, F. Lovis, and C. P. W. Zebitz. 2001. The green leafhopper *Empoasca vitis* Goethe population dynamics in different zones of foliation and effects of insecticide treatments in vineyards. IOBC/WPRS Bull. 24 (7): 231–235.

Lentini, A., G. Serra, S. Ortu, and G. Delrio. 2008. Seasonal abundance on distribution of *Planococcus ficus* on grape vine in Sardinia. IOBC/WPRS Bull. 36: 267–272.

Lo, P. L., and J. T. S. Walker. 2011. Soil applications of two neonicotinoid insecticides to control mealybugs (Pseudococcidae) in in vineyards. N. Z. Plant. Protect. Res. 64: 101–106.

Martín-Vertedor, D., J. J. Ferrero-Garcia, and L. M. Torres-Vila. 2010. Global warming affects phenology and voltinism of *Lobesia botrana* in Spain. Agric. For. Entomol. 12:169–176.

Martinson, T. E., R. Dunst, A. Lakso, and G. English-Loeb. 1997. Impact of feeding injury by eastern grape leafhopper (Homoptera: Cicadellidae) on yield and juice quality of Concord grapes. Am. J. Enol. Vitic. 48: 291–302.

Mazzoni, V., G. Anfora, C. Ioriatti, and A. Lucchi. 2008. Role of winter host plants in vineyard colonization and phenology of *Zygina rhamni* (Hemiptera: Cicadellidae: Typhlocybinae). Ann. Entomol. Soc. Am. 101: 1003–1009.

Mori, N., A. Bressan, M. Martini, M. Guadagnini, V. Girolami, and A. Bertaccini. 2002. Experimental transmission by *Scaphoideus titanus* Ball of two Flavescence dorée-type phytoplasmas. Vitis 41: 99–102.

Mori, N., D. Tonello, G. Posenato, A. Pozzebon, and C. Duso. 2014. Efficacy of biopesticides against *Scaphoides titanus* Ball in different experimental conditions. IOBC/WPRS Bull. 105, 45–48.

Moschos, T. 2006. Yield loss quantification and economic injury level estimation for the carpophagous generations of the European grapevine moth *Lobesia botrana* Den. et Schiff. (Lepidoptera: Tortricidae). Int. J. Pest. Manag. 52:141–147.

Moutous, G., and A. Fos. 1971. Essais de lutte chimique contre la Cicadelle de la Vigne *Empoasca flavescens* Fabr. Résultats 1970. Revue de Zoologie Agricole et de Pathologie Végétale 70 (1): 48–56.

Nielsen, D. G. 1990. Evaluation of biorational pesticides for use in arboriculture. J. Arboric.. 16: 82–88.

Pavan, F. 2001. The role of *Rubus* bushes in the life cycle of two typhlocybinae infesting European vineyards. Redia 83: 47–60.

Pavan, F., C. Floreani, P. Barro, P. Zandigiacomo, and L. Dalla Montà. 2013. Occurrence of two different development patterns in *Lobesia botrana* (Lepidoptera: Tortricidae) larvae during the second generation. Agric. For. Entomol. 14: 398–406.

Pavan, F., E. Cargnus, G. Bigot, and P. Zandigiacomo. 2014. Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies. Bull. Insectol. 67: 273–280.

Pavan, F., E. Cargnus, S. Kiaeianmoosavi, G. Bigot, F. Tacoli, and P. Zandigiacomo. 2016. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* and grey mould. Bull. Insectol. 69: 107–115.

Pavan, F., G. Stefanelli, A. Villani, P. Gasparinetti, G. Colussi, D. Mucignat, D. Del Cont Bernard, and P. Mutton. 2000. Danni da *Empoasca vitis* (Göthe) (Homoptera: Cicadellidae) in vigneti dell'Italia nord-orientale e soglie d'intervento. Frustula Entomologica 21: 109–124.

Pavan, F., V. Girolami, and G. Sacilotto. 1998. Second generation of grape berry moths, *Lobesia botrana* (Den. & Schiff.) (Lep., Tortricidae) and *Eupoecilia ambiguella* (Hb.) (Lep., Cochylidae): spatial and frequency distributions of larvae, weight loss and economic injury level. J. Appl. Entomol. 122: 361–368.

Pimentel, D., L. McLaughlin, A. Zepp, B. Lakitan, T. Kraus, P. Kleinman, F. Vancini, W. J. Roach, E. Graap, W. S. Keeton, and G. Selig. 1993. Environmental and economic effects of reducing pesticide use in agriculture. Agric. Ecosys. Environ. 46: 273–288.

Posenato, G., M. Pasini, L. Tosi, and E. Tescari. 2004. Efficacia di Laser (Spinosad) su *Drepanothrips reuteri* (Uzel) - 3 anni di sperimentazione. Atti Giornate Fitopatologiche 2004, vol. 1: 91–94.

Prabhaker, N., C. Gispert, and S. J. Castle. 2012. Baseline susceptibility of *Planococcus ficus* (Hemiptera: Pseudococcidae) from California to select insecticides. J. Econ. Entomol. 105: 1392–1400.

Salgado, V. L., J. J. Sheets, G. B. Watson, and A. L. Schmidt. 1992. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pest. Biochem. Physiol. 60: 91–102.

Sarwar, M., and M Salman. 2015. Success stories of Eco-friendly organically acceptable insecticides as natural products discovery. Int. J. Mater. Chem. Phys. 1: 388–394.

Schmutter, H. 1990. Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35: 271–297.

Schvester, A., P. Carle, and G. Moutous. 1963. Transmission de la Flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. (Homopt. Jassidae). Ann. Épiphyties 14: 175–198.

Schvester, D., G. Moutous, J. Bonfilis, P. Carle. 1962. Étude biologique des cicadelles de la vigne dans le sud-ouest de la France. Ann. Épiphyties 13: 205–237.

Schvester, D., G. Moutous, J. Bonfils, and P. Carle. 1962. Étude biologique des cicadelles de la vigne dans le sud-ouest de la France. Ann. Epiphyties 13: 205–237.

Tsai, C., A. Rowhani, D. A. Golino, K. M. Daane, and R. P. P. Almeida. 2010. Mealybug transmission of grapevine leafroll viruses: an analysis of virus-vector specificity. Phytopathology 100: 830–834.

Van Steenwyk, R. A., N. C. Toscano, G. R. Ballmer, K. Kido, and H. T. Reynolds. 1975. Increases of *Heliothis spp*. in cotton under various insecticide treatment regimes. Environ. Entomol. 4: 993–996.

Vartholomaiou, A. N., E. I. Navrozidis, C. C. Payne, and G. A. Salpiggidis. 2008. Agronomic techniques to control. *Lobesia botrana*. Phytoparasitica 36: 264–271.

Vassiliou, V. A. 2011. Effectiveness of insecticides in controlling the first and second generations of the *Lobesia botrana* (Lepidoptera: Tortricidae) in table grapes. J. Econ. Entomol. 104: 580–585.

Vega, F. E., N. V. Meyling, J. J. Luangsa-ard, and M. Blackwell. 2012. Fungal Entomopathogens. *In* Vega, F. E., and H. K. Kaya (eds), pp. 171–206, *Insect Pathology.* Academic Press, San Francisco.

Vidano, C. 1963. Alterazioni provocate da insetti in *Vitis* osservate, sperimentate e comparate. Annali della Facoltà di Scienze agrarie, Universita di Torino 1: 513–644.

Vidano, C. 1964. Scoperta in Italia dello *Scaphoideus littoralis* Ball cicalina americana collegata alla "Flavescence dorée" della vite. L'Italia Agricola 101: 1031–1049.

Vidano, C., and A. Arzone. 1983. Biotaxonomy and epidemiology of Typhlocybinae on vine, pp. 75–85. *In* Knight, W. J., N. C. Pant, T. S. Robertson, and M. R. Wilson (eds) Proceedings of the 1st International workshop on biotaxonomy, classification and biology of leafhoppers and planthoppers (Auchenorrhyncha) of economic importance, London, 4-7 October 1982, Commonwalth Institute of Entomology, London, UK.

Walton, V. M., and K. L. Pringle. 2004. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A review. S. Afr. J. Enol. Vitic. 25: 54–62.

Zoubiri, S., and A. Baaliouamer. 2014. Potentiality of plants as source of insecticide principles. J. of Saudi Chem. Soc. 18: 925–938.

Chapter 2 - Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in vineyards

Chapter 2 is formatted according to the journal author guidelines and was published as:
Federico Tacoli, Francesco Pavan, Elena Cargnus, Elisabetta Tilatti, Alberto Pozzebon, Pietro Zandigiacomo (2017). Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in vineyards. Journal of Economic Entomology, 110 (3): 1164-1178. DOI: 10.1093/jee/tox105

Abstract

During 2015, the influence of kaolin applications and bunch-zone leaf removal on the grapevine leafhoppers, *Empoasca vitis* (Göthe) and *Zygina rhamni* Ferrari, and their egg parasitoids (*Anagrus* spp.) were tested in four vineyards of northeastern Italy. The mode of action of kaolin on *E. vitis* nymphs was also investigated in the laboratory. In the treated plots kaolin was applied at a rate of 2% W/V on two occasions separated by 5-6 days. In two vineyards it was applied either on the whole canopy or the bunch zone at the beginning of the *E. vitis* second-generation (preventive criterion), and in the other two vineyards it was applied to the whole canopy at the peak of the *E. vitis* third-generation (curative criterion). Both the preventive and curative kaolin applications caused a significant decrease in the populations of *E. vitis* and *Z. rhamni* nymphs. The effect of the preventive applications was persistent and was associated with reduced *E. vitis* leaf symptoms. Kaolin did not influence the activity of *Anagrus* spp. Bunch-zone leaf removal did not affect leafhopper populations. Laboratory experiments showed that inhibition of feeding was the main mode of action through which kaolin affected nymph populations. Based on these outcomes, kaolin could be a valuable alternative to synthetic insecticides in controlling grapevine leafhoppers.

1. Introduction

Empoasca vitis (Göthe) and *Zygina rhamni* Ferrari (Hemiptera: Cicadellidae) are the most common and widespread leafhoppers in European vineyards (Vidano and Arzone 1983). *E. vitis* is a polyphagous species that overwinters as adults on evergreen plants and can complete from one to four generations a year in different European grape-growing areas (Schvester et al. 1962, Vidano 1963, Baggiolini et al. 1968, Cerutti et al. 1988). This leafhopper is a phloem feeder of grapevine leaf veins, and the leaf margins can become reddish or yellowish and then desiccate (Vidano 1963, Carle and Moutous 1965). Leaf symptoms are associated with physiological damage such as reductions in photosynthesis, mesophyll conductance and transpiration rate (Candolfi et al. 1993), which can lead to economic damage (i.e., yield losses and sugar content reduction of berries) when the infestation exceeds one or two nymphs per leaf (Moutous and Fos 1971, Baillod et al. 1993, Pavan et al. 2000, Lehmann et al. 2001).

The leafhopper *Z. rhamni* is an oligophagous species that in northern Italy overwinters mostly on *Rubus* spp., on which it can complete a generation in early spring before migrating towards vineyards where it has three generations per year (Vidano 1963, Pavan 2001, Mazzoni et al. 2008). It is a mesophyll feeder leaving white dots on the upper surface of leaves where cell contents were extracted. Relatively to mesophyll-feeder grapevine leafhoppers, economic damage is reported for American species (Jubb et al. 1983, Martinson et al. 1997). For *Z. rhamni* strong symptoms are described (Vidano 1963) and an economic threshold of two-three nymphs per leaf was suggested for northern Italy (Girolami et al. 1989).

Synthetic insecticides are usually applied to control *E. vitis* (Lavezzaro et al. 2006, Posenato et al. 2006, Pozzebon et al. 2011). In the context of IPM, other control approaches have been proposed, such as conservation biological control to promote the activity of the egg parasitoid *Anagrus atomus* L. (Hymenoptera: Mymaridae) and other natural enemies (Cerutti et al. 1991, van Helden and Decante 2001, Ponti et al. 2005, Zanolli and Pavan 2011), planting of grapevine cultivars of low susceptibility (Pavan and Picotti 2009, Fornasiero et al. 2016), use of copper compounds (Pavan 1994), and nitrogen fertilization and irrigation management to reduce plant vigor (Decante and van Helden 2001, Decante et al. 2009) or induce a moderate water stress (Costello 2008, Fornasiero et al. 2012).

As an alternative to synthetic insecticides, kaolin particle film technology was proposed for arthropod pest control (Glenn et al. 1999). Kaolin is a white-colored clay (aluminosilicate mineral) that is chemically inert, fine-grained, nonporous, low abrasive, non-expandable and easily dispersible into water (Harben 1995). As a water suspension it can be sprayed on plants, creating a particle film that changes the tactile and visual features of the leaf and fruit surfaces making the plant repellent to arthropods. Insect activities like egg-laying, feeding on and grasping the host plant surfaces can be compromised by the clay particles that stick to their bodies (Glenn et al. 1999, Glenn and Puterka 2005). To date, kaolin has been found to be effective against sap feeders such as lace bugs (Marcotegui et al. 2015), thrips (Tyler-Julian et al. 2014), aphids (Markó et al. 2008, Sleezer et al. 2011, Nateghi et al. 2013, Pissinati and Ventura 2015), psyllids (Puterka et al. 2005, Butler et al. 2011) and scales (Pascual et al. 2010), and against chewing pests such as tephritid fruit flies (Caleca et al. 2010, Pascual et al. 2010), tortricid moths (Knight et al. 2001, Markó et al. 2008, Pease et al. 2016) and blossom weevils (Markó et al. 2008).

About the influence of kaolin on *E. vitis*, Markó et al. (2008) reported that 12 kaolin applications on apple trees, from late March to early August, significantly reduced the captures of adults in late September. Kaolin has also been applied against other sap feeders belonging to the Hemiptera, Cicadomorpha and Fulgoromorpha. It was found to be effective in controlling the planthopper *Ommatissus lybicus* Bergevin (Hemiptera: Tropiduchidae) on date palm (Mahmoudi et al. 2014). On grapevines treated with kaolin a deterrent effect and a higher mortality rate were observed for the sharpshooter *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), which is a vector of *Xylella fastidiosa* Wells, the causal agent of Pierce's disease (Wood and McBride 2001, Puterka et al. 2003, Tubajika et al. 2007). Kaolin was reported as effective in controlling *Empoasca fabae* (Harris) (Hemiptera: Cicadellidae) on aubergine (Maletta et al. 2004) but not on potatoes (Maletta et al. 2006). The populations of *Typhlocyba pomaria* McAtee (Hemiptera: Cicadellidae) were significantly reduced on apple trees submitted to ten or more kaolin applications (Knight et al. 2001, Bostanian and Racette 2008).

Because in Markó et al. (2008) data on *E. vitis* nymph infestation during the kaolin applications were not reported and adult reduction was obtained with high number of applications, the information reported seems inadequate to understand whether kaolin could be effectively and profitably used to control *E. vitis* in vineyards. Based on these considerations, the aim of this study was to assess if only two applications of kaolin can be an alternative to synthetic insecticides in controlling *E. vitis* and *Z. rhamni* nymphs on grapevines. Kaolin could be of profitable if used mostly in organic vineyards, where the only options against leafhoppers are pyrethrum (Mori et al. 2004), whose efficacy is prejudiced by its low persistence, or copper products, whose amount applied per hectare is limited by legal measures due to environmental issues (Mazzini 2010). Towards this end, the efficacy of kaolin applications was tested against grapevine leafhoppers with both preventive and curative approaches. Under laboratory conditions, the mode of action of kaolin against *E. vitis* nymphs was also studied. Moreover, because some negative effects of kaolin on natural enemies have been reported, e.g. on minute pirate bugs (Bengochea et al. 2010), green lacewings (Porcell et al. 2011, Bengochea et al. 2014), ladybirds (Pascual et al. 2010), hymenopteran parasitoids (Bengochea et al. 2013) and spiders (Sackett et al. 2007, Markó et al. 2010), its side effect on *Anagrus* spp., which are egg parasitoids of the grapevine leafhoppers, was tested.

Since bunch-zone leaf removal in vineyards is known to reduce *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae) and *Botrytis cinerea* Persoon ex Fries (Pavan et al. 2016), and greater plant vigor and leaf density favor *E. vitis* infestation (Decante and van Helden 2001, Decante et al. 2009, Pavan and Picotti 2009), the second aim of this paper was to evaluate the influence of two different leaf densities in the bunch zone on grapevine leafhoppers.

2. Materials and methods

2.1. Field trials

In the 2015 growing season four trials were carried out in four vineyards of northeastern Italy. In two trials kaolin was applied with a preventive criterion and in the other two with a curative criterion.

2.1.1. Preventive application trials

In preventive applications, kaolin was sprayed when *E. vitis* nymph populations were very low. In this period, the development of the first generation was finishing and the first newly-hatched nymphs of the second generation, the most dangerous in the grape-growing area of northeastern Italy, were observed. The preventive criterion was adopted in two vineyards (A and B). Vineyard A (locality Cormons, Gorizia district, 45°57’51”N, 13°26’49”E, 56 m a.s.l., cultivar Pinot Gris) is a 10-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.5 m and 0.8 m, respectively. Vineyard B (locality Cormons, Gorizia district, 45°57’20”N, 13°26’50”E, 50 m a.s.l., cultivar Pinot Gris) is a 30-year-old organic vineyard with grapevines growing using the double arched Guyot training system with distances between and along rows of 2.8 m and 1 m, respectively. In both vineyards standard fungicide programs were followed and no insecticide was sprayed before and during sampling period.

The treatments under comparison in both vineyards were: control, kaolin (Surround WP, Tessenderlo Kerley Inc., Phoenix, Arizona, USA, 2% W/V, Surround WP/water) sprayed on the whole canopy (hereafter called “kaolin WC”, at a rate of 1000 L/ha) or localized on the bunch-zone (hereafter called “kaolin BL”, at a rate of 500 L/ha). In both vineyards kaolin was applied on 18 and 24 June using a backpack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano RE, Italy). In both trials, a randomized block design with four replicates was adopted. Each block (row) was divided into three plots of 20 (vineyard A) or 28 (vineyard B) grapevines. All the plots were divided into two subplots of 10 (vineyard A) and 14 (vineyard B) grapevines, respectively, that were subjected or not to bunch-zone leaf removal on 17 June. Bunch-zone leaf removal consisted of removing all leaves that covered the bunches.

For both vineyards (A and B) weather data were obtained from a weather station distant about five kilometers (Regional Meteorological Observatory; http://www.osmer.fvg.it/OSMER).

In all treatments of both trials, nymphs of *E. vitis* and *Z. rhamni* were sampled weekly on leaves from 11 June, a few days before the first kaolin application, to 20 July, about a month after the second kaolin application. Because there were four different combinations between kaolin application (whole canopy and bunch localized) and bunch-zone leaf removal (yes and no), two canopy zones were considered for leaf sampling: the bunch zone (lower part of the canopy) and the vegetative zone (upper part of the canopy). On each subplot 100 mature leaves were sampled (25 per row side and canopy zone).

In the control and kaolin WC treatments of both trials, adults of *E. vitis* and *Z. rhamni*, and *Anagrus* spp. of the '*atomus'* group (*sensu* Chiappini et al., 1996) were sampled using yellow sticky traps replaced weekly from 4 June (2-wk before the first kaolin application) to 18 August (about two months after the second kaolin application). From 4 June to 18 June alone, the traps were left in the vineyards for more than a week as a consequence of seven consecutive rainy days that forced to postpone the planned kaolin-application of 11 June to 18 June. One trap (20 × 10 cm) per subplot (with or without leaf removal), smeared with glue (Temo-O-Cid®, Kollant Srl, Vigonovo VE, Italy), was hung on the horizontal wires of the grapevine trellis at about 1.5 m from the ground level so as to be inside the canopy, but not covered by leaves. In the laboratory the two leafhoppers and *Anagrus* spp. of '*atomus*' group were identified (Vidano 1958, Viggiani 1988, Chiappini et al. 1996, Chiappini and Mazzoni 2000) and counted.

In both trials the influence of kaolin on leafhopper egg-laying and egg parasitization rate by *Anagrus* spp. was assessed in subplots without bunch-zone leaf removal of the control and kaolin WC. Fifty mature leaves per subplot were collected in the vegetative zone on 3 August, before the beginning of third generation egg hatching, and taken to the laboratory. On each leaf the emergence holes of leafhopper nymphs (hatched eggs) and those of *Anagrus* spp. adults (parasitized eggs) on leaf veins were counted under a dissection microscope (Picotti and Pavan 1993). Because the eggs of *E. vitis* and *Z. rhamni* are indistinguishable it was not possible to associate the emergence holes to either species. To obtain the total number of leafhopper eggs laid, the hatched and parasitized eggs were summed. The percentage of parasitized eggs was calculated from the ratio between the parasitized eggs and the total number of leafhopper eggs laid. Since mature leaves collected in the vegetative zone were absent or still very small when overwintering females laid eggs, the emergence holes observed on these leaves were almost all associable with the second generation.

In the vineyard B, where symptom expression by *E. vitis* feeding-activity was very evident at vintage time, the percentage of symptomatic leaf surface (change in color and/or drying out) was estimated in subplots without bunch-zone leaf removal of the control and kaolin WC treatments according to eight injury classes (0, 1, 5, 10, 25, 50, 75, and 100%) on the basis of reference patterns (Pavan et al. 2000). This procedure was applied to 200 leaves per subplot (800 leaves per treatment). To avoid subjective choices, on the 10 central grapevines of the subplots 10 basal leaves of both proximal and distal shoots of the canes were sampled.

Data on nymph densities on leaves and adult captures on yellow sticky traps were analyzed using a repeated measures analysis of variance (ANOVA) with the MIXED procedure of SAS (v 9.2, SAS Institute, Cary, NC). In these analyses nymph densities and adult captures were considered as a dependent variable with observation made at different time (sampling dates) in separated analyses. For data on nymphs, kaolin application (control, kaolin WC and kaolin BZ), bunch-zone leaf removal (yes vs. no), canopy zone (vegetative vs. bunch), time and their interactions were considered as sources of variation and their effects were tested with a F test ($\alpha = 0.05$). For data on adults, kaolin application (control and kaolin WC), bunch-zone leaf removal (yes vs. no), time and their interactions were considered as sources of variation and their effects were tested with a F test ($\alpha = 0.05$). Differences among treatments were evaluated using a t-test to the least-square means with Bonferroni adjustment of the p-values ($\alpha = 0.05$). The Kenward-Roger method was used for degrees of freedom estimation (Littell et al. 2006). This method can produce not integer value for degree of freedom. In all analyses and according to Akaike's Information Criterion, first-order autoregressive was chosen as best fitting covariance structure for correlating different sampling dates (Littell et al. 2006). Data were checked for analysis assumptions and were log $(x+1)$ transformed prior to the analyses.

Data on egg densities were square root transformed and analyzed using a two-way ANOVA with kaolin and leaf removal as independent variables. Data on percentages of parasitized eggs were analyzed by using Fisher's exact test. After arcsine transformation, two-way ANOVA was used to compare the percentage of leaf surface with *E. vitis* symptoms in the two treatments (control and kaolin WC) and in the two cane positions (proximal and distal).

2.1.2. Curative application trials

In curative applications, kaolin was sprayed when the *E. vitis* nymphs of the third generation had already reached high population levels. This control strategy was adopted in two vineyards (C and D). Vineyard C (locality Cormons, Gorizia district, 45°57’53”N, 13°30’19”E, 59 m a.s.l., cultivar Sauvignon Blanc) is a 25-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.6 m and 0.75 m, respectively. Vineyard D (locality Buttrio, Udine district, 46°1’12”N, 13°21’19”E, 50 m a.s.l., cultivar Tocai Friulano) is a 15-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.8 m and 1.2 m, respectively. In both vineyards, standard fungicide programs had been adopted and pyrethrum (Asset, Copyr S.p.A., Milano, Italy, 0.7 L/ha) was applied once in late June against *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Neither insecticides nor fungicides were applied during the trials. The treatments under comparison in vineyard C and D were control and kaolin WC (Surround WP, 2% W/V at rate of 1000 L/ha). In both vineyards a randomized block design with four replicates was used. Each block (row) was divided into two plots of six (vineyard C) and 12 (vineyard D) grapevines. In both trials kaolin was applied on 19 and 24 August.

Weather data for vineyards C and D were obtained from the two nearest weather stations of the Regional Meteorological Observatory (data from http://www.osmer.fvg.it/OSMER).

In both trials, on 18 and 24 August, respectively before the first and the second kaolin applications, and on 28 August, 4-d after the second kaolin application, nymphs of *E. vitis* and *Z. rhamni* were sampled on 100 mature leaves (50 per each row side) per replicate chosen on the middle-basal part of main shoots.

Data on nymph densities in the two treatments were analyzed with a t-test after logarithmic transformation.

2.2. Laboratory experiments

Two laboratory experiments were carried out in late summer 2015 to investigate the mode of action of kaolin against *E. vitis* nymphs. In experiment 1 the survival of first and second instar nymphs was compared in two treatments: (i) nymphs placed on kaolin-untreated leaves (control), and (ii) nymphs placed on kaolin-treated leaves (kaolin-on-leaves). In experiment 2 the survival of third to fifth instar nymphs was compared in three treatments: (i) nymphs placed on kaolin-untreated leaves (control), (ii) nymphs placed on kaolin-treated leaves (kaolin-on-leaves), (iii) and nymphs sprayed with kaolin and then placed on kaolin-untreated leaves (kaolin-on-nymphs). In both experiments, each treatment was replicated 40 times.

For both experiments, 40 non-infested grapevine leaves per treatment were collected in a Carménère vineyard contiguous to vineyard D. The grapevines before leaf collection were submitted to a single insecticide application (pyrethrum, Asset, 0.7 L/ha) against *S. titanus* in late

June. In the laboratory each leaf was visually checked for absence of leafhoppers and, after being sprayed with kaolin or not, individually inserted into a transparent self-sealing plastic bag (20 × 33 cm). Kaolin (Surround WP) was used at 4% W/V, to obtain the same residue as in field trials, and applied with a hand sprayer to run-off. *E. vitis* nymphs used in the experiments came from infested leaves collected in same vineyard. In the laboratory first and second instar nymphs (experiment 1) or third to fifth instar nymphs (experiment 2) were randomly chosen on these leaves and induced to move to non-infested leaves so to have one nymph per bagged leaf. In the kaolin-on-nymphs treatment, nymphs were induced to move onto the kaolin-untreated leaves after they had been covered with kaolin by spraying infested leaves. In the following days the bags were checked to note whether nymphs were alive or dead. The day after the beginning of the experiments (1-d) the nymph position (on leaf or bag surfaces) was also recorded. Samplings were carried out after 1-d and 3-d in the first experiment, and daily, as long as nymphs alive were observed in all treatments, in the second experiment.

Data on percentage of nymph mortality and percentage of nymphs recorded on the bag surface at 1-d were compared by using Fisher's exact test (experiment 1) and Ryan's multiple range test (Ryan 1960) (experiment 2).

3. Results

3.1. Preventive application trials

3.1.1. Weather conditions

During the sampling period two important rainfalls on 23 June (46.7 mm) and 8 July (21.9 mm) were recorded for vineyards A and B. After the rainfall that occurred on 23 June (6-d from the first kaolin-application) kaolin coverage was restored and up to 8 July there was no more rainfall. In any case the kaolin leaf coverage was little affected by the weather conditions because kaolin was still visible on leaves at the end of the sampling period.

3.1.2. *Empoasca vitis* nymphs on leaves

Vineyard A. Kaolin application determined a reduction of *E. vitis* nymph densities (Table 1; Fig. 1A and B). A significant interaction "time*kaolin application" was found because the effect of kaolin emerged from the second sampling date onwards. Higher densities were observed in the vegetative zone than the bunch zone. The interactions "canopy zone*kaolin application" and "canopy zone*kaolin application*time" were significant indicating that the effect of kaolin was different between the two canopy zones. In the vegetative zone, *E. vitis* nymph densities were lower in the kaolin WC than the other treatments (vs. control: *t* = 15.65; df = 71.8; *P* < 0.001; vs. kaolin BL: *t* = 14.25; df = 71.8; *P* < 0.001), whereas no differences were observed between control and kaolin BL (*t* = 7.4; df = 71.8; *P* = 1.000). In the bunch zone, nymph densities were lower in both the kaolin treatments than the control (vs. kaolin WC: *t* = 11.14; df = 71.8; *P* < 0.001; vs. kaolin BL: *t* = 13.03; df = 71.8; *P* < 0.001), whereas no differences emerged between the two kaolin treatments (*t* = 1.89; df = 71.8; *P* = 1.000). No effect of bunch-zone leaf removal was observed on *E. vitis* nymph densities (Table 1; Fig. 2A and B).

Vineyard B. Kaolin application determined a reduction of *E. vitis* nymph densities Table 1; Fig. 1C and D). A significant interaction "time*kaolin application" was found because the effect of kaolin emerged from the third sampling date onwards. Higher densities were observed in the vegetative zone than the bunch zone. The interactions "canopy zone*kaolin application" and "canopy zone*kaolin application*time" were significant indicating that the effect of kaolin applications were different between the two canopy zones. In the vegetative zone, *E. vitis* nymph densities were lower in the kaolin WC than the other treatments (vs. control: *t* = 13.11; df = 68.7; *P* < 0.001; vs. kaolin BL: *t* = 11.28; df = 68.7; *P* < 0.001), whereas no differences were observed between control and kaolin BL (*t* = 1.82; df = 68.7; *P* = 1.000). In the bunch zone, nymph densities were lower in both the kaolin treatments than the control (vs. kaolin WC: *t* = 10.61; df = 68.7; *P* < 0.001; vs. kaolin BL: *t* = 8.47; df = 68.7; *P* < 0.001), whereas no differences emerged between the two kaolin treatments (*t* = 2.15; df = 68.7; *P* = 0.52). No effect of bunch-zone leaf removal was observed on *E. vitis* nymph densities (Table 1; Fig. 2C and D).

3.1.3. *Zygina rhamni* nymphs on leaves

Vineyard A. Kaolin application determined a reduction of *Z. rhamni* nymph densities (Table 1; Fig. 3A and B). A significant interaction "time*kaolin application" was found because the effect of kaolin emerged from the second sampling date onwards. Higher densities were observed in the vegetative zone than the bunch zone. The interactions "canopy zone*kaolin application" and "canopy zone*kaolin application*time" were significant indicating that the effect of kaolin applications were different between the two canopy zones. In the vegetative zone, *Z. rhamni* nymph densities were lower in the kaolin WC than the other treatments (vs. control: *t* = 6.48; df = 88.5; *P* < 0.001; vs. kaolin BL: *t* = 6.54; df = 88.5; *P* < 0.001), whereas no differences were observed between control and kaolin BL (*t* = 0.07; df = 88.5; *P* = 1.000). In the bunch zone, nymph densities were lower in both the kaolin treatments than the control (vs. kaolin WC: *t* = 6.49; df = 88.5; *P* < 0.001; vs kaolin BL: *t* = 5.99; df = 88.5; *P* < 0.001), whereas no differences emerged between the two kaolin treatments (*t* = 0.49; df = 88.5; *P* = 1.000). No effect of bunch-zone leaf removal was observed on *Z. rhamni* nymph densities (Table 1; Fig. 4A and B).

Vineyard B. Kaolin application determined a reduction of *Z. rhamni* nymph densities (Table 1; Fig 3 C and D). A significant interaction “time*kaolin application” was found because the effect of kaolin emerged from the third sampling date onwards. Higher densities were observed in the bunch zone than the vegetative zone where densities were always lower than 2 individuals per 100 leaves. The interaction “canopy zone*kaolin application” was significant indicating that the effect of kaolin applications were different between the two canopy zones. In the vegetative zone, *Z. rhamni* nymph densities were not different among the three treatments (always $P > 0.05$). In the bunch zone, nymph densities were lower in both the kaolin treatments than the control (vs. kaolin WC: $t = 6.48$; df = 78.9; $P < 0.001$; vs kaolin BL: $t = 7.25$; df = 78.9; $P < 0.001$), whereas no differences emerged between the two kaolin treatments ($t = 0.77$; df = 78.9; $P = 1.000$). No effect of bunch-zone leaf removal was observed on *Z. rhamni* nymph densities (Table 1; Fig. 4C and D).

3.1.4. *Empoasca vitis* adults on traps

In both vineyards *E. vitis* captures were greatest in the sampling before the first kaolin-application and then rapidly decreased (Table 2; Fig. 5A and C). In both vineyards *E. vitis* captures were lower in the kaolin WC treatment than the control and emerged in mid-June after the first kaolin-application. In both vineyards, bunch-zone leaf removal did not substantially influence the amount of *E. vitis* captures (Table 2; Fig. 5B and D).

3.1.5. *Zygina rhamni* adults on traps

In vineyard A no effect of kaolin application was observed on *Z. rhamni* captures (Table 2; Fig. 6A), whereas a significant effect of bunch-zone leaf removal emerged with lower captures on plots with leaf removal as compared to those without leaf removal (Table 2; Fig. 6B). In vineyard B *Z. rhamni* captures were lower in the kaolin WC treatment than the control and these differences emerged from mid-July onwards (Table 2; Fig. 6C). No effect of bunch-zone leaf removal was observed (Table 2; Fig. 6D).

3.1.6. *Anagrus* spp. adults on traps

In both vineyards *Anagrus* spp. captures were lower in the kaolin WC treatment than the control, but no significant differences emerged (Table 2; Fig. 7A and C). In both vineyards, the captures were not significantly different between subplots with or without leaf removal (Table 2; Fig. 7B and D).

3.1.7. Leafhopper eggs and parasitization by *Anagrus* spp.

In both vineyards, kaolin application had no significant influence on the amount of leafhopper eggs laid, nor the amount of eggs hatched or the percentage of eggs parasitized by

Anagrus spp. (Table 3). In both vineyards bunch-zone leaf removal had no influence on the amount of leafhopper eggs laid, nor the amount of eggs hatched or the percentage of eggs parasitized by *Anagrus* spp. In both vineyards the interaction between the two factors (kaolin and bunch-zone leaf removal) was never significant for the three variables considered (vineyard A: $F \leq 1.12$; df = 1, 12; $P \geq 0.31$; vineyard B: $F \leq 0.09$; df = 1, 12; $P \geq 0.77$).

3.1.8. *Empoasca vitis* symptoms on leaves

In vineyard A, the percentage of leaf surface with symptoms was lower in the kaolin WC treatment than the control (0.83±0.74% vs. 13.31±3.00%) ($F = 172.11$; df = 1, 12; $P < 0.0001$). No differences in the incidence of symptoms was recorded between leaves of proximal and distal shoots of the canes (7.87±7.73% vs. 6.27±6.11%) ($F = 2.83$; df = 1, 12; $P = 0.12$).

3.2. Curative application trials

3.2.1. Weather conditions

During the sampling period in vineyards C and D, four important rainfalls were recorded on 19 (C 17.3 mm, D 33.3 mm), 20 (C 14.8 mm, D 24 mm), 24 (C 41 mm, D 52 mm) and 25 August (C 23.3 mm, D 5.4 mm). Kaolin was applied after the rainfalls that occurred on 19 August and the coverage was restored after the rainfalls on 24 August. In any case the kaolin leaf coverage was little affected by the weather conditions because also at the end of the sampling period kaolin was visible on leaves.

3.2.2. *Empoasca vitis* nymphs on leaves

In the sampling conducted 1-d before the first kaolin-application (18 August), no differences in *E. vitis* nymph densities between the two treatments were found for either vineyard (vineyard C: $t = 0.46$; df = 6; $P = 0.67$; vineyard D: $t = 1.78$; df = 6; $P = 0.13$) (Fig. 8A and C).

In the sampling conducted 5-d after the first kaolin-application and 6-h before the second one (24 August), *E. vitis* nymph densities were about three times lower in the kaolin WC than the control in both vineyards (vineyard C: $t = 2.68$; df = 6; $P = 0.04$; vineyard D: $t = 6.94$; df = 6; $P = 0.0004$).

In the sampling conducted 4-d after the second kaolin application (28 August), the differences in nymph densities between kaolin WC and the control increased in both vineyards (vineyard C: $t = 3.795$; df = 6; $P = 0.01$; vineyard D: $t = 7.79$; df = 6; $P = 0.0002$).

3.2.3. *Zygina rhamni* nymphs on leaves

In the sampling conducted 1-d before the first kaolin-application (18 August), no differences between the two treatments were found for *Z. rhamni* nymph density in either vineyard (vineyard C: *t* = 0.23; df = 6; *P* = 0.83; vineyard D: *t* = 1.28; df = 6; *P* = 0.25) (Fig. 8B and D).

In the sampling conducted 5-d after the first kaolin-application and 6-h before the second one (24 August), *Z. rhamni* nymph populations were lower in the kaolin WC than the control in both vineyards (vineyard C: *t* = 2.47; df = 6; *P* = 0.05; vineyard D: *t* = 2.81; df = 6; *P* = 0.03).

In the sampling conducted 4-d after the second kaolin-application (28 August), the differences in nymph density between the two treatments increased in both vineyards (vineyard C: *t* = 3.78; df = 6; *P* = 0.01; vineyard D: *t* = 4.48; df = 6; *P* = 0.004).

3.3. Laboratory experiments

3.3.1. Experiment 1. Effect of kaolin on *E. vitis* first and second instar nymphs

At 1-d sampling a significantly higher mortality rate of *E. vitis* nymphs was observed in the kaolin-on-leaves treatment than the control (*P* < 0.0001) (Fig. 9A). At 3-d sampling this significant difference was confirmed with almost all nymphs dead in the kaolin-on-leaves treatment but only one third of them dead in the control (*P* < 0.0001).

At 1-d sampling, the proportion on nymphs on the bag surface was very high in the kaolin-on-leaves treatment (88%) and very low in the control (10%) (*P* < 0.0001).

3.3.2. Experiment 2. Effect of kaolin on *E. vitis* third to fifth instar nymphs

Mortality of *E. vitis* nymphs in the kaolin-on-leaves treatment increased more rapidly than both the control and kaolin-on-nymphs treatment, in which nymphs were sprayed with kaolin before being placed on untreated leaves (Fig. 9B). The differences were significant from 1-d to 7-d samplings (*P* ≤ 0.05) when almost all nymphs in the kaolin-on-leaves treatment were dead but about half of the nymphs in both the control and the kaolin-on-nymphs treatment were still alive.

At 1-d sampling, the proportion of nymphs on the bag surface was significantly higher in the kaolin-on-leaves treatment (65%) than in the control (10%) and kaolin-on-nymphs (0%) (*P* = 0.01).

4. Discussion

4.1. Effect of kaolin on leafhopper populations

Kaolin caused a decrease in the field populations of *E. vitis* and *Z. rhamni* nymphs at very low levels when sprayed under both the preventive and curative criteria. The effect of kaolin in the preventive applications was persistent as differences from the control remained for up to four weeks. The reduction in *E. vitis* nymph densities was associated with a more than 10-fold reduction in leaf surface area showing symptoms from leafhopper feeding activity. The level of *E. vitis* control is comparable to that obtained with the most effective synthetic insecticides (Lavezzaro et al. 2006, Posenato et al. 2006, Pozzebon et al. 2011).

4.2. Mode of action of kaolin against leafhoppers

According to Glenn et al. (1999), kaolin could affect *E. vitis* and *Z. rhamni* populations by reducing egg laying, by exerting a direct toxicity towards motile forms or by inhibiting their feeding activity. From the theoretical point of view, kaolin could also reduce the leafhopper population by favoring the activity of natural enemies (e.g., *Anagrus* spp. egg-parasitoids and generalist predators).

In the present study, the amount of leafhopper egg laying was not significantly lower in the kaolin treatment than control. This result is in accordance with other studies that reported a decrease in leafhopper/planthopper populations without a significant effect on oviposition (Knight et al. 2001, Mahmoudi et al. 2014). The data on the amount of eggs seem at odds with the lower captures on the grapevines sprayed with kaolin. This may be due to a lower attractiveness of the traps that resulted from the light reflecting properties of kaolin particle film that could alter leafhoppers perception of the leaves (Glenn et al. 1999, Glenn and Puterka 2005) without affecting egg-laying activity. However, because on grapevine Puterka et al. (2003) reported that a decrease in captures of the sharpshooter *H. coagulata* on yellow sticky traps was associated with a significant oviposition-deterrent effect, another hypothesis is that kaolin had a repellent effect on adults but that this did not result in a lower number of eggs because egg laying may have almost ended when the kaolin was applied. Two observations agree with this possibility. First, adult captures also decreased rapidly in the control after the first kaolin-application and this could be associated with a decrease in egg laying independent of kaolin. Second, the increase in the nymph population ended in late June (Vineyard A) and early July (Vineyard B) indicating that fewer eggs hatched after late June-early July. In fact, considering that at average June temperatures (20.7 °C) the eggs hatch in about 15-d

(Cerutti et al. 1990), the newly-hatched nymphs from eggs laid before the first kaolin application (18 June) would be recorded no later than early July. Based on this prediction, in vineyard A negligible egg laying or very high egg parasitization can be hypothesized because nymph densities did not substantially increase after 29 June, whereas in vineyard B, where nymph densities still increased significantly from 29 June to 6 July, a significant amount of egg-laying can be presumed after the first kaolin application. In agreement with these data, in this last vineyard the total number of eggs was lower by 20% in kaolin treatments than the control, although the difference was not statistically significant. However, if an egg-laying deterrent effect of kaolin cannot be excluded, its role in the leafhopper control did not appear crucial considering both the curative activity of kaolin in the field and the high nymph mortality in the laboratory.

Nymph mortality due to contact of kaolin with the insect integument would seem feasible from the knock-down effect observed in the curative application trials. At this purpose, the effect of spraying kaolin directly on insects has not been investigated by other recent studies, but a contact toxicity was observed mainly due to absorption of epicuticular wax (Ebeling 1971) and the abrasion of the epicuticle (Alexander 1944a, 1944b; David and Gardiner 1950). The loss of epicuticle integrity could imply water loss and thus death by desiccation. However, our laboratory data excluded with certainty kaolin contact toxicity on *E. vitis* because the mortality of the nymphs sprayed with kaolin and placed on kaolin-untreated leaves was not different from that on the control.

Inhibition of leafhopper feeding activity due to kaolin particle films has been suggested by previous studies (Knight et al. 2001, Glenn and Puterka 2005, Tubajika et al. 2007, Mahmoudi et al. 2014). In our study this hypothesis is supported by the fact that in the laboratory the nymphs died only when they were confined to leaves covered with kaolin. Moreover, under laboratory conditions, the day after the beginning of the two experiments most of the nymphs in the kaolin-on-leaves treatment were recorded on the bag surface rather than on the leaf surface. Our data would suggest that this anti-feeding effect is greater in younger nymphs (first and second instars) than older ones (third to fifth instars). In the laboratory a higher and more rapid mortality rate was observed in experiment 1 than experiment 2, in which nymphs of the older instars were placed on kaolin-treated leaves. In the field, a higher efficacy of kaolin was observed in the preventive application trials than the curative application trials when many nymphs of the older instars were present on the leaves. Without feeding, younger nymph instars could die earlier and in a higher proportion than the older ones because they have a less favorable ratio between body surface and volume, and then a lower desiccation tolerance.

The absence of a difference in leafhopper egg parasitization between kaolin and control treatments, and with even lower captures of *Anagrus* spp. adults in kaolin treatment, clearly indicates that kaolin did not favor the activity of *Anagrus* spp. adults. Also, a greater predation of leafhopper nymphs seems unlikely on the basis of the data reported in the literature as well as to our preliminary data because the amount of generalist predators (e.g., spiders, minute pirate bugs) not only did not increase in the kaolin covered plots, but in some cases significantly decreased (Sackett et al. 2007, Bengochea et al. 2010, Markó et al. 2010, Pascual et al. 2010).

4.3. Effect of bunch-zone leaf removal on *Empoasca vitis*

Bunch-zone leaf removal did not reduce *E. vitis* nymph and adult densities. This would seem in contradiction with data from the literature that has reported greater infestations in plots or cultivars with greater plant vigor (Decante and van Helden 2001, Decante et al. 2009, Pavan and Picotti 2009). However, it must be considered that removing a small quantity of leaves from the bunch zone does not affect plant vigor and the leaf density in the vegetative zone where the highest *E. vitis* populations were observed also in the plots without leaf removal.

4.4. *Empoasca vitis* nymph spatial distribution

In the two trials on kaolin preventive-applications, a variation in the spatial distribution of *E. vitis* nymphs was observed over the sampling period. In fact, in the first sampling the leaves of the bunch zone and in the subsequent samplings those of the vegetative zone were preferred by *E. vitis*, respectively. Since the leaves of the bunch and vegetative zones are, respectively, the basal and the median leaves of the shoots, a shift in egg-laying preference by females from older leaves (basal) to younger ones (median) can be supposed. In fact, in the first sampling, when nymphs were mostly observed in the bunch zone, many older-instar nymphs belonging to the first generation were still observed, whereas in the following samplings, when nymphs were mostly observed in the vegetative zone, many younger-instar nymphs belonging to the second generation were found (data not reported). This shift in the occurrence of nymphs from basal to median leaves, from the first to the second generation, has been observed previously by Fos et al. (1997).

Data on the spatial distribution of *E. vitis* nymphs in the kaolin BL treatment suggest that nymphs were not able to move from kaolin-covered leaves in the bunch zone to the kaolin-free leaves of the vegetative zone. In fact, the infestation levels recorded in the vegetative zone of the kaolin BL treatment did not differ from the vegetative zones of the control over the whole season. Therefore, during the same generation it is not necessary to cover leaves that emerge after the kaolin application because the nymphs already present, and those that will hatch by eggs already laid, are not able to move from kaolin-covered leaves to the kaolin-free leaves. For this reason we think that the number of treatments necessary to ensure high *E. vitis* control is lower than reported in other studies (Markó et al. 2008). The high level of effectiveness achieved in this study with just two applications of kaolin spaced 5-6 days apart is evidence of this fact. A strategy based on a reduced number of kaolin applications also seems possible because kaolin exhibited good resistance to water washout. A practical consequence of the preference of *E. vitis* second-generation for leaves of the vegetative zone is the necessity to apply kaolin over the entire canopy.

4.5. Kaolin as an alternative to synthetic insecticides in viticulture

Kaolin, which is commercialized as a natural crop protectant, demonstrated insecticidal activity against grapevine leafhoppers. The high efficacy achieved with only two applications, the negligible impact on *Anagrus* parasitoids and the resistance to water washout are features that support kaolin as a candidate for controlling *E. vitis* in vineyards, mostly in organic farms where very effective insecticides are not currently available. Moreover, the possibility of using kaolin with curative criterion allows applying the product only when economic threshold is exceeded in agreement with IPM principles.

The use of kaolin would be a valuable option only if negative side effects on grapevine production did not occur. Kaolin applied to grapevines submitted to bunch-zone leaf removal reduced sunburn damage, did not influence yield, reduced bunch sugar content, increased must total acidity and enhanced wine sensory attributes (Coniberti et al. 2013). The authors suggested that, in kaolin covered berries, decreased sugar may be explained by lower water loss and increased acidity by lower berry temperatures. In fact, no differences in sugar content and must total acidity were detected compared to grapevines not submitted to bunch-zone leaf removal. In other conditions, kaolin was effective in reducing grapevines water stress without influencing sugar content and must total acidity (Glenn et al. 2010).

5. References

Alexander, P., J. A. Kitchener, and H. V. A. Briscoe. 1944a. Inert dust insecticides: Part 1. Mechanisms of action. Ann. Appl. Biol. 31: 143–149.

Alexander, P., J. A. Kitchener, and H. V. A. Briscoe. 1944b. Inert dust insecticides: Part 2. Mechanisms of action: The nature of effective dusts. Ann. Appl. Biol. 31: 150–156.

Baggiolini, M., V. Canevascini, Y. Tencalla, R. Caccia, G. Sobrio, and S. Cavalli. 1968. La cicadelle verte, *Empoasca flavescens* F. (Homopt., Typhlocybidae), agent d'altérations foliaires sur vigne. Recherche agronomique en Suisse 7 (1): 43–69.

Baillod, M., M. Jermini, P. Antonin, C. Linder, C. Mittaz, E. Carrera, V. Udry, and A. Schmid. 1993. Stratégies de lutte contre la cicadelle verte de la vigne, *Empoasca vitis* (Göthe). Efficacité des insecticides et problématique liée à la nuisibilité. Revue suisse de Viticulture, Arboriculture, Horticulture 25: 133–141.

Bengochea, P., S. Hernando, R. Saelices, Á. Adán, F. Budia, M. González-Núñez, E. Viñuela, and P. Medina. 2010. Side effects of kaolin on natural enemies found on olive crops. IOBC/WPRS Bull. 55: 61–67.

Bengochea, P., F. Amor, R. Saelices, S. Hernando, F. Budia, A. Adán, and P. Medina. 2013. Kaolin and copper-based products applications: Ecotoxicology on four natural enemies. Chemosphere 91: 1189–1195.

Bengochea, P., R. Saelices, F. Amor, Á. Adán, F. Budia, P. del Estal, E. Viñuela, and P. Medina. 2014. Non-target effects of kaolin and coppers applied on olive trees for the predatory lacewing *Chrysoperla carnea*. Biocontrol Sci. Technol. 24: 625–640.

Bostanian, N. J., and G. Racette. 2008. Particle films for managing arthropod pests of apple. J. Econ. Entomol. 101: 145–150.

Butler, C. D., F. J. Byrne, M. L. Keremane, R. F. Lee, and J. T. Trumble. 2011. Effects of insecticides on behavior of adult *Bactericera cockerelli* (Hemiptera: Triozidae) and transmission of *Candidatus* Liberibacter psyllaurous. J. Econ. Entomol. 104: 586–594.

Caleca, V., G. Lo Verde, V. Lo Verde, M. Palumbo Piccionello, and R. Rizzo. 2010. Control of *Bactrocera oleae* and *Ceratitis capitata* in organic orchards: use of clays and copper products. Acta Hortic. 873: 227–233.

Candolfi, M. P., M. Jermini, E. Carrera, and M. C. Candolfi-Vasconcelos. 1993. Grapevine leaf exchange, plant growth, yield, fruit quality and carbohydrate reserves influenced by the grape leafhopper, *Empoasca vitis*. Entomol. Exp. Appl. 69: 289–296.

Carle, P., and G. Moutous. 1965. Observation sur le mode de nutrition sur vigne de quatre espèces de cicadelles. Annales des Epiphyties 16: 333–354.

Cerutti, F., J. Baumgärtner, and V. Delucchi. 1988. Ricerche sull'ecosistema "vigneto" nel Ticino: I. Campionamento delle popolazioni di *Empoasca vitis* Goethe (Hom., Cicadellidae, Typhlocybinae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 61: 29–41.

Cerutti, F., J. Baumgärtner, and V. Delucchi. 1990. Ricerche sull'ecosistema 'vigneto' in Ticino: III. Biologia e fattori di mortalità di *Empoasca vitis* Goethe (Homoptera, Cicadellidae, Typhlocybinae). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 63: 43–54.

Cerutti, F., J. Baumgärtner, and V. Delucchi. 1991. The dynamics of grape leafhopper *Empoasca vitis* Göthe populations in Southern Switzerland and the implications for habitat management. Biocontrol Sci. Techn. 1: 177–194.

Chiappini, E., and E. Mazzoni. 2000. Differing morphology and ultrastructure of the male copulatory apparatus in species-groups of *Anagrus* Haliday (Hymenoptera: Mymaridae). J. Nat. Hist. 34: 1661–1676.

Chiappini, E., S. V. Triapitsyn, and A. Donev. 1996. Key to the Holarctic species of *Anagrus* Haliday (Hymenoptera: Mymaridae) with a review of the Nearctic and Palaearctic (other than European) species and descriptions of new taxa. J. Nat. Hist. 30: 551–595.

Coniberti A., V. Ferrari, E. Dellacassa, E. Boido, F. Carrau, V. Gepp, and E. Disegna. 2013. Kaolin over sun-exposed fruit affects berry temperature, must composition and wine sensory attributes of Sauvignon blanc. Europ. J. Agronomy 50: 75–81.

Costello, M. J. 2008. Regulated deficit irrigation and density of *Erythroneura* spp. (Hemiptera: Cicadellidae) on grape. J. Econ. Entomol. 101: 1287–1294.

David, W. A. L., and B. O. C. Gardiner. 1950. Factors influencing the action of dust insecticides. Bull. Entomol. Res. 41: 1–61.

Decante, D., and M. van Helden. 2001. Répartition intraparcellaire des cicadelles ravageurs du vignoble bordelais. IOBC/WPRS Bull. 24 (7): 203–210.

Decante, D., C. van Leeuwen, and M. van Helden. 2009. Influence of plot characteristics and surrounding vegetation on the intra-plot spatial distribution of *Empoasca vitis*. Agr. Forest Entomol. 11: 377–388.

Ebeling, W. 1971. Sorptive dusts for pest control. Annu. Rev. Entomol. 16: 123–158.

Fornasiero, D., C. Duso, A. Pozzebon, D. Tomasi, F. Gaiotti, and F. Pavan. 2012. Effects of irrigation on the seasonal abundance of *Empoasca vitis* in North-Italian vineyards. J. Econ. Entomol. 105: 176–185.

Fornasiero, D., F. Pavan, A. Pozzebon, P. Picotti, and C. Duso. 2016. Relative infestation level and sensitivity of grapevine cultivars to the leafhopper *Empoasca vitis* (Hemiptera: Cicadellidae). J. Econ. Entomol. 109: 416–425.

Fos, A., L. Delbac, P. Lecharpentier, V. Lecharpentier, and J. Stockel. 1997. Etude de la répartition spatiale d'*Empoasca vitis* Goethe (Homoptera, Typhlocybinae) et apports pour l'échantillonnage. I – Répartition sur le cep de vigne. Journal International des Sciences de la Vigne et du Vin 31: 119–125.

Girolami, V., C. Duso, E. Refatti, and R. Osler. 1989. Lotta integrata in viticoltura. Malattie della vite. IRIPA – COLDIRETTI, Treviso, Italy.

Glenn, D. M., and G. J. Puterka. 2005. Particle films: a new technology for agriculture. Hortic. Rev. 31: 1–44.

Glenn, D. M., G. Puterka, T. Vanderzwet, R. E. Byers, and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92: 759–771.

Glenn, D. M., N. Cooley, R. Walker, P. Clingeleffer, and K. Shellie. 2010. Impact of kaolin particle film and water deficit on wine grape water use efficiency and plant water relations. HortScience 45: 1178–1187.

Harben, P. W. 1995. The industrial minerals handbook: a guide to markets, specifications & prices. Arby Industrial Minerals Division Metal Bulletin. PLC, London.

Jubb, G. L., L. Danko, and C. W. Haeseler. 1983. Impact of *Erythroneura comes* Say (Homoptera: Cicadellidae) on caged 'Concord' grapevines. Environ. Entomol. 12: 1576–1580.

Knight, A. L., B. A. Christianson, T. R. Unruh, G. Puterka, and D. M. Glenn. 2001. Impact of seasonal kaolin particle films on apple pest management. Can. Entomol. 133: 413–428.

Lavezzaro, S., A. Morando, and G. Gallesio. 2006. Un quadriennio di prove di lotta contro la cicalina verde della vite in Piemonte. Atti Giornate Fitopatologiche 1: 117–122.

Lehmann, F., K.-J. Schirra, F. Lovis, and C. P. W. Zebitz. 2001. The green leafhopper *Empoasca vitis* Goethe – population dynamics in different zones of foliation and effects of insecticide treatments in vineyards. IOBC/WPRS Bull. 24 (7): 231–235.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models, 2nd ed., SAS Institute Inc, Cary NC.

Mahmoudi, M., A. Sahragard, H. Pezhman, and M. Ghadamyari. 2014. Efficacy of biorational insecticides against Dubas bug, *Ommatissus lybicus* (Hem.: Tropiduchidae) in a date palm orchard and evaluation of kaolin and mineral oil in the laboratory. J. Entomol. Soc. Iran 33 (4): 1–10.

Maletta, M., W. Tietjen, G. Ghidiu, K. Holmstorm, and W. Cowgill. 2004. Evaluation of controls for flea beetle on eggplant in an organic production system. Acta Hortic. 638: 341–346.

Maletta, M., M. Henninger, and K. Holmstorm. 2006. Potato leafhopper control and plastic mulch culture in organic potato production. HortTechnology 16: 199–204.

Marcotegui, A., I. Sánchez-Ramos, S. Pascual, C. E. Fernández, G. Cobos, I. Armendáriz, A. Cobo, and M. González-Núñez. 2015. Kaolin and potassium soap with thyme essential oil to control *Monosteira unicostata* and other phytophagous arthropods of almond trees in organic orchards. J. Pest Sci. 88: 753–765.

Markó, V., L. H. M. Blommers, S. Bogya, and H. Helsen. 2008. Kaolin particle films suppress many apple pests, disrupt natural enemies and promote woolly apple aphid. J. Appl. Entomol. 132: 26–35.

Markó, V., S. Bogya, E. Kondorosy, and L. H. M. Blommers. 2010. Side effects of kaolin particle films on apple orchard bug, beetle and spider communities. Int. J. Pest Manage. 56: 189–199.

Martinson, T. E., R. Dunst, A. Lakso, and G. English-Loeb. 1997. Impact of feeding injury by eastern grape leafhopper (Homoptera: Cicadellidae) on yield and juice quality of Concord grapes. Am. J. Enol. Vitic. 48: 291–302.

Mazzini, F. 2010. Rame, come cambiano le regole di impiego. L'Informatore Agrario 66 (20): 47–50.

Mazzoni, V., G. Anfora, C. Ioriatti, and A. Lucchi. 2008. Role of winter host plants in vineyard colonization and phenology of *Zygina rhamni* (Hemiptera: Cicadellidae: Typhlocybinae). Ann. Entomol. Soc. Am. 101: 1003–1009.

Mori, N., N. Bottura, G. Posenato, G. P. Sancassani, and V. Girolami. 2004. Lotta contro *Scaphoideus titanus* Ball nei vigneti a conduzione biologica. Atti Giornate Fitopatologiche 1: 111–116.

Moutous, G., and A. Fos. 1971. Essais de lutte chimique contre la Cicadelle de la Vigne *Empoasca flavescens* Fabr. Résultats 1970. Revue de Zoologie Agricole et de Pathologie Végétale 70 (1): 48–56.

Nateghi, M., F. Paknejad, and M. Moarefi. 2013. Effect of concentrations and time of kaolin spraying on wheat aphid. J. Biol. Environ. Sci. 7: 163–168.

Pascual, S., G. Cobos, E. Seris, and M. González-Núñez. 2010. Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove. J. Pest. Sci. 83: 121–133.

Pavan, F. 1994. Contributo allo studio delle cause di pullulazione di *Empoasca vitis* (Göthe) nei vigneti: influenza dei trattamenti antiperonosporici. Rivista di Viticoltura e di Enologia 47 (2): 39–47.

Pavan, F. 2001. The role of *Rubus* bushes in the life cycle of two typhlocybinae infesting European vineyards. Redia 83: 47–60.

Pavan, F., and P. Picotti. 2009. Influence of grapevine cultivars on the leafhopper *Empoasca vitis* and its egg parasitoids. BioControl 54: 55–63.

Pavan, F., G. Stefanelli, A. Villani, P. Gasparinetti, G. Colussi, D. Mucignat, D. Del Cont Bernard, and P. Mutton. 2000. Danni da *Empoasca vitis* (Göthe) (Homoptera: Cicadellidae) in vigneti dell'Italia nord-orientale e soglie d'intervento. Frustula Entomologica 21 (1998): 109–124.

Pavan, F., E. Cargnus, F. Kiaeian Moosavi, G. Bigot, F. Tacoli, and P. Zandigiacomo. 2016. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould. Bull. Insectol. 69: 107–115.

Pease, C. E., J. F. López-Olguín, I. Pérez-Moreno, and V. Marco-Mancebón. 2016. Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). J. Econ. Entomol. 109: 740–745.

Picotti, P., and F. Pavan. 1993. Studi su *Anagrus atomus* (Linnaeus) (Hymenoptera, Mymaridae) parassitoide oofago di *Empoasca vitis* (Goethe) (Homoptera, Cicadellidae) su vite. 1. Dinamica di popolazione in assenza di trattamenti insetticidi. Bollettino del Laboratorio di Entomologia agraria "Filippo Silvestri" 48: 105–115.

Pissinati, A., and M. U. Ventura. 2015. Control of cabbage aphid, *Brevicoryne brassicae* (L.) using kaolin and neem oil. J. Entomol. 12: 48–54.

Ponti, L., C. Ricci, F. Veronesi, and R. Torricelli. 2005. Natural hedges as an element of functional biodiversity in agroecosystems: the case of a Central Italy vineyard. Bull. Insectol. 58: 19–23.

Porcel, M., B. Cotes, and M. Campos. 2011. Biological and behavioral effects of kaolin particle film on larvae and adults of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Biol. Control. 59: 98–105.

Posenato, G., E. Marchesini, and N. Mori. 2006. Efficacia di thiamethoxam su *Empoasca vitis* a confronto con lambda-cyhalothrin, abamectina, indoxacarb e chlorpyrifos. Atti Giornate Fitopatologiche 1: 111–116.

Pozzebon, A., M. Pederiva, R. Moret, and C. Duso. 2011. The impact of a number of insecticides on *Empoasca vitis* populations in north-eastern Italy. IOBC/WPRS Bull. 67: 285–288.

Puterka, G. J., M. Reinke, D. Luvisi, M. A. Ciomperik, D. Bartels, L. Wendel, and D. M. Glenn. 2003. Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. Online. Plant Health Progress doi: 10.1094/PHP-2003-0321-01-RS.

Puterka, G. J., D. M. Glenn, and R. C. Pluta. 2005. Action of particle films on the biology and behavior of pear psylla (Homoptera: Psyllidae). J. Econ. Entomol. 98: 2079–2088.

Ryan, T. A. 1960. Significance tests for multiple comparison of proportions, variances, and other statistics. Psychol. Bull. 57: 318–328.

Sackett, T. E., C. M. Buddle, and C. Vincent. 2007. Effects of kaolin on the composition of generalist predator assemblages and parasitism of *Choristoneura rosaceana* (Lep., Tortricidae) in apple orchards. J. Appl. Entomol. 131: 478–485.

Schvester, D., G. Moutous, J. Bonfils, and P. Carle. 1962. Étude biologique des cicadelles de la vigne dans le sud-ouest de la France. Annales des Epiphyties 13: 205–237.

Sleezer, S., D. T. Johnson, B. Lewis, F. Goggin, C. Rothrock, and M. Savin. 2011. Foliar grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), seasonal biology, predictive model and management in the Ozarks region of the United States. Acta Hortic. 904: 151–156.

Tubajika, K. M., E. L. Civerolo, G. J. Puterka, J. M. Hashim, and D. A. Luvisi. 2007. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. Crop Prot. 26: 92–99.

Tyler-Julian, K., J. Funderburk, G. Frantz, and C. Mellinger. 2014. Evaluation of a push-pull strategy for the management of *Frankliniella bispinosa* (Thysanoptera: Thripidae) in bell peppers. Environ. Entomol. 43: 1364–1378.

van Helden, M., and D. Decante. 2001. The possibilities for conservation biocontrol as a management strategy against *Empoasca vitis*. IOBC/WPRS Bull. 24 (7): 291–297.

Vidano, C. 1958. Le cicaline italiane della vite. Hemiptera Typhlocibinae. Bollettino di Zoologia agraria e di Bachicoltura 1: 61–115.

Vidano, C. 1963. Alterazioni provocate da insetti in *Vitis* osservate, sperimentate e comparate. Annali della Facolta di Scienze agrarie, Universita di Torino 1: 513–644.

Vidano, C., and Arzone A. 1983. Biotaxonomy and epidemiology of Typhlocybinae on vine, pp. 75-85. *In* Knight, W. J., N. C. Pant, T. S. Robertson, and M. R. Wilson (eds) Proceedings of the 1st International workshop on biotaxonomy, classification and biology of leafhoppers and planthoppers (Auchenorrhyncha) of economic importance, London, 4-7 October 1982, Commonwalth Institute of Entomology, London, UK.

Viggiani, G. 1988. A preliminary classification of the Mymaridae (Hymenoptera: Chalcidoidea) based on the external male genitalia characters. Bollettino del Laboratorio di Entomologia agraria "Filippo Silvestri" 45: 141–148.

Wood, M., and McBride J. 2001. Scientists sharpen strategies to sabotage: glassy-winged sharpshooter. Agricultural Research Magazine 49 (11): 20–22.

Zanolli, P., and F. Pavan. 2011. Autumnal emergence of *Anagrus* wasps, egg parasitoids of *Empoasca vitis*, from grapevine leaves and their migration towards brambles. Agric. Forest Entomol. 13: 423–433.

Table 1. Preventive application trials. Results of repeated measures ANOVA performed on *Empoasca vitis* and *Zygina rhamni* nymph densities observed on leaves.

Source of variation	**Vineyard A**			**Vineyard B**		
	F	df	*P*	*F*	df	*P*
Empoasca vitis						
Kaolin application	179.78	2, 71.8	< 0.001	141.51	2, 68.7	< 0.001
Leaf removal	0.13	1, 71.8	0.720	0.19	1, 68.7	0.668
Kaolin app.*Leaf rem.	2.54	2, 71.8	0.086	1.79	2, 68.7	0.174
Canopy zone	75.17	1, 71.8	< 0.001	35.19	1, 68.7	< 0.001
Canopy zone*Kaolin app.	69.37	2, 71.8	< 0.001	22.30	2, 68.7	< 0.001
Canopy zone*Leaf rem.	0.03	1, 71.8	0.868	0.75	1, 68.7	0.389
Canopy zone*Kaolin app.*Leaf rem.	0.69	2, 71.8	0.504	0.25	2, 68.7	0.782
Time	53.48	5, 173	< 0.001	33.60	5, 168	< 0.001
Time*Kaolin app.	22.07	10, 181	< 0.001	15.14	10, 177	< 0.001
Time*Leaf rem.	1.21	5, 173	0.309	1.65	5, 168	0.150
Time*Kaolin app.*Leaf rem.	0.94	10, 181	0.502	1.24	10, 177	0.268
Canopy zone*Time	29.58	5, 173	< 0.001	16.40	5, 168	< 0.001
Canopy zone*Kaolin app.*Time	7.54	10, 181	< 0.001	4.98	10, 177	< 0.001
Canopy zone*Leaf rem.*Time	1.02	5, 173	0.410	0.15	5, 168	0.981
Canopy zone*Kaolin app.*Leaf rem.*Time	1.32	10, 181	0.220	0.62	10, 177	0.799
Zygina rhamni						
Kaolin application	38.88	2, 88.5	< 0.001	18.75	2, 78.9	< 0.001
Leaf removal	0.61	1, 88.5	0.438	1.94	1, 78.9	0.167
Kaolin app.*Leaf rem.	0.49	2, 88.5	0.616	1.02	2, 78.9	0.364
Canopy zone	5.50	1, 88.5	0.021	31.19	1, 78.9	< 0.001
Canopy zone*Kaolin app.	15.45	2, 88.5	< 0.001	13.51	2, 78.9	< 0.001
Canopy zone*Leaf rem.	0.12	1, 88.5	0.733	3.95	1, 78.9	0.051
Canopy zone*Kaolin app.*Leaf rem.	0.22	2, 88.5	0.803	0.86	2, 78.9	0.428
Time	27.81	5, 173	< 0.001	5.04	5, 165	< 0.001
Time*Kaolin app.	4.62	10, 182	< 0.001	4.02	10, 176	< 0.001
Time*Leaf rem.	1.63	5, 173	0.154	1.21	5, 165	0.309
Time*Kaolin app.*Leaf rem.	0.54	10, 182	0.859	1.38	10, 176	0.192
Canopy zone*Time	5.14	5, 173	< 0.001	1.47	5, 165	0.204
Canopy zone*Kaolin app.*Time	3.62	10, 182	< 0.001	1.31	10, 176	0.227
Canopy zone*Leaf rem.*Time	0.72	5, 173	0.606	0.56	5, 165	0.729
Canopy zone*Kaolin app.*Leaf rem.*Time	1.10	10, 182	0.366	1.28	10, 176	0.245

Table 2. Preventive application trials. Results of repeated measures ANOVA performed on *Empoasca vitis*, *Zygina rhamni, Anagrus* spp. adult captures on yellow sticky traps.

	Vineyard A			**Vineyard B**		
	F	df	*P*	*F*	df	*P*
Empoasca vitis						
Kaolin application	24.57	1, 21.1	< 0.001	4.41	1, 23	0.047
Leaf removal	0.81	1, 28.1	0.225	0.00	1, 23	0.979
Kaolin app.*Leaf rem.	0.58	1, 28.1	0.577	1.15	1, 23	0.295
Time	8.94	9, 98.9	< 0.001	125.24	9, 101	< 0.001
Time*Kaolin app.	0.90	9, 98.9	< 0.001	4.96	9, 101	< 0.001
Time*Leaf rem.	0.46	9, 98.9	0.761	1.22	9, 101	0.293
Time*Kaolin app.*Leaf rem.	0.76	9, 98.9	0.702	0.95	9, 101	0.485
Zygina rhamni						
Kaolin application	2.93	1, 28	0.098	12.62	1, 22.8	0.002
Leaf removal	6.68	1, 28	0.015	0.02	1, 22.8	0.903
Kaolin app.*Leaf rem.	0.06	1, 28	0.816	0.00	1, 22.8	0.952
Time	47.77	9, 99.6	< 0.001	23.96	9, 100	< 0.001
Time*Kaolin app.	1.66	9, 99.6	0.108	2.34	9, 100	0.019
Time*Leaf rem.	0.75	9, 99.6	0.660	0.55	9, 100	0.838
Time*Kaolin app.*Leaf rem.	0.36	9, 99.6	0.953	0.56	9, 100	0.827
***Anagrus* spp.**						
Kaolin application	3.01	1, 28.1	0.094	2.45	1, 32.8	0.127
Leaf removal	0.81	1, 28.1	0.375	0.23	1, 32.8	0.638
Kaolin app.*Leaf rem.	0.58	1, 28.1	0.453	0.00	1, 32.8	0.979
Time	8.94	9, 98.9	< 0.001	15.36	9, 95.2	< 0.001
Time*Kaolin app.	0.90	9, 98.9	0.524	0.61	9, 95.2	0.784
Time*Leaf rem.	0.46	9, 98.9	0.898	0.40	9, 95.2	0.934
Time*Kaolin app.*Leaf rem.	0.76	9, 98.9	0.655	0.53	9, 95.2	0.846

Table 3. Preventive application trials. Results of two-way ANOVA performed on leafhopper eggs laid and hatched on 100 leaves (mean±standard deviation) and the percentage of parasitized eggs.

	Kaolin			Leaf-removal		
	Control	Kaolin WC	ANOVA	With	Without	ANOVA
Vineyard A						
Eggs laid	216.5±54.9	214.5±50.3	*F* = 0.006; df = 1, 12; *P* = 0.94	201.3±46.5	229.8±54.0	*F* = 1.2; df = 1, 12; *P* = 0.30
Hatched eggs	102.5±43.6	116.5±35.6	*F* = 0.47; df = 1, 12; *P* = 0.51	103.3±43.6	115.8±35.6	*F* = 0.37; df = 1, 12; *P* = 0.55
Percentage of parasitized eggs	53.4±7.1	46.7±8.7	*F* = 2.44; df = 1, 12; *P* = 0.14	50.0±9.8	50.2±7.5	*F* = 0.001; df = 1, 12; *P* = 0.98
Vineyard B						
Eggs laid	126.8±55.5	102.0±21.6	*F* = 1.2; df = 1, 12; *P* = 0.30	113.8±52.9	115.0±33.2	*F* = 0.003; df = 1, 12; *P* = 0.96
Hatched eggs	76.8±37.4	68.0±20.1	*F* = 0.27; df = 1, 12; *P* = 0.61	74.3±37.4	70.5±24.4	*F* = 0.05; df = 1, 12; *P* = 0.83
Percentage of parasitized eggs	40.8±12.4	33.5±12.8	*F* = 1.18; df = 1, 12; *P* = 0.30	35.7±9.8	38.6±15.8	*F* = 0.22; df = 1, 12; *P* = 0.65

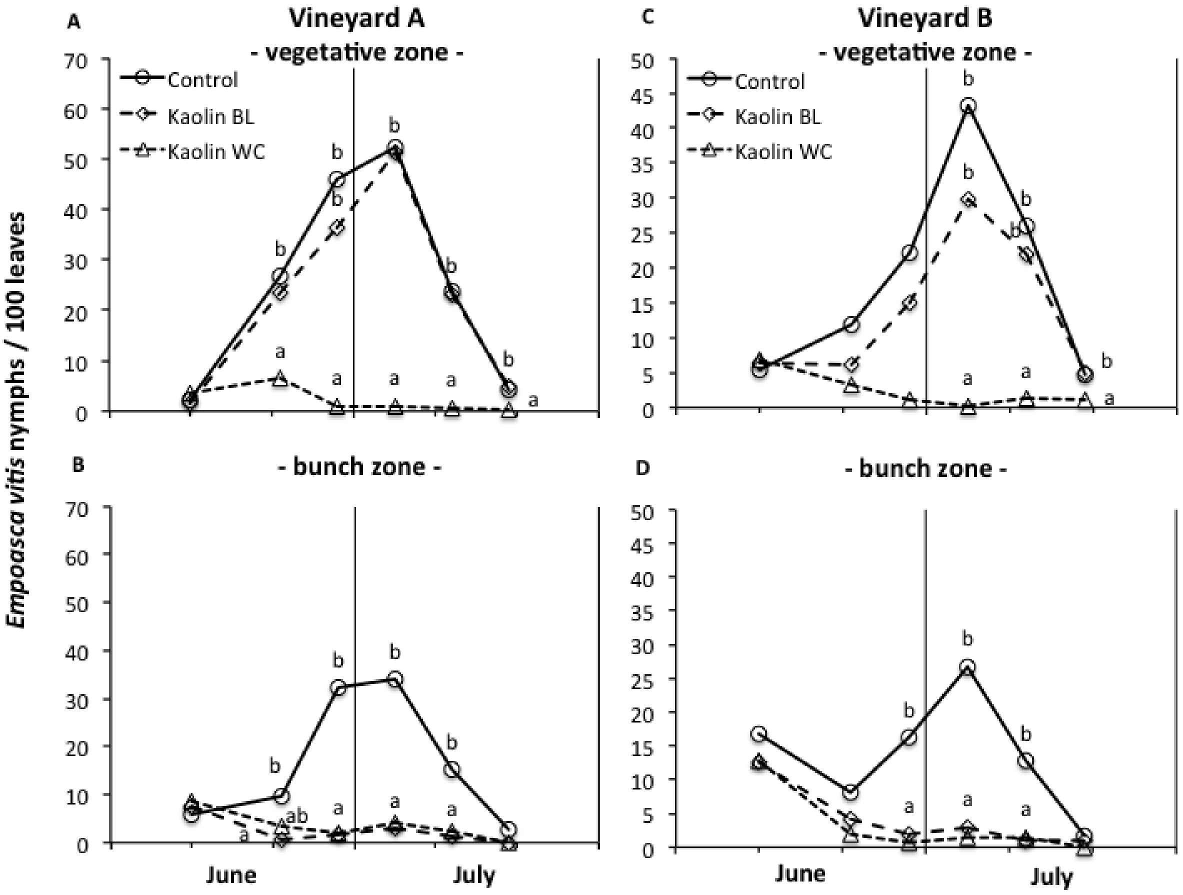

Fig. 1. (A–D) Preventive application trials. *Empoasca vitis* nymphs recorded during the sampling period in two vineyards, in two canopy-zones and under three treatments. Kaolin was applied on 18 and 24 June. Different letters among treatments at the same date indicate significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

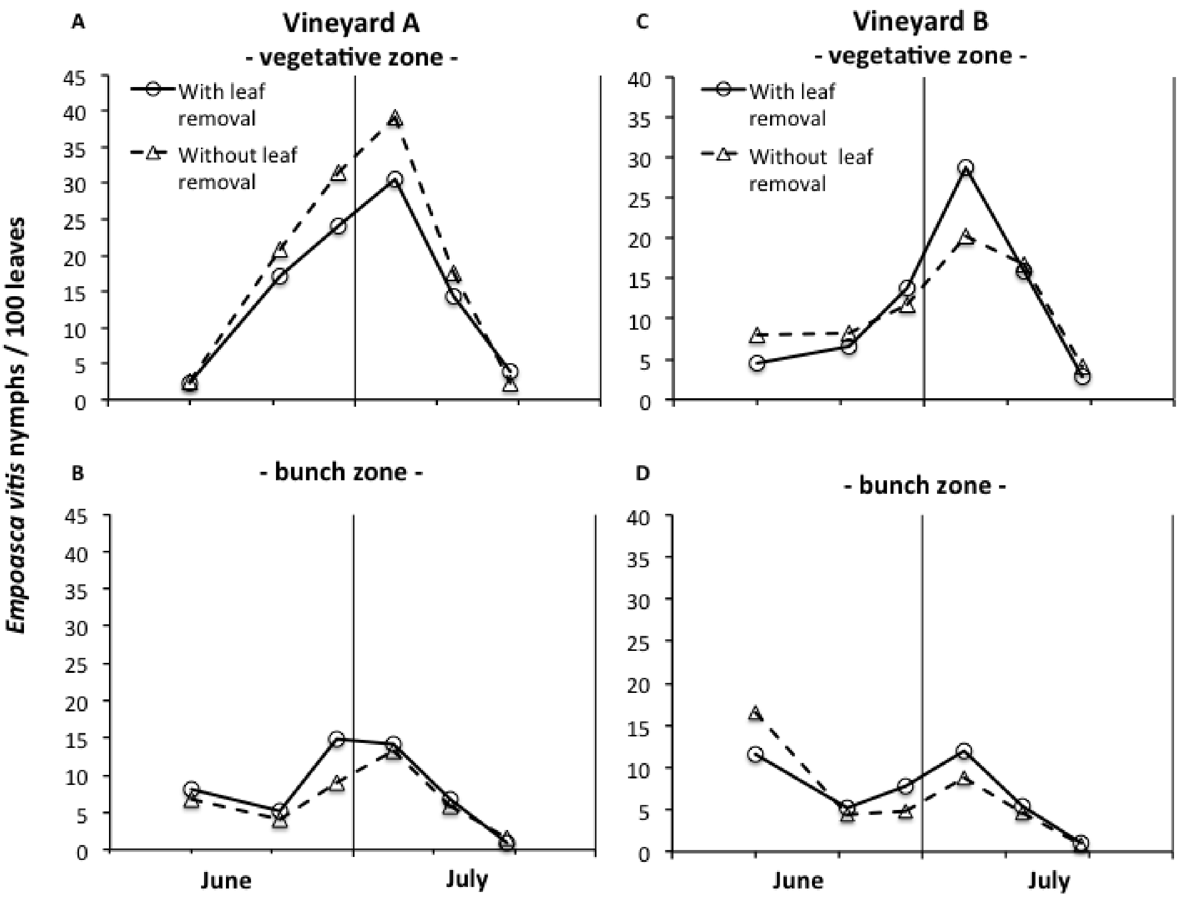

Fig. 2. (A–D) Preventive application trials. *Empoasca vitis* nymphs recorded during the sampling period in two vineyards, in the two leaf position and under two treatments. Bunch-zone leaf removal was performed on 17 June. Different letters among treatments at the same date indicate significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

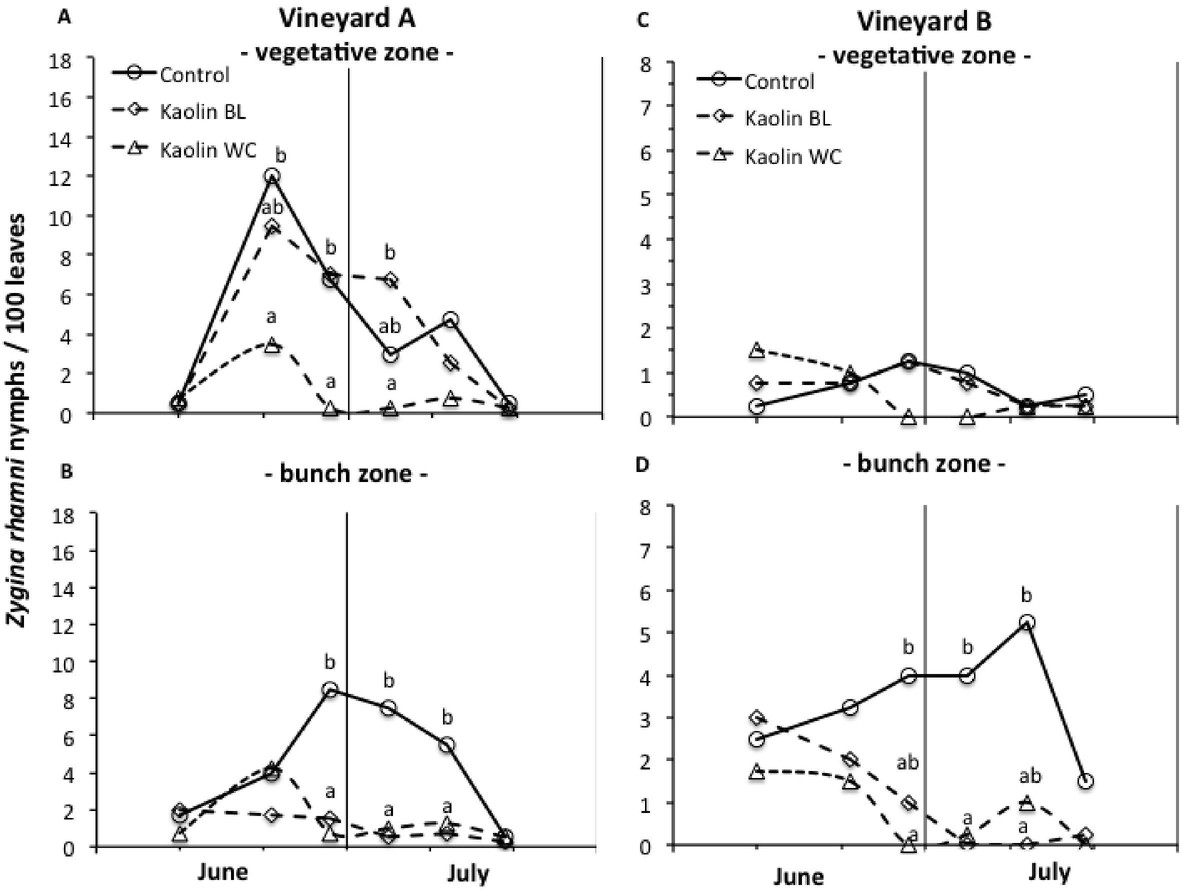

Fig. 3. (A–D) Preventive application trials. *Zygina rhamni* nymphs recorded during the sampling period in two vineyards, in two canopy-zones and under three treatments. Kaolin was applied on 18 and 24 June. Different letters among treatments at the same date indicate significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

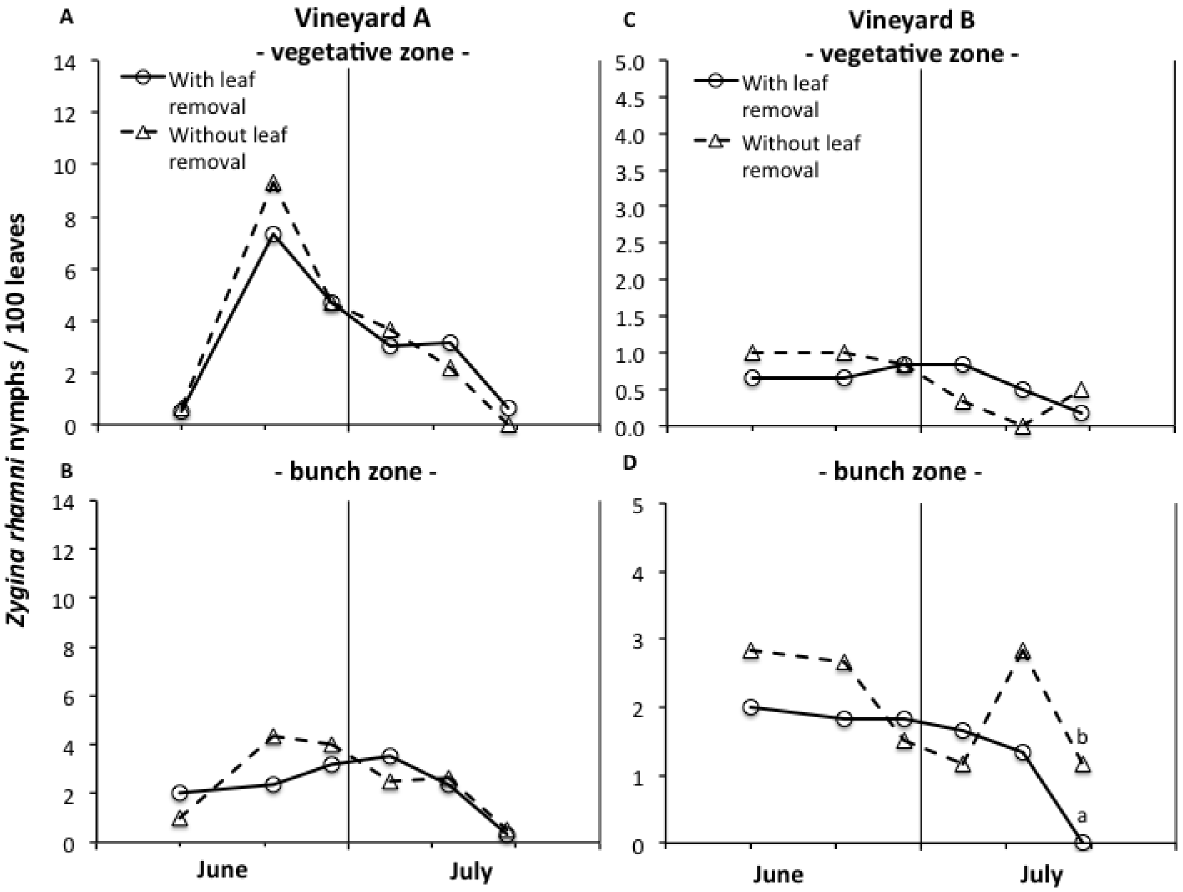

Fig. 4. (A–D) Preventive application trials. *Zygina rhamni* nymphs recorded during the sampling period in two vineyards, in the two leaf position and under two treatments. Bunch-zone leaf removal was performed on 17 June. Different letters among treatments at the same date indicate significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

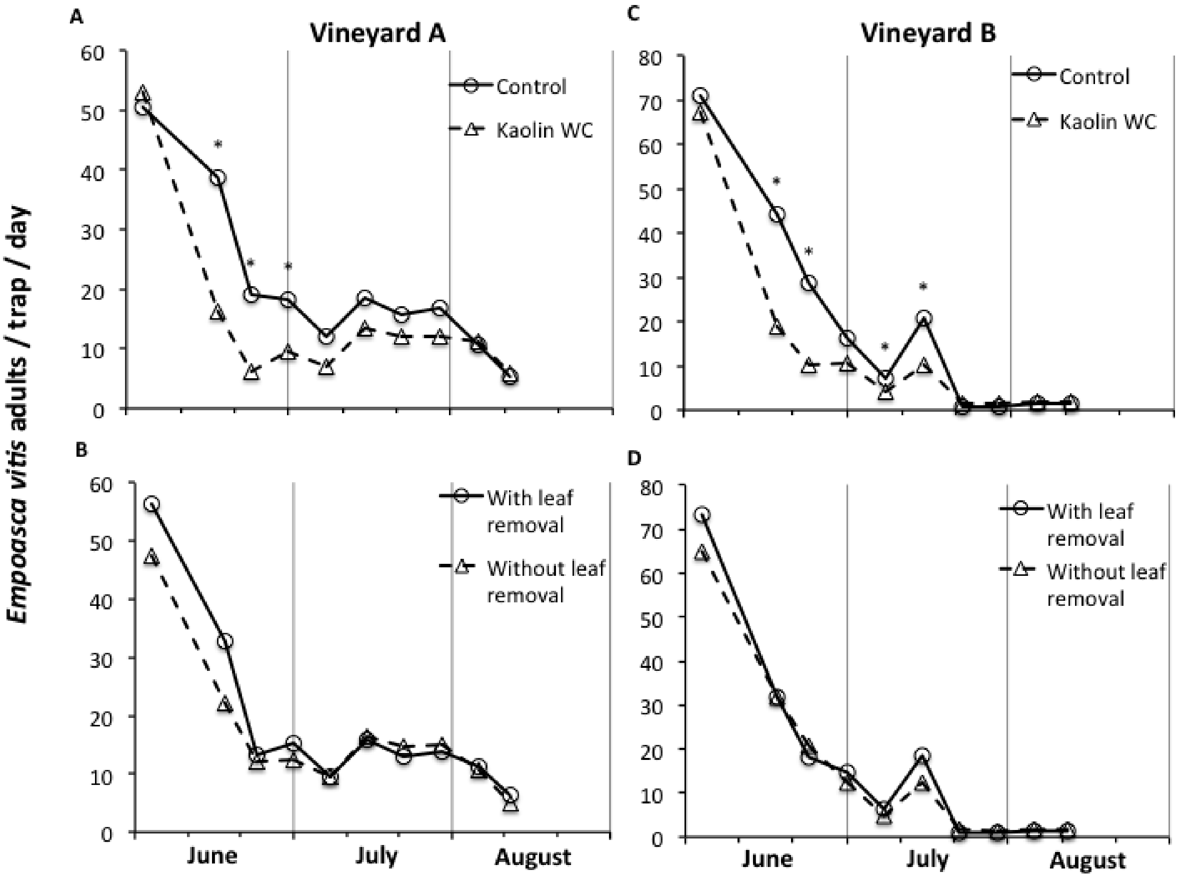

Fig. 5. (A–D) Preventive application trials. Captures of *Empoasca vitis* by yellow sticky traps recorded from June to the middle of August in two vineyards, in the control and kaolin whole-canopy (WC) and in plots with or without bunch-zone leaf removal. Kaolin was applied on 18 and 24 June and the leaf removal on 17 June. Asterisks * indicate dates with significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

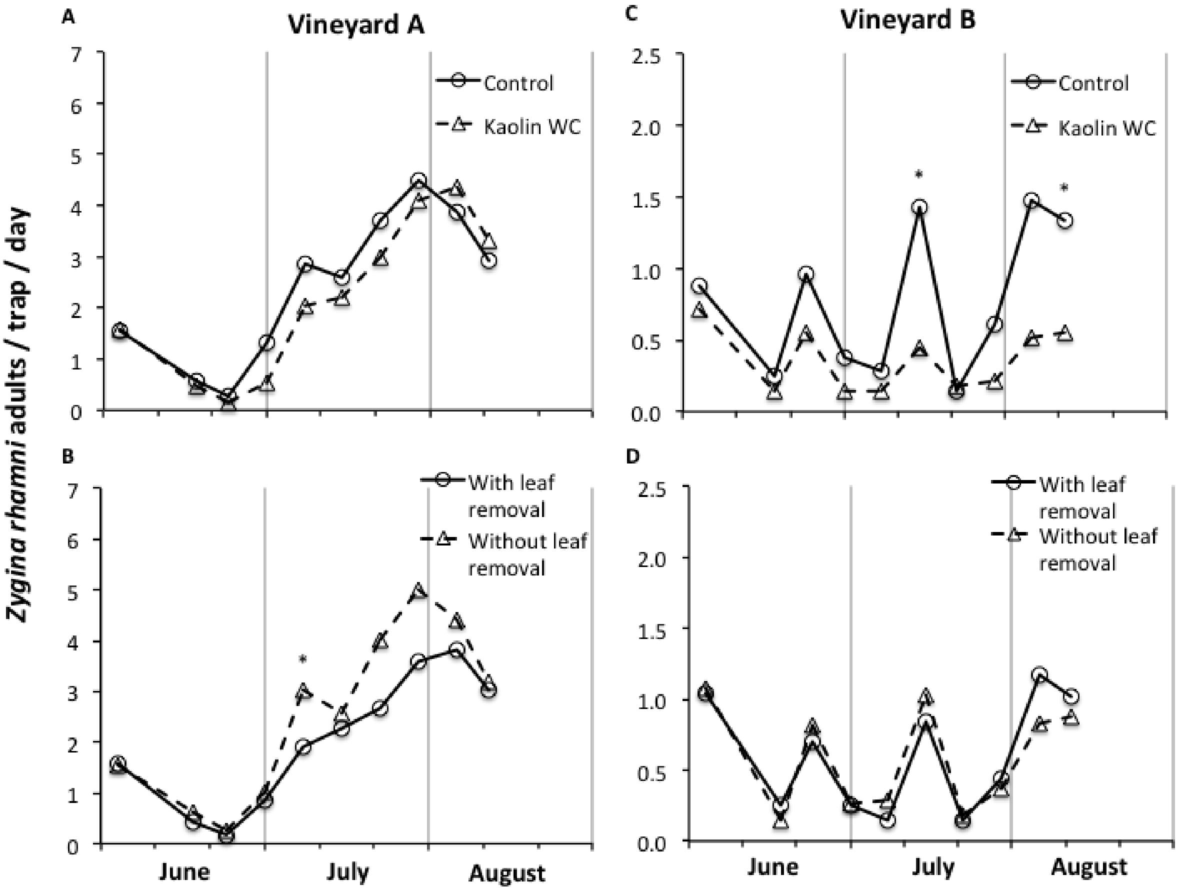

Fig. 6. (A–D) Preventive application trials. Captures of *Zygina rhamni* by yellow sticky traps recorded from June to early August in two vineyards, in the control and kaolin whole-canopy (WC) and in plots with or without bunch-zone leaf removal. Kaolin was applied on 18 and 24 June and the leaf removal on 17 June. Asterisks * indicate dates with significant differences according to t-test on the least square mean with Bonferroni correction (α = 0.05).

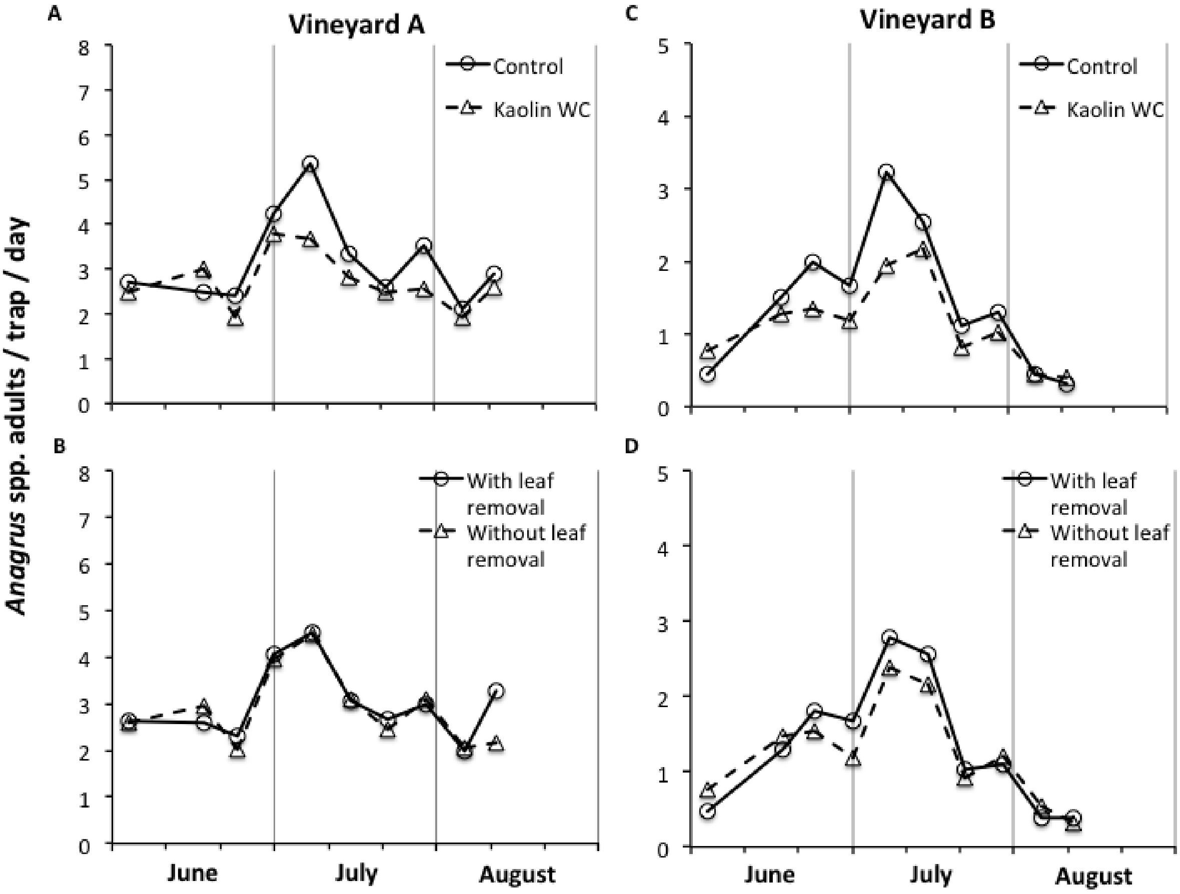

Fig. 7. (A–D) Preventive application trials. Captures of *Anagrus* spp. by yellow sticky traps recorded from June to early August in two vineyards, in the control and kaolin whole-canopy (WC) and in plots with or without bunch-zone leaf removal. Kaolin was applied on 18 and 24 June. Asterisks * indicate dates with significant differences according to t-test on the least square mean with Bonferroni correction ($\alpha = 0.05$).

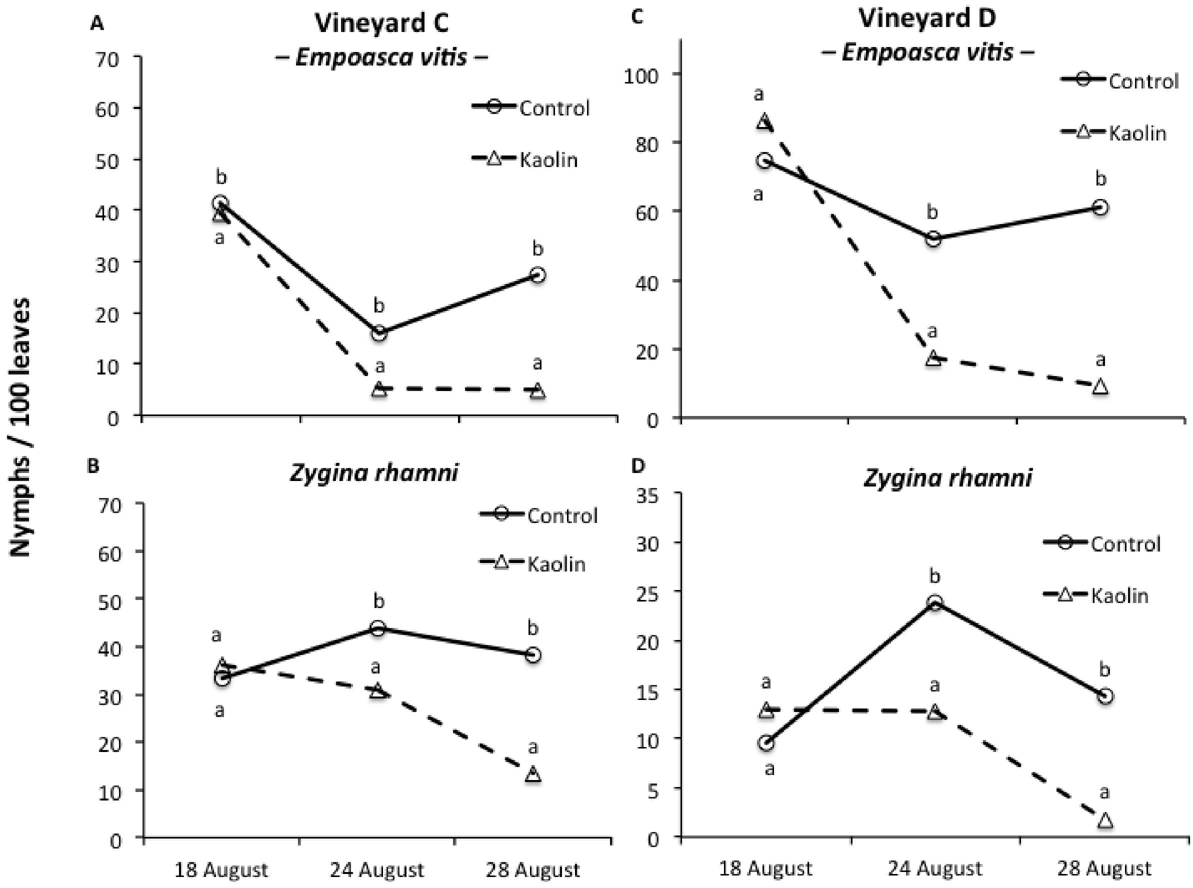

Fig. 8. (A–D) Curative application trials. *Empoasca vitis* and *Zygina rhamni* nymphs recorded in two vineyards and in the control and kaolin. Kaolin was applied on 19 and 24 August. Different small letters among treatments at the same date indicate significant differences according to a t-test ($\alpha = 0.05$).

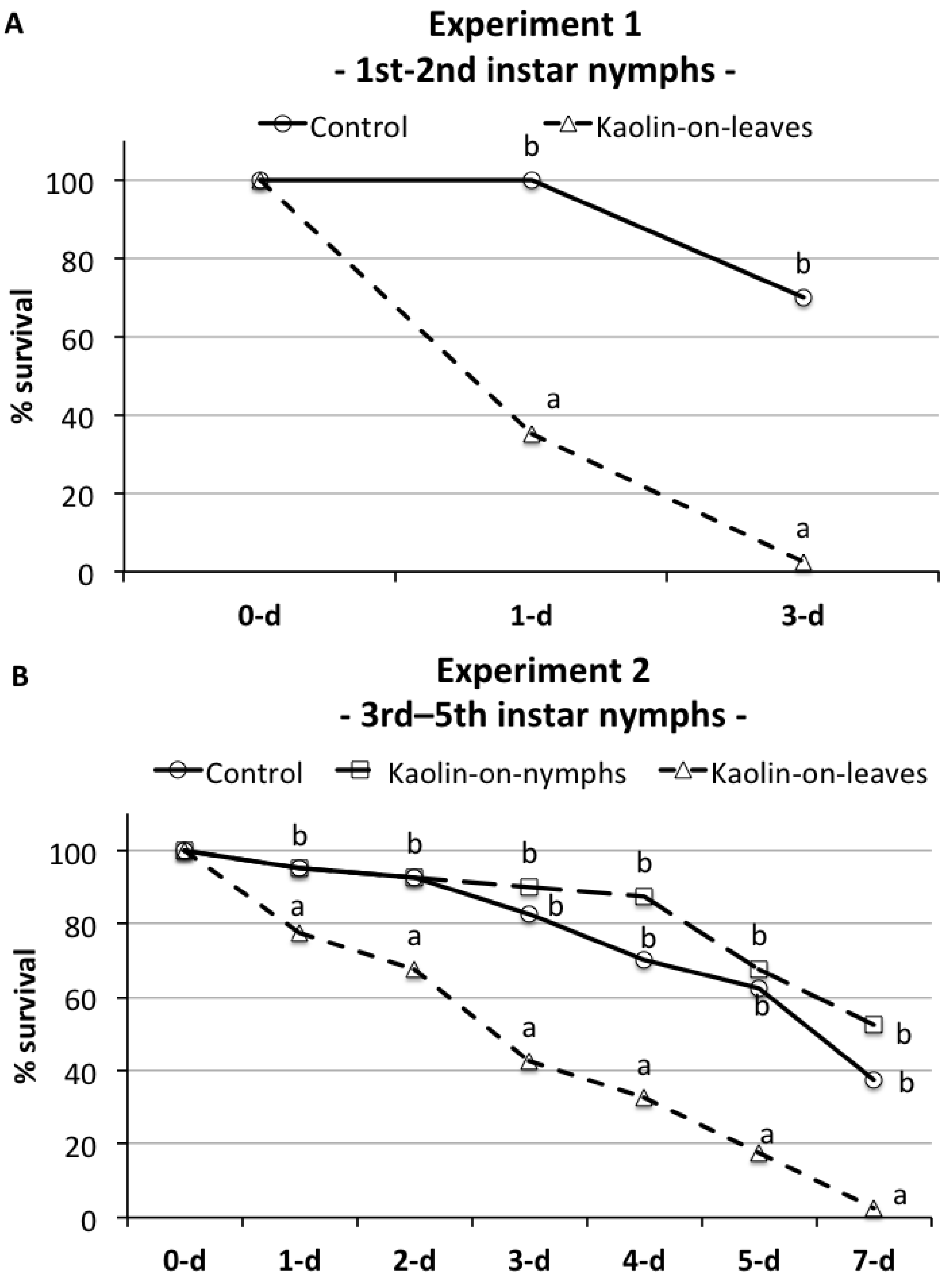

Fig. 9. (A–B) Laboratory experiments. Survival of *Empoasca vitis* nymphs in different treatments and days (d). Different small letters among treatments in the same time period indicate significant differences according to Fisher's exact test (A) or Ryan's test (B) ($\alpha = 0.05$), respectively.

Chapter 3 - Control of *Scaphoideus titanus* (Hemiptera: Cicadellidae) with natural products in organic vineyards

Chapter 3 is formatted according to the journal author guidelines and was published as:

Federico Tacoli, Nicola Mori, Alberto Pozzebon, Elena Cargnus, Sarah Da Vià, Pietro Zandigiacomo, Carlo Duso and Francesco Pavan (2017). Control of *Scaphoideus titanus* (Hemiptera: Cicadellidae) with natural insecticides in organic vineyards. Insects, 8, 129. DOI: 10.3390/insects8040129

Abstract

The leafhopper *Scaphoideus titanus* is the vector of '*Candidatus* Phytoplasma vitis', the causal agent of Flavescence dorée (FD) a key disease for European viticulture. In organic vineyards, the control of *S. titanus* relies mostly on the use of pyrethrins that have suboptimal efficacy. During 2016, three field trials were conducted to evaluate the efficacy of kaolin, orange oil, insecticidal soap and spinosad against *S. titanus* nymphs, in comparison with pyrethrins. The activity of kaolin was evaluated also in the laboratory. In all field trials, kaolin had an efficacy against nymphs comparable to pyrethrins, while the other products were not effective. Laboratory results confirmed that kaolin increased nymph mortality. In organic vineyards, kaolin and pyrethrins are valuable tools in the management of FD. Nevertheless, their efficacy is lower compared to that of the synthetic insecticides used in conventional viticulture. Therefore, further research should be conducted in order to identify alternatives to synthetic insecticides for *S. titanus* control in the context of organic viticulture.

1. Introduction

The leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) is the vector of '*Candidatus* Phytoplasma vitis', the causal agent of Flavescence dorée (FD), which is a Grapevine Yellows Disease (GYD) that causes severe damage in European vineyards [1–4].

Flavescence-dorée phytoplasma is a quarantine disease in the European and Mediterranean Plant Protection Organization (EPPO) region [5] and control measures are mandatory in some European countries [6]. The FD control strategy in vineyards relies mostly on *S. titanus* control and on the roguing of symptomatic grapevines, from which the vector can acquire the phytoplasma [6–9]. However, since insecticide applications are poorly effective against infected adults migrating into vineyards [10–12], the strategy adopted is effective only if external sources of infected individuals (i.e., infected vineyards, both abandoned or cultivated but untreated against *S. titanus*, and wild American grapevines growing in hedgerows and groves) are previously removed.

In Italian conventional vineyards, one to two insecticide applications against *S. titanus* can keep the populations of this vector and the percentage of FD-symptomatic grapevines at acceptable levels [11,13,14]. Infected grapevines can also die or recover, but this latter capability varies across cultivars. In cultivars with a high incidence of recovery, the chemical control of *S. titanus* can bring the percentage of symptomatic grapevines back to acceptable levels without their roguing [15]. The high efficacy of insecticide applications in vineyards is due to the biology of *S. titanus*: (i) it is monophagous on *Vitis* sp. plants, therefore external sources can be easily removed, and (ii) it completes only one generation a year after being overwintered as eggs [6,16,17]. The control strategy aims to kill nymphs at the completion of the latency period, that is, before they have reached the fourth-fifth instars, which is the point that they become infective [13,18]. The need for more than one insecticide application derives from the prolonged egg-hatching period, which lasts for over 45 days [17]. The timing of insecticide applications is usually based on nymph samplings. In Italy, the first insecticide application is performed at the appearance of third instar nymphs and the second application occurs after two or three weeks to kill the nymphs hatched after the first application [13].

In conventional vineyards, an effective control of *S. titanus* nymphs is ensured by the use of organophosphates, pyrethroids and neonicotinoids [19–23]. All these insecticides are characterized by a long residual activity.

In organic vineyards, the control of *S. titanus* is difficult and often higher population levels are observed as compared to conventional vineyards, despite several insecticide applications [11]. The most effective insecticides in organic viticulture are pyrethrins, which are used alone or in combination with piperonyil butoxide, mineral oil or sesame oil [21,22,24–29]. However, the effectiveness of pyrethrins is lower than synthetic insecticides [11,22], because the former exert a marked knock-down effect when nymphs are directly hit, but the effectiveness declines to low levels when nymphs are kept in contact with fresh residues [26]. The absence of residual activity in pyrethrins mandates many applications to cover the entire egg-hatching period adequately. Mineral oil, spinosad, azadirachtin and *Beauveria bassiana* are considered less effective than pyrethrins or totally ineffective [22,24–28]. The need for repeated pyrethrin applications to overcome the low persistence of this class of insecticides may result in detrimental effects on predatory mites of the Phytoseiidae family [26,28,30]. Considering both the low efficacy and the toxicity to non-target organisms of pyrethrins, the identification of other natural products is necessary.

Recently, natural products such as kaolin, essential oils and insecticidal soap (i.e., potassium salts of fatty acids) have been found to be effective against leafhoppers or other Hemiptera [31–35]. In particular, a high efficacy of kaolin against the leafhoppers *Empoasca vitis* (Göthe) and *Zygina rhamni* Ferrari (Hemiptera: Cicadellidae) was observed both in vineyards and in the laboratory [32]. In the laboratory, plant essential oils have been found to exhibit a toxic activity against mealybugs (Hemiptera: Pseudococcidae) [33]. Insecticidal soap was effective in the control of some Pentatomidae under field conditions [34] and *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in the laboratory [35]. In the present study, the efficacy of kaolin, orange oil, insecticidal soap and spinosad against *S. titanus* nymphs was compared to pyrethrins in field trials. To evaluate if kaolin had any effects on egg laying by females, nymph population levels in the kaolin and control were assessed in the year following the kaolin applications. The activity of kaolin on nymphs was also evaluated in the laboratory.

2. Materials and methods

2.1. Field trials

In 2016, three field trials were carried out in vineyards located in north-eastern Italy to evaluate the efficacy of five natural products (Table 1) against *S. titanus* in comparison to an untreated control. Vineyard I (Togliano, Udine district, 46°06”45”N, 13°24’40”E, 140 m a.s.l., cultivar Merlot) is a 15-yr-old conventional vineyard with grapevines growing under the Guyot training system with distances between and along the rows of 2.4 m and 0.7 m, respectively. Vineyard II (Nimis, Udine district, 46°11’34”N, 13°15’42”E, 200 m a.s.l., cultivar Verduzzo Friulano) is a 15-yr-old conventional vineyard with grapevines growing under the Guyot training system with distances between and along the rows of 2.9 m and 0.8 m, respectively. Vineyard III

(Lonigo, Vicenza district, 45°24’04”N, 11°23’26”E, 31 m a.s.l., cultivar Garganega) is a 20-yr-old organic vineyard with grapevines growing under the “Pergola” training system with distances between and along the rows of 4.0 m and 1.0 m, respectively. In all vineyards, a standard fungicide program was followed and no insecticides were applied during the growing season.

In all trials, a randomized block design with four replicates was adopted. Each block (row) was divided into 6 plots of 20 (vineyard I) or 16 (vineyard II) or 15 (vineyard III) grapevines and product applications were planned as described in Table 1. The timing of the applications was based on the appearance of different *S. titanus* instar nymphs, in particular: (A) first-instar nymphs (26 May in vineyard I, 25 May in vineyard II and 26 May in vineyard III); (B) second-instar nymphs (1 June in vineyard I, 3 June in vineyard II and 1 June in vineyard III); (C) third-instar nymphs (8 June in vineyard I, 8 June in vineyard II and 10 June in vineyard III); and (D) fourth-instar nymphs (14 June in vineyard I, 14 June in vineyard II and 15 June in vineyard III). All products were applied using a backpack sprayer (M1200, Cifarelli s.p.a., Voghera, PV, Italy) at a rate of 1000 L/ha spraying the canopy and the suckers growing along the vertical trunk.

In all vineyards, *S. titanus* nymphs were sampled before application timing A (25 May in vineyard I, 3 June in vineyard II and 26 of May in vineyard III) and weekly up to one week after application timing D (i.e., 28 June in vineyard I, 6 July in vineyard II and 30 June in vineyard III). Sampling was conducted on the 10 central grapevines of each plot. *Scaphoideus titanus* nymphs were counted on sucker leaves. Suckers were chosen as sampling units because in spring they host the highest nymph density [13,36]. In vineyards I and II, five sucker leaves per grapevine were sampled for a total of 200 leaves per treatment. In vineyard III, all leaves of 10 suckers per plot were sampled for a total of 40 suckers per treatment being the population density too low to use leaf as sampling unit.

In vineyards I and II, *S. titanus* nymphs were also sampled in early June of 2017 in the plots that in 2016 belonged to the control and the kaolin. In vineyard III, this sampling was not done because a late frost heavily damaged suckers.

2.2. Laboratory bioassay

A laboratory bioassay was carried out to evaluate the efficacy of kaolin against *S. titanus* nymphs. Mortality of first-instar and second-instar nymphs was compared in two treatments: (i) nymphs placed on kaolin-treated leaves (kaolin); and (ii) nymphs placed on water-treated leaves (control). The sample size was equal to 50 leaves per treatment. For this purpose, 100 insecticide-free grapevine leaves were collected from vineyard I. In the laboratory, each leaf was visually checked to ensure the absence of *S. titanus* individuals. Fifty leaves were sprayed with kaolin and

50 with water, and each were inserted individually into transparent self-sealing plastic bags (20 × 33 cm). The kaolin application was done at a 4% W:V (Surround WP:water) concentration with a hand sprayer to run-off. The *S. titanus* nymphs used in the bioassay were collected from insecticide-free leaves picked in the same vineyard. First- and second-instar nymphs were randomly chosen on these leaves and a single individual was induced to move onto each bagged leaf with a brush. After 1, 2, 3, 6 and 9 days from the beginning of the bioassay, the bags were checked to note whether nymphs were alive or dead.

2.3. Statistical analyses

Statistical analyses were performed with Microsoft Excel 2013 for Windows (Microsoft Corporation 2013, Redmond, WA, USA) and SAS (v 9.4, SAS Institute, Cary, NC, USA).

Data collected in the field trials in 2016 were analyzed using mixed linear models performed with the PROC MIXED (SAS Institute 9.4). In modelling treatment, time and their interactions were considered as sources of variation and F tests were used to evaluate their effects ($\alpha = 0.05$). Numbers of *S. titanus* nymphs were considered as response variable with repeated measures made at different times, i.e. sampling dates. Treatments were compared using a *t*-test to the least-square means with Bonferroni adjustment of the *p*-values ($\alpha = 0.05$). Data collected in the spring of 2017 were compared with a *t*-test. Data were log $(x + 1)$ transformed prior to the analyses.

Data collected in the laboratory bioassay were compared with a Fisher's exact test and the mortality percentage of nymphs was calculated following Abbott [37].

3. Results

3.1. Field trials

Vineyard I. During the sampling period, significant differences were recorded among treatments ($F_{5,19.7} = 9.52$, $p < 0.0001$) (Figure. 1). Considering the overall trial period, only kaolin and pyrethrins significantly reduced *S. titanus* nymph densities compared to the control. The time effect was significant ($F_{5,74.2} = 167.51$, $p < 0.0001$) because *S. titanus* numbers were low at the beginning of the trial, increased until the fourth sampling date and were very low on the last sampling date. A significant interaction time*treatment was found ($F_{25,76.5} = 7.22$, $p < 0.0001$) because the efficacy of the treatments varied over time. In the pyrethrin-treated plots, population densities dropped to low numbers after the first application (timing C), but on the subsequent sampling dates they rose to levels similar to the other treatments, despite a second application (timing D). Kaolin-treated plots showed nymph densities lower than the control on the three sampling dates after the first application (timing A), but not on the last sampling date.

In the spring of 2017, no significant difference in nymph populations was observed between the plots treated with kaolin in the previous year and the control (mean ± SD nymphs per leaf, 1.12 ± 0.41 vs. 1.48 ± 0.18) ($t_6 = 1.59, p = 0.16$).

Vineyard II. During the sampling period, significant differences were recorded among treatments ($F_{5,36.4} = 6.13$, $p = 0.0003$) (Figure. 2). Considering the overall trial period, only kaolin significantly reduced *S. titanus* nymph densities compared to the control. The time effect was significant ($F_{5,83.1} = 37.9$, $p < 0.0001$) because *S. titanus* numbers were low at the beginning of the trial, increased until the fourth sampling date and were very low on the last sampling date. The time*treatment interaction was not significant ($F_{25,84.9} = 0.85, p = 0.67$).

In the spring of 2017, no significant difference in nymph populations was observed between the plots treated with kaolin in the previous year and the control (mean ± SD nymphs per leaf, 0.20 ± 0.04 vs. 0.18 ± 0.10) ($t_6 = 0.72, p = 0.50$).

Vineyard III. During the sampling period significant differences were recorded among treatments ($F_{5,40.1} = 4.29$, $p = 0.0032$) (Figure. 3). Considering the overall trial period, only kaolin and pyrethrins significantly reduced *S. titanus* nymph densities compared to the control. The time effect was significant ($F_{5,86.1} = 34.34$, $p < 0.0001$) because *S. titanus* numbers were low at the beginning of the trial, increased until the second sampling date and were very low on the last sampling date. The time*treatment interaction was not significant ($F_{25,87.1} = 0.91, p = 0.59$).

3.2. Laboratory bioassay

One day after the beginning of the bioassay, a significantly higher mortality rate of first-instar and second-instar nymphs of *S. titanus* was observed in the kaolin-treated leaves compared to the control (Figure. 4). This difference persisted until the end of the bioassay. The Abbott efficacy calculated for kaolin at the end of this experiment was 46%.

4. Discussion and conclusions

Among the natural products tested in the current study, only kaolin showed an efficacy comparable to that of pyrethrins in the control of *S. titanus* nymphs in vineyards. Based on laboratory data, kaolin increased the mortality of *S. titanus* nymphs. A previous laboratory study showed that kaolin applications increase the mortality of the nymphs of the grapevine leafhopper *E. vitis* [32]. Because kaolin acted against *E. vitis* as a feeding inhibitor, a similar mode of action might be involved with the *S. titanus* nymphs. However, the Abbott mortality of first-instar and second-instar nymphs feeding on kaolin-treated leaves for three days was much lower for *S. titanus* (43.2% in this study) than for *E. vitis* (96.4% in [32]). The lower susceptibility of *S. titanus* can be explained with its larger body size because also for *E. vitis* a lower mortality (48.8% at the third day) occurred when older and then larger nymphs were tested [32]. Moreover, based on nymph samplings carried out in the year following the kaolin applications, the plants coated with this product did not appear to have had any oviposition-deterrent effect.

In all field trials, three kaolin applications against *S. titanus* had an efficacy comparable to two pyrethrin applications (considered as standard procedure in organic Italian vineyards). In these trials, kaolin and pyrethrins reduced nymph numbers with a suboptimal efficacy. Neither of the natural products had significant efficacy in late June when egg hatching was almost complete, and when the aged nymphs disperse along the growing suckers and are able to colonize the upper parts of the grapevine canopy.

In organic vineyards, a kaolin-based control strategy against *S. titanus* should be preferred to a pyrethrin-based one, particularly when the effects on other pests are considered. Indeed, unlike pyrethrins, kaolin showed a high efficacy against *E. vitis* and *Z. rhamni* [32] and a moderate effect against *L. botrana* both in the laboratory [38] and in the field (Tacoli et al., unpublished data) [39]. Kaolin also has some application advantages over pyrethrins, such as greater persistence and an absence of application-timing issues, due to its use as a preventive control measure from the beginning of *S. titanus* egg hatching. On the other hand, kaolin has been associated with agronomical benefits in vineyards such as reductions in berry-sunburn damage and higher efficiency in water use [40,41]. Nevertheless, side effects of kaolin and pyrethrins on natural enemies should be considered [42,43].

In conventional viticulture, some of the abovementioned benefits of kaolin seem to be less appealing. First of all, the efficacy of kaolin against *S. titanus* is probably much lower than that of synthetic insecticides, as suggested by some studies [23,44]. However, the negative effects of some synthetic insecticides towards natural enemies [45] could counterbalance this gap.

The moderate effect of three kaolin applications on *S. titanus* suggests that kaolin alone is not enough to achieve the same level of FD control in organic viticulture as obtained with synthetic insecticides in conventional viticulture. Therefore, further research on *S. titanus* control strategies is needed, and in an Integrated Pest Management (IPM) context, alternatives to chemical control should also be considered, such as: (i) conservation and augmentative biological control [6]; (ii) mating disruption based on vibrational disturbance [46–49]; (iii) symbiotic control based on bacteria that damage the vector or its ability to transmit the phytoplasma causal agent [50,51]; and

(iv) push-and-pull strategies [6,52]. Moreover, because *S. titanus* eggs are laid under the bark of two- or more-year-old wood [36], the following cultural practices can be used to reduce *S. titanus* populations: (i) the removal of two-year-old wood from vineyards after winter pruning; and (ii) the removal of suckers growing along the vertical trunk, which are abundantly colonized by nymphs that hatch from the eggs laid into the bark of the trunk [53,54]. In organic vineyards, the integration of these control tools with kaolin applications could increase the efficacy of *S. titanus* management.

5. References

1. Schvester, A.; Carle, P.; Moutous, G. Transmission de la Flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. (Homopt. Jassidae). *Ann. Épiphyt.* **1963**, *14*, 175–198. (In French)
2. Carraro, L.; Loi, N.; Kuszala, C.; Clair, D.; Boudon-Padieu E.; Refatti E. On the ability-inability of *Scaphoideus titanus* Ball to transmit different grapevine yellow agents. *Vitis* **1994**, *33*, 231–234.
3. Bianco, P.A.; Alma, A.; Casati, P.; Scattini, G.; Arzone, A. Transmission of 16SrV phytoplasmas by *Scaphoideus titanus* Ball in Northern Italy. *Plant Protect. Sci.* **2001**, *37*, 49–56.
4. Mori, N.; Bressan, A.; Martini, M.; Guadagnini, M.; Girolami, V.; Bertaccini, A. Experimental transmission by *Scaphoideus titanus* Ball of two Flavescence dorée-type phytoplasmas. *Vitis* **2002**, *41*, 99–102.
5. EPPO/CABI. *Quarantine Pests for Europe*. CAB International: Wallingford, UK, 1997.
6. Chuche, J.; Thiéry, D. Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: A review. *Agron. Sustain. Dev.* **2014**, *34*, 381–403, doi:10.1007/s13593-014-0208-7
7. Caudwell, A. La flavescence dorée de la vigne en France. *Phytoma-La Défense des végétaux* **1981**, *325*, 16–19. (In French)
8. Barba, M. La lotta obbligatoria alla flavescenza dorata e al suo vettore *Scaphoideus titanus*. In *Flavescenza dorata e altri giallumi della vite in Toscana e in Italia*; Bertaccini A., Braccini P., Eds; ARSIA Regione Toscana: Firenze, Italy; Quaderno ARSIA 2005, Volume *3*, pp. 135–138. (In Italian)
9. Weintraub, P.G.; Beanland, L. Insect vectors of phytoplasmas. *Annu. Rev. Entomol.* **2006**, *51*, 91–111, doi: 10.1146/annurev.ento.51.110104.151039
10. Carle, P.; Schvester, D. Nouvelle mise au point su la lutte contre *Scaphoideus titanus* Ball, cicadelle vectrice de la Flavescence dorée de la vigne. *Rev. Zool. Agric. Appl.* **1964**, *63(7-9)*, 107–114. (In French)
11. Pavan, F.; Mori, N.; Bigot, G.; Zandigiacomo, P. Border effect in spatial distribution of Flavescence dorée affected grapevines and outside source of *Scaphoideus titanus* vectors. *Bull. Insectol.* **2012**, *65*, 281–290.
12. Lessio, F.; Portaluri, A.; Paparella, F.; Alma, A. A mathematical model of flavescence dorée epidemiology. *Ecol. Model.* **2015**, *312*, 41–53, doi: 10.1016/j.ecolmodel.2015.05.014
13. Pavan, F.; Stefanelli, G.; Villani, A.; Mori, N.; Posenato, G.; Bressan, A.; Girolami, V. Controllo della flavescenza dorata attraverso la lotta contro il vettore *Scaphoideus titanus* Ball. In *Flavescenza dorata e altri giallumi della vite in Toscana e in Italia*; Bertaccini A., Braccini

P., Eds; ARSIA Regione Toscana: Firenze, Italy; Quaderno ARSIA 2005, Volume 3, pp. 91–108. (In Italian)

14. Bosco, D.; Mori, N. Flavescence dorée vector control in Italy. *Phytopathogenic Mollicutes* **2013**, *3*, 40–43, doi: 10.5958/j.2249-4677.3.1.009
15. Pavan, F.; Mori, N.; Bressan, S.; Mutton, P. Control strategies for grapevine phytoplasma diseases: Factors influencing the profitability of replacing symptomatic plants. *Phytopathol. Mediterr.* **2012**, *51*, 11–22.
16. Schvester, D.; Moutous, G.; Bonfilis, J.; Carle, P. Étude biologique des cicadelles de la vigne dans le sud-ouest de la France. *Ann. Épiphyt.* **1962**, *13*, 205–237. (In French)
17. Vidano, C. Scoperta in Italia dello *Scaphoideus littoralis* Ball cicalina americana collegata alla "Flavescence dorée" della vite. *L'Italia Agricola* **1964**, *101,* 1031–1049. (In Italian)
18. Schvester, D.; Carle, P.; Moutous, G. Nouvelles données sur la transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. *Ann. Zool. Écol. Anim.* **1969**, *1*, 445–465. (In French)
19. Mori, N.; Posenato, G.; Sancassani, G.; Tosi, L.; Girolami, V. Insetticidi per il controllo delle cicaline nei vigneti. *L'Informatore Agrario* **1999**, *55(15)*, 93–97. (In Italian)
20. Posenato, G.; Mori, N.; Bressan, A.; Stefanelli, G.; Pavan, F.; Girolami, V. Valutazione dell'attività di alcuni insetticidi nei confronti degli adulti di *Scaphoideus titanus* Ball e *Metcalfa pruinosa* (Say). *Atti Giornate Fitopatologiche*, **2002**, *1*, 459–162. (In Italian)
21. Mazzoni, E.; Colla, R.; Chiusa, B.; Ciampitti, M.; Cravedi, P. Experiences for vector control of grape golden flavescence in Lombardia and Emilia Romagna (Northern Italy) vineyards. *IOBC/WPRS Bull.* **2003**, *26(8)*, 221–225.
22. Jermini, M.; Gusberti, M.; Schaub, L.; Linder, C.; Gugerli, P.; Schärer, S.; Emery, S. Flavescence dorée and *Scaphoideus titanus*: Distribution and control in Switzerland. *IOBC/WPRS Bull.* **2008**, *36*, 107–111.
23. Žežlina, I.; Škvarč, A.; Bohinc, T.; Trdan, S. Testing the efficacy of single applications of five insecticides against *Scaphoideus titanus* on common grapevines. *Int. J. Pest Manage.* **2013**, *59*, 1–9, doi: 10.1080/09670874.2012.735378
24. Caobelli, R.; Carcereri, G. Lotta biologica alla cicalina della vite. *L'Informatore Agrario* **1995**, *51(33)*, 75–77. (In Italian)
25. Bottura, N.; Mori, N.; Posenato, G.; Sancassani, G.P.; Girolami, V. Lotta alle cicaline nei vigneti a conduzione biologica. *L'Informatore Agrario* **2003**, *59(15)*, 75–79. (In Italian)

26. Mori, N.; Bottura, N.; Posenato, G.; Sancassani, G.P.; Girolami V. Lotta contro *Scaphoideus titanus* Ball nei vigneti a conduzione biologica. *Atti Giornate Fitopatologiche*, **2004**, *1*, 111–116. (In Italian)
27. Mori, N.; Tonello, D.; Posenato, G.; Pozzebon, A.; Duso, C. Efficacy of biopesticides against *Scaphoides titanus* Ball in different experimental conditions. *IOBC/WPRS Bull.* **2014**, *105*, 45–48.
28. Gusberti, M.; Jermini, M.; Wyss, E.; Linder, C. Efficacité d'insecticides contre *Scaphoideus titanus* en vignobles biologiques et effets secondaires. *Rev. Suisse Vitic. Arboric. Hortic.* **2008**, *40*, 173–177. (In French)
29. Constant, N. L'utilisation du pyrèthre naturel pour lutter contre la cicadelle de la flavescence dorée en viticulture biologique. *Progrès Agricole et Viticole* **2009**, *126*, 334–340. (In French)
30. Duso, C.; Angeli, G.; Castagnoli, M.; Liguori, M.; Facchin, P.; Malagnini, V.; Pozzebon, A. Pesticides and phytoseiid mites: A synopsis of research carried out on grapes, apples and vegetables in northern and central Italy. Acarology XI: Proceedings of the International Congress; Merida, Mexico, September 8–13, 2002; Morales-Malacara J.B., Behan-Pelletier V., Ueckermann E., Pérez T.M., Estrada E., Gispert C., Badii M., Eds; Merida, Instituto de Biololgía, UNAM; Facultad de Ciencias, UNAM; Sociedad Latinoamericana de Acarología: Ciudada Universitaria, Mexico, 2007; pp. 325–337.
31. Tubajika, K.M.; Civerolo, E.L.; Puterka, G.J.; Hashim, J.M.; Luvisi, D.A. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. *Crop Prot.* **2007**, *26*, 92–99, doi: 10.1016/j.cropro.2006.04.006
32. Tacoli, F.; Pavan, F.; Cargnus, E.; Tilatti, E.; Pozzebon, A.; Zandigiacomo P. Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in vineyards. *J. Econ. Entomol.* **2017**, *110*, 1164–1178, doi: 10.1093/jee/tox105
33. Karamaouna, F.; Kimbaris, F.; Michaelakis, A.; Papachristos D.; Polissiou, M.; Papatsakona, P.; Tsora, E. Insecticidal activity of plant essential oils against the vine mealybug, *Planococcus ficus*. *J. Insect Sci.* **2013**, *13*, 142, doi: 10.1673/031.013.14201
34. Trdan, S.; Žnidarčič, D.; Valič, N. Field efficacy of three insecticides against cabbage stink bugs (Heteroptera: Pentatomidae) on two cultivars of white cabbage. *Int. J. Pest Manage*. **2006**, *52*, 79–87, doi: 10.1080/09670870600568212
35. Lee, D.H.; Short, B.D.; Nielsen, A.L.; Leskey, T.C. Impact of organic insecticides on the survivorship and mobility of *Halyomorpha halys* (Hemiptera: Pentatomidae) in the laboratory. *Florida Entomol.* **2014**, *97*, 414–421, doi: 10.1653/024.097.0211

36. Bagnoli, B.; Gargani, E. Survey on *Scaphoideus titanus* egg distribution on grapevines. *IOBC/WPRS Bull.* **2011**, *67*, 233–237.

37. Abbott, W.S. A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925,** *18*, 265–267.

38. Pease, C.E.; López-Olguín, J.F.; Pérez-Moreno, I.; Marco-Mancebón, V. Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *J. Econ. Entomol.* **2016***, 109*, 740–745, doi: 10.1093/jee/tov400

39. Tacoli, F.; Cargnus E.; Kiaeian Moosavi, F.; Zandigiacomo P.; Pavan, F. University of Udine, Udine, Italy. Unpublished Data, 2017.

40. Coniberti, A.; Ferrari, V.; Dellacassa, E.; Boido, E.; Carrau, F.; Gepp, V.; Disegna, E. Kaolin over sun-exposed fruit affects berry temperature, must composition and wine sensory attributes of Sauvignon blanc. *Eur. J. Agron.* **2013***, 50*, 75–81, doi: 10.1016/j.eja.2013.06.001

41. Brillante, L.; Belfiore, N.; Gaiotti F.; Lovat, L.; Sansone, L.; Poni, S.; Tomasi, S. Comparing kaolin and pinolene to improve sustainable grapevine production during drought. *PLoS ONE* **2016** *11(6)*, e0156631, doi: 10.1371/journal.pone.0156631

42. Jansen, J.P.; Defrance, T.; Warnier, A.M. Effect of organic-farming-compatible insecticides on four aphid natural enemy species. *Pest Manag. Sci*. **2010**, *66*, 650–656, doi: 10.1002/ps.1924

43. Pascual, S.; Cobos, G.; Seris, E.; González-Núñez, M. Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove. *J. Pest Sci.* **2010**, *83*, 121–133, doi: 10.1007/s10340-009-0278-5

44. Pavan, F.; Bellomo, C.; Vidoni, F.; Bigot, G.; Ostan, M.; Boccalon, W.; Bressan, S.; Mutton, P.; Frausin, C.; De Biasio, A.C.; et al. Efficacia della lotta insetticida contro *Scaphoideus titanus* Ball in Friuli Venezia Giulia. *Supplemento. Notiziario. ERSA* **2005**, *17(5-6)*, 11–21. (In Italian)

45. Pozzebon, A.; Tirello, P.; Moret, R.; Pederiva, M.; Duso, C. A fundamental step in IPM on grapevine: Evaluating the side effects of pesticides on predatory mites. *Insects* **2015**, *6*, 847–857, doi: 10.3390/insects6040847

46. Mazzoni, V.; Prešern, J.; Lucchi, A.; Virant-Doberlet, M. Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). *Bull. Entomol. Res.* **2009**, *99*, 401–413, doi: 10.1017/S0007485308006408

47. Eriksson, A.; Anfora, G.; Lucchi, A.; Lanzo, F.; Virant-Doberlet, M.; Mazzoni, V. Exploitation of insect vibrational signals reveals a new method of pest management. *PLoS ONE* **2012**, *7(3)*, e32954, doi: 10.1371/journal.pone.0032954

48. Polajnar, J.; Eriksson, A.; Rossi Stacconi, M.V.; Lucchi, A.; Anfora, G.; Virant-Doberlet, M.; Mazzoni, V. The process of pair formation mediated by substrate-borne vibrations in a small insect. *Behav. Process.* **2014**, *107*, 68–78, doi: 10.1016/j.beproc.2014.07.013
49. Polajnar, J.; Eriksson, A.; Virant-Doberlet, M.; Mazzoni, V. Mating disruption of a grapevine pest using mechanical vibrations: From laboratory to the field. *J. Pest Sci.* **2016**, *89*, 909–921, doi: 10.1007/s10340-015-0726-3
50. Alma, A.; Daffonchio, D.; Gonella, E.; Raddadi, N. Microbial symbionts of Auchenorrhyncha transmitting phytoplasmas: A resource for symbiotic control of phytoplasmoses. In *Phytoplasmas: genomes, plant hosts and vectors*; Weintraub P.G., Jones P., Eds; CABI: Wallingford, UK, 2010; pp. 272–292.
51. Gonella, E.; Crotti, E.; Rizzi, A.; Mandrioli, M.; Favia, G.; Daffonchio, D.; Alma, A. Horizontal transmission of the symbiotic bacterium *Asaia* sp. in the leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). *BMC Microbiol.* **2012**, *12(Suppl. 1)*, S4, doi: 10.1186/1471-2180-12-S1-S4
52. Mazzoni, V.; Ioriatti, C.; Trona, F.; Lucchi, A.; Cristofaro, A. de; Anfora, G. Study on the role of olfaction in host plant detection of *Scaphoideus titanus* (Hemiptera: Cicadellidae) nymphs. *J. Econ. Entomol.* **2009**, *102*, 974–980, doi: 10.1603/029.102.0316
53. Cara, C.; Trivellone, V.; Linder, C.; Junkert, J.; Jermini, M. Influence de la gestion des repousses du tronc et du bois de taille sur les densités de *Scaphoideus titanus*. *Rev. Suisse Vitic. Arboric. Hortic.* **2013**, *45*, 114–119. (In French)
54. Trivellone, V.; Jermini, M.; Posenato, G.; Mori, N. Influence of pruning wood management and suckering on *Scaphoideus titanus* Ball density in two distinct wine-growing area. In Book of Abstracts IOBC-WPRS meeting of the Working Group on "Integrated Protection and Production in Viticulture", Vienna, Austria, 20–23 October 2015; IOBC-WPRS: Vienna, Austria, 2015; p. 11.

Table 1. Natural products tested in the vineyards against *Scaphoideus titanus*.

Active Constituent	Commercial Product		Application Rate in Water	Application Timing *
	Name	Formulation		
Kaolin	Surround WP (Tessenderlo Kerley Inc., Phoenix, Arizona, USA)	WP (wettable powder), 95% kaolin	2% w/v	A, B, C
Orange oil	Prev-Am Plus (Nufarm Italia, Milano, Italy)	SL (soluble liquid), 5.88% orange oil	0.5% v/v	C, D
Insecticidal soap	Flipper (Dow Agrosciences Italia, Milano, Italy)	SL (soluble liquid), 47.8% potassium salts of fatty acids	2% v/v	C, D
Spinosad	Laser (Dow Agrosciences Italia, Milano, Italy)	SC (suspension concentrate), 44.2% pure spinosad	0.02% v/v	C, D
Pyrethrins	Biopiren Plus (Copyr, Milano, Italy)	EC (emulsifiable concentrate), 2% pure pyrethrins	0.16% v/v	C, D

(*) A, B, C, D refer to the appearance of first-instar, second-instar, third-instar and fourth-instar nymphs of *S. titanus*, respectively.

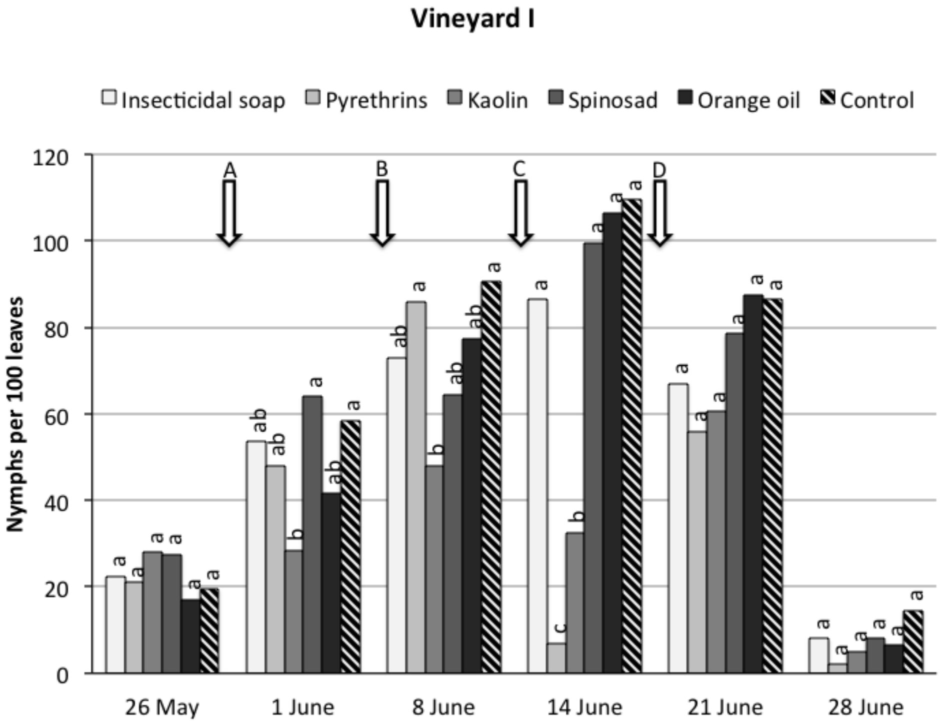

Figure 1. *Scaphoideus titanus* nymphs recorded on grapevine suckers during the sampling period in vineyard I under six different treatments. Within the same date, different small letters above bars indicate significant differences according to *t*-tests on the least square means with Bonferroni correction ($\alpha = 0.05$). The arrows indicate the application timings of the natural products.

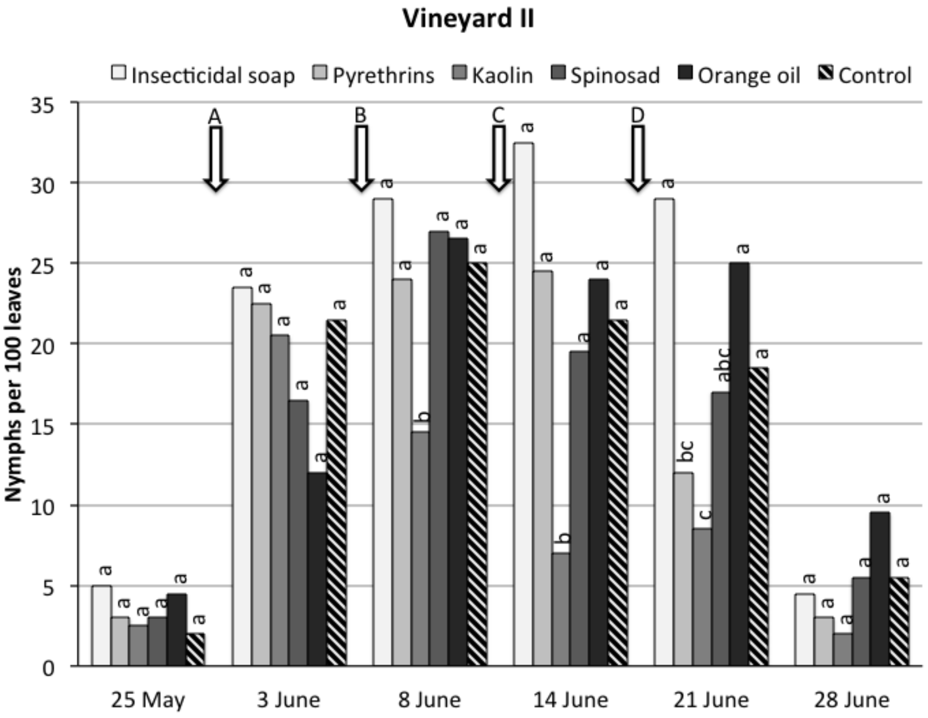

Figure 2. *Scaphoideus titanus* nymphs recorded on grapevine suckers during the sampling period in vineyard II under six different treatments. Within the same date, different small letters above bars indicate significant differences according to *t*-tests on the least square means with Bonferroni correction (α = 0.05). The arrows indicate the application timings of the natural products.

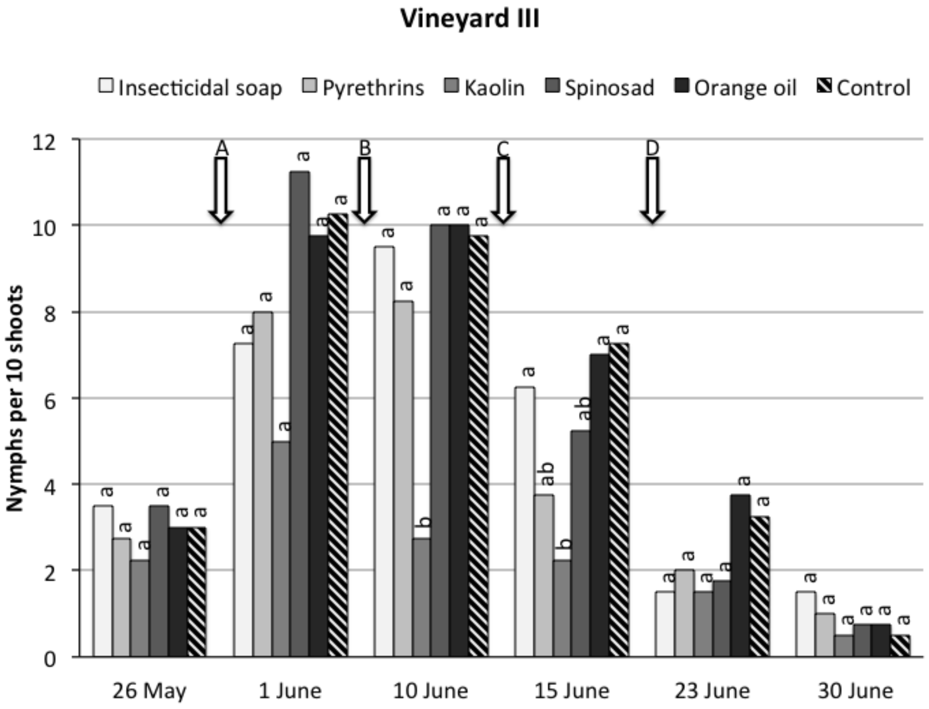

Figure 3. *Scaphoideus titanus* nymphs recorded on grapevine suckers during the sampling period in vineyard III under six different treatments. Within the same date, different small letters above bars indicate significant differences according to *t*-tests on the least square means with Bonferroni correction (α = 0.05). The arrows indicate the application timings of application of the natural products.

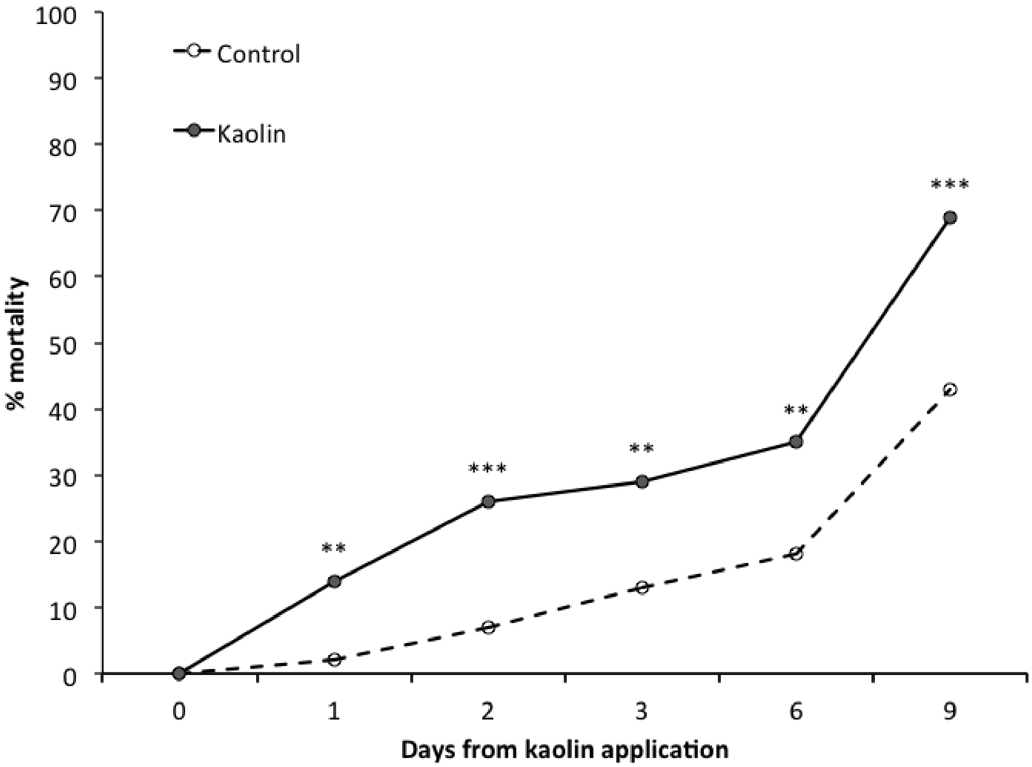

Figure 4. *Scaphoideus titanus* nymph mortality recorded in the laboratory in the kaolin and control. ’**’ and ‘***’ indicate significant differences for α = 0.01 and α = 0.001, respectively, according to Fisher’s exact test.

Chapter 4 - Activity and mode of action of kaolin against *Lobesia botrana* (Lepidoptera: Tortricidae) on grapevines

Chapter 4 is formatted according to the journal author guidelines and has been submitted to Pest Management Science as:

Federico Tacoli, Elena Cargnus, Fatemeh Kiaeian Moosavi, Pietro Zandigiacomo and Francesco Pavan. Activity and mode of action of kaolin against *Lobesia botrana* (Lepidoptera: Tortricidae) on grapevines.

Abstract

BACKGROUND: The control of *Lobesia botrana* in vineyards presents some critical issues when alternative strategies to synthetic insecticides are adopted as environmentally safe tools. During 2015–2017, laboratory and field bioassays on the activity of kaolin against *L. botrana* were carried out and the efficacy of kaolin and *Bacillus thuringiensis* (BT) with or without bunch-zone leaf removal (LR) was assessed in four vineyards of north-eastern Italy.

RESULTS: In laboratory bioassays, berries covered by kaolin were less preferred by *L. botrana* females for egg laying by 53%, and reduced their fecundity and survival by 82% and 22%, respectively. Kaolin coverage reduced egg hatching by 14%. Berries covered by kaolin were less preferred by larvae for settlement by 72%, but their survival and development were not affected. In field bioassays, kaolin reduced the female egg laying by 84%. In the field trials, kaolin, BT and LR reduced *L. botrana* infestation significantly. When the products were combined with LR, the efficacy of kaolin increased and was not significantly different from BT.

CONCLUSION: In the context of IPM strategies in vineyards, kaolin can have an important role due to its efficacy against *L. botrana* and other grapevine pests and a positive synergy with LR.

1. Introduction

The European grapevine moth *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) is the most important carpophagous pest in European vineyards and has recently spread to the Nearctic region.[1] Dependent on the different grape-growing areas and years, the moth may complete two to four generations per year.[2,3] The larvae from the second generation are carpophagous and can cause yield losses and favour the spread of the bunch rot *Botrytis cinerea* Pers. Fr. [4–7]

The control of *L. botrana* is typically achieved by synthetic insecticides, but concern about environmental and health problems and the occurrence of insect resistance to some active constituents[8,9] are leading to different approaches in the control of this pest.

In the context of IPM strategies, mating disruption[10] and *Bacillus thuringiensis* Berliner[11] are the main alternatives to synthetic insecticides against *L. botrana,* particularly in organic vineyards. However, despite the positive eco-toxicological profile of these control tools, they are not always satisfactory for farmers. Mating disruption does not provide a good control of this moth in cases of high population densities or small and irregularly-shaped or windy vineyards. Moreover, farmers can incur additional costs in order to control other grapevine pests such as carpophagous tortricids (e.g. *Eupoecilia ambiguella* (Hb.)), leafhoppers or scales, otherwise usually controlled by the broad-spectrum insecticides used against *L. botrana.*

On the other hand, *B. thuringiensis* does not usually meet the needs of farmers due to its lower both persistence, which requires two applications per generation, and efficacy in comparison with synthetic insecticides.[12] In addition, cultivar choice[13–17] and cultural practices[18,19] can reduce infestations and damage by *L. botrana*. In particular, bunch-zone leaf removal affects larval population levels by increasing egg and newly-hatched larvae mortalities due to the high temperatures reached by sunlight-exposed bunches.[19–21]

Kaolin particle film technology has been widely studied as arthropod pest control tool in orchards and field crops. Under laboratory and field conditions kaolin has been demonstrated to be effective in the control of several insects and mites.[22–26] Plants coated with kaolin become unrecognizable as a host for arthropod pests due to alterations in visual and tactile features and insect activities can be impaired by the particles that stick to their bodies while they move on the clay film.[27,28] For these reasons, oviposition and feeding deterrence can occur, with this last effect associated with a lower survival rate and a longer developmental time.[27,29–32] Kaolin can also exert a direct toxicity toward motile forms.[27]

Until today, the effect of kaolin on *L. botrana* was studied only under laboratory conditions.[33] In particular, these authors reported that kaolin reduced egg laying, egg hatching and larval survival. The activity of kaolin has also been evaluated against other lepidopteran tortricids. For *Cydia pomonella* it has been observed that kaolin reduced egg laying and larval settlement in the laboratory and infestation in apple orchards.[26,34–36] Kaolin also reduced the population levels of *Grapholita molesta* (Busck) in peach orchards.[37] Kaolin reduced field population levels of some leafroller tortricids, in agreement with laboratory data showing negative effects on egg laying and larval feeding.[35,36,38–40]

The aim of the present study was to investigate the influence of kaolin on *L. botrana* performance both in laboratory and field bioassays as well as to test for the first time the efficacy of kaolin in reducing larval infestations in field trials.

2. Materials and methods

2.1. *Lobesia botrana* mass rearing

Lobesia botrana individuals used in the laboratory and field bioassays were derived from mass rearing of the moth conducted in a climatic chamber at 24±1°C temperature, 70±5% RH and a 16:8 (L:D) daily cycle. Larvae were fed on an artificial diet[41] and females laid eggs on transparent polyethylene (PE) bags. The rearing originated from larvae collected in May 2013 from a Pinot Gris vineyard located in north-eastern Italy (Corona di Mariano del Friuli, Gorizia district, 45°55'30"N, 13°29'44"E, 40 m a.s.l.) and was refreshed each year in order to prevent inbreeding effects. The grape-growing area of larval collection was the same as the field trials.

2.2. Laboratory bioassays

In the laboratory bioassays, grapevine berries of the cultivars Pinot Gris (BBCH 75, Lorenz *et al.* [42]) or Italia (BBCH 89) from organic vineyards were used. Before the bioassays the berries were washed in a 4% methanol-water solution. All bioassays were carried out at the same climatic conditions described for mass rearing.

Surround WP (Tessenderlo Kerley Inc., Phoenix, Arizona, USA) kaolin formulation was used in all bioassays at the rate of a 2% (W/V) water suspension. The product was applied on berries with a hand-sprayer until run off.

2.2.1 Influence of kaolin on female egg laying

The oviposition response of *L. botrana* females to the presence of kaolin on the surface of berries (cv Italia) was evaluated in both two-choice and no-choice bioassays.

The two-choice bioassay was carried out to assess the influence of kaolin on the egg-laying preferences of *L. botrana* females. Before the start of the bioassays, one-day-old adults from the rearing were placed in PE bags (30 cm × 15 cm) for mating. Females were collected after 48 h and used for the bioassay. Berries sprayed with kaolin (kaolin-covered berries) and water (control) were offered to females as follows. Four rubber rings (1.5 cm external diameter × 1 cm internal diameter × 0.5 cm height) were fixed on the border of the lid of a glass Petri dish (8 cm diameter) at the same distance from each other. Lid and rings were covered by tulle. After spraying, two berries per treatments were arranged in alternate positions on the rubber rings. The lid with berries was placed in polystyrene boxes (16 cm × 9 cm × 8 cm) lined with black felt and closed at the top with tulle and a transparent polycarbonate cover.[43] Felt and tulle were used to avoid oviposition on any surface other than the berries. Finally, two females were released into each box. The eggs laid on kaolin-covered and control berries were counted after five days. The bioassay was replicated 8 times.

The no-choice bioassay was carried out to evaluate the influence of kaolin on *L. botrana* female fecundity. Before the start of this bioassay, one-day-old adults from the rearing were placed in PE bags (30 cm × 15 cm) for mating. After 48 h females were collected and individually confined for a further 24 h inside in glass tubes (3 cm diameter × 10 cm height). Only females that had laid eggs inside the tube were used. The same polystyrene boxes and glass Petri lids with rubber rings as above described were used. Each box contained four berries sprayed with kaolin (kaolin-covered berries) or with water (control). Finally, one female was released into each box. Females were randomly subdivided between the two treatments. At intervals of 24 h, the eggs laid by each female on kaolin-covered and control berries were counted and all berries replaced until female death. To establish the fecundity of each female, also the number of eggs previously laid inside the tubes was counted. The bioassay was replicated 14 times for both treatments.

2.2.2. Influence of kaolin on egg hatching

A bioassay was carried out to establish the influence of kaolin on *L. botrana* egg hatching through a comparison of three treatments, i.e. untreated eggs on kaolin-covered berries, kaolin-covered eggs and control. For this bioassay, kaolin and control berries with eggs from the previous bioassays (see 2.2.1) were used and, to obtain kaolin-covered eggs, a number of control berries was treated with kaolin within a maximum of 24 h after eggs being laid. For the three treatments, there were 127, 366 and 377 eggs, respectively. Berries were placed into polystyrene boxes (5 cm diameter × 1.8 cm height) and were checked after 10 days for egg hatching.

2.2.3. Influence of kaolin on larval settlement

A two-choice bioassay was conducted to assess whether any feeding-deterrent effect on *L. botrana* newly-hatched larvae occurred when berries were covered in kaolin. For this purpose, polystyrene boxes (9 cm × 6 cm × 1.8 cm) black-head stage eggs laid on PE bags were used. Two eggs were placed on the middle of the box floor with four berries, two sprayed with kaolin (kaolin) and two with water (control), at its corners. To guarantee air exchange and avoid excess relative humidity inside the boxes, the lid of each box was drilled to create a breathe hole (2.5 cm diameter) that was then closed with tulle fixed with hot glue. Each box was checked after 18 h to see which berries the newly hatched larvae had settled on. This bioassay was replicated 40 times.

2.2.4. Influence of kaolin on larval survival and development

A bioassay was conducted to assess whether any lethal or sub-lethal effect occurred when *L. botrana* larvae fed on kaolin-covered berries. Cylindrical polystyrene boxes (5 cm diameter × 1.8 cm height) were provided with breath holes in the lid as described for the rectangular polystyrene boxes. One black-head-stage egg laid on PE bag was placed with two berries in each box. Half of the boxes contained kaolin-treated berries and the other half contained water-treated berries (control). The boxes were checked daily for larval development without opening them to avoid any external interference. The presence of excrement around the larval entrance hole into the berry was considered a valid signal of larval feeding activity. The boxes were opened only after pupation or when traces of larval activity were not observed anymore. To evaluate larval performance several parameters were considered. Larval mortality and development time were recorded. After pupation, the fifth instar head capsules of larvae were collected and mounted on slides in Berlese's liquid to measure the length of the left mandible, as larval size parameter.[3] The sex of the pupae was determined[44] and pupal weight was measured with a precision balance (Sartorius CP2P: capacity 2.1 g; readability 0.001 mg). This bioassay was replicated 40 times per treatment.

2.3. Field bioassay on egg-laying preference

A two-choice bioassay was carried out in late August 2016 (BBCH 89) in a 8-year-old IPM vineyard (Bicinicco, Udine district, 45°55'59"N, 13°13'60"E, 35 m a.s.l., cultivar Chardonnay) with grapevines growing using the Guyot training system and with distances between and along rows of 2.5 m and 0.8 m, respectively. In the vineyard, a standard fungicide programme was followed but no insecticide against *L. botrana* was sprayed before or after the kaolin application. Buprofezin (Applaud Plus, Sipcam, Milano, Italy) was applied on 17 June for the control of *Scaphoideus titanus* Ball. Shoots holding two bunches of similar size and not in contact with each other were chosen. Bunches were also checked for the absence of *L. botrana* eggs. One bunch on each shoot was sprayed with kaolin using a hand-sprayer to run off, while the other one (control), was protected from the spray by covering it in a plastic bag until the kaolin suspension had dried. Then the shoots were trimmed and inserted into cylindrical transparent tulle cages (15 cm diameter × 25 cm length) and four one-day-old mated females were released into each cage. After five days, the cages were removed and bunches harvested. In the laboratory, all berries were checked under a dissecting microscope to count the eggs laid. The bioassay was replicated 20 times.

2.4. Field trials

During 2015–2017, four trials on the influence of kaolin and bunch-zone leaf removal (LR) on larval infestation of the *L. botrana* second generation were carried out in vineyards located in north-eastern Italy (a total of four trials). Vineyard A (Cormons, Gorizia district, 45°57’51”N, 13°26’49”E, 56 m a.s.l., N70°E-S20°W oriented rows, cultivar Pinot Gris) is a 10-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.5 m and 0.8 m, respectively. Vineyard B (Cormons, Gorizia district, 45°57’20”N, 13°26’50”E, 50 m a.s.l., N20°W-S70°E oriented rows, cultivar Pinot Gris) is a 30-year-old organic vineyard with grapevines growing using the double arched Guyot training system with distances between and along rows of 2.8 m and 1 m, respectively. Vineyard C (Cormons, Gorizia district, 45°58’02”N, 13°31’31”E, 53 m a.s.l., N25°W-S65°E oriented rows, cultivar Pinot Gris) is a 15-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.2 m and 0.7 m, respectively. Vineyard D (Cormons, Gorizia district, 45°56’32”N, 13°27’23”E, 44 m a.s.l., N25°W-S65°E oriented rows, cultivar Pinot Gris) is a 10-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.4 m and 0.7 m, respectively. In all vineyards, standard fungicide programmes were followed and no insecticide was sprayed before or after the trial applications. The trials were conducted in 2015 in vineyards A and B, in 2016 in vineyard C and in 2017 in vineyard D.

In all vineyards kaolin and *B. thuringiensis* (Dipel DF, Sumitomo Chemical Agro Europe S.A.S, Saint Didier au Mont d’Or, FR, 1% W/V, Dipel DF/water) were compared with an untreated control. Sprayings were applied at the occurrence of definite *L. botrana* phenological stages as expected on the basis of male flight, determined with pheromone traps, and the average air temperatures. Kaolin was applied two or three times per year, depending on washing off by rain, starting from the beginning of egg laying so as to ensure a satisfactory berry coverage up to the end of egg hatching (18, 24 June in 2015; 10, 20, 27 June in 2016; 19, 22 June, 3 July in 2017). *B. thuringiensis* was applied twice, at the beginning of egg hatching and then a week later (24 June, 1

July in 2015; 27 June, 4 July in 2016; 22 June, 3 July in 2017). The products were sprayed using a backpack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano, RE, Italy) at a rate of 1000 L/ha.

In all trials, a randomized block design with four replicates was adopted. Each block (row) was divided into three plots of 28 (vineyard A) or 20 (vineyard B) or 24 (vineyard C and D) grapevines. In order to test the efficacy of kaolin and *B. thuringiensis* combined with bunch-zone leaf removal, plots of all trials were divided into two subplots of 14 or 10 or 12 grapevines, respectively, and were subjected or not to manual removal of all leaves covering the bunches (17 June 2015, 10 June 2016, 19 June 2017).

The infestation of *L. botrana* was estimated at about 40 days from the beginning of the second-generation flight. In each subplot, 100 bunches were sampled on 10 (vineyard A, C, and D) or 8 (vineyard B) grapevines, excluding edge plants. The sampling was based on an *a priori* scheme[5], to avoid the subjective choice of the sampled bunches. On each bunch the number of larval nests was assessed.

2.5. Statistical analyses

A repeated *G*-test of goodness-of-fit was used for the two-choice bioassays and a two-sample *t*-test was used for the no-choice bioassay. Fisher's exact test and Ryan tests were used for proportions comparison. A paired-sample *t*-test was used for over-time comparison. A log rank test was used to compare *L. botrana* female survival.[45]

To compare data in the field trials, a three-way ANOVA with Bonferroni confidence interval adjustment and Tukey's post-hoc tests were used.

Statistical analyses were performed with GraphPad InStat Version 3.1 for Macintosh (GraphPad Software 2001) and Microsoft Excel 2013 for Windows (Microsoft Corporation 2013).

3. Results

3.1. Laboratory bioassays

3.1.1. Influence of kaolin on female egg-laying

In the two-choice bioassay, *L. botrana* females laid significantly fewer eggs in the kaolin-covered berries than control (Table 1). On average, kaolin reduced female preference for egg laying by 53%.

3.1.2. Influence of kaolin on female fecundity and survival

The day before the beginning of the no-choice bioassay, the mean (±SD) number of eggs laid inside the glass tubes was not significantly different between the two groups of females used in the kaolin-covered berries (29.2±14.4 eggs/female) and control (26.0±13.0 eggs/female) (t = 0.62, df = 26, P = 0.54). During the no-choice bioassay, females laid significantly fewer eggs in kaolin-covered berries than control (Table 1). On average kaolin reduced female fecundity by 82%.

The over-time egg-laying pattern was also different between the kaolin-covered berries and control (Fig. 1). In kaolin-covered berries, at T1 there was already a significantly lower number of eggs laid per female than at T0 (t = 2.80, df = 13, P = 0.015), whereas in the control female fecundity was not significantly different from T0 up to T5 [(T1), t = 1.516, df = 13, P = 0.15; (T2-4), t = 0.025, df = 13, P = 0.98; (T5), t = 2.15, df = 13, P = 0.051; (T6), t = 2.44, df = 13, P = 0.03; (T7), t = 4,716, df = 13, P = 0.0004; (T8) t = 6.15, df = 13, P < 0.0001]. In the kaolin-covered berries, no females laid eggs from T6, whereas in the control some females laid eggs up to T8. Because of the different egg-laying pattern between treatments, from T1 the number of eggs laid per female was significantly lower in the kaolin-covered berries than control [(T1), t = 3.13, df = 26, P = 0.0043; (T2-4); t = 4.58, df = 26, P = 0.0001; (T5), t = 3.81, df = 26, P = 0.0008].

In the kaolin-covered berries, females lived significantly fewer days (7.9±0.9) than control (10.1±1.4) (χ^2 = 15,370, df = 1, P < 0.001) with a 22% reduction in survival.

3.1.3. Influence of kaolin on egg hatching

The egg-hatching rate was significantly lower in the kaolin-covered eggs than in both the kaolin-covered berries and control (Table 1). On average, kaolin coverage reduced egg hatching by 14%.

3.1.4. Influence of kaolin on larval settlement

The number of settled larvae was significantly lower in the kaolin-covered berries than control (Table 1). On average, kaolin reduced larval preference for settlement by 72%.

3.1.5. Influence of kaolin on larval survival and development

No differences in larval mortality were observed between treatments (Table 2).

None of the considered development parameters showed statistically significant differences between larvae reared on the kaolin or control berries (Table 2). The results were no different if males were considered separately from females.

3.2. Field bioassay on egg laying

L. botrana laid significantly fewer eggs on berries of the kaolin-covered bunches than control bunches (G = 344.12, df = 1, $P < 0.0001$) (Fig. 2). On average, kaolin reduced female preference for egg laying by 84%.

3.3. Field trials

In field trials, significant differences were observed for all the three effects, i.e., treatment, bunch-zone leaf removal and vineyard (Table 3; Fig. 3a and 3b). Among treatments, both kaolin and *B. thuringiensis* significantly reduced the number of *L. botrana* larval nests than control, but *B. thuringiensis* showed higher efficacy (around 60%) than kaolin (around 40%) (P = 0.004, Tukey's post-hoc test) (Fig. 3a).

The interaction treatment*leaf removal was significant indicating that bunch-zone leaf removal influenced the efficacy of treatments (Table 3). In particular, bunch-zone leaf removal significantly reduced the number of larval nests in the control and kaolin, but not in the *B. thuringiensis* (Fig. 3c). Also the interactions treatment*vineyard and leaf removal*vineyard were significant, indicating that the effects of treatments and bunch-zone leaf removal on population levels were influenced by the vineyard effect.

4. Discussion and conclusion

4.1. Effect of kaolin on female performance

In the two-choice bioassays, kaolin was deterrent to egg laying by *L. botrana* females and this effect was more marked in the field than in the laboratory bioassays. This result is in accordance with other laboratory studies on *L. botrana*[33] and *C. pomonella*.[34]

In the no-choice bioassay, kaolin reduced female fecundity and survival, and decreased egg laying as early as the first day. For *L. botrana* a reduction in oviposition in no-choice bioassays has been reported previously, but female fecundity was not considered.[33] Other studies on tortricid moths have also evidenced an adverse effect of kaolin on oviposition in no-choice bioassays.[34,38,40]

Kaolin could impair *L. botrana* ovipositional behaviour by making the host unrecognizable.[27,46] The change in colour of kaolin-coated berries could affect the moth visual cues and the kaolin physical barrier could also alter the insect tactile and chemical perception of the host. Regarding this, it is known that *L. botrana* females lay eggs on several types of substrates (i.e., fruits of different colour and species, glass marbles and plastic surfaces that have the common feature of being smooth.[17,20,43,47] Therefore, kaolin could hinder egg laying by changing the berry surface from smooth to dusty and irregular. Kaolin powder could also stick to the chemo- and mechanoreceptors of the tarsi and the ovipositor, disturbing the ability of females to recognize the substrate as suitable for egg laying. For example, on blueberry fruits covered with kaolin the braconid *Diachasma alloeum* (Muesebeck) no longer responds to synomones released by fruits infested by *Rhagoletis mendax* Curran eggs.[48]

The effect of kaolin on female survival has been investigated for *C. rosaceana*, but only minimal differences between treatments were found.[38] In our study, the lower *L. botrana* survival cannot be considered the only reason for the reduction in fecundity because the decrease in egg laying was already evident when all females were still alive. It could be argued that both egg laying and survival reductions are consequences of stress induced in the females by the presence of kaolin.

4.2. Effect of kaolin on egg hatching, and larval behaviour and performance

In our study, kaolin reduced the hatching rate of *L. botrana* eggs only when they were directly covered with the product. In contrast to our results, in another study on *L. botrana* a reduction in egg hatching has been observed also for eggs laid on kaolin-covered berries.[33] Assuming that there may have been a kaolin contact toxicity due to absorption of epicuticular lipids[49], it can be suggested that the higher rate of kaolin suspension used in the other study[33] compared to ours (4% versus 2%) also increased mortality of eggs when they were laid on kaolin-covered berries. No reduction in hatching for eggs laid on kaolin-treated leaves has been also reported for *C. pomonella*, *C. rosaceana* and *C. fumiferana*.[34,38,40] However, unlike our study, for *C. pomonella* has not been reported any reduction in hatching also when eggs were directly sprayed with kaolin.[34]

In the two-choice bioassay on the settlement of *L. botrana* newly-hatched larvae, kaolin-covered berries were less preferred. This result is in accordance with a study on *C. pomonella*[34] that highlighted reduced larval entry into the fruit, due to lower larval dispersal and host recognition, and a study on *C. rosaceana* where kaolin negatively affected the larval dispersal behaviour.[39] Effects of kaolin on *L. botrana* larvae are likely to be due to disruption in orientation, caused by the physical barrier covering the berries. Under field conditions, kaolin may also increase *L. botrana* newly-hatched-larvae wandering time on the berry surface, as suggested by a on average longer although not significant development time, and then the risk of mortality due to predation and exposure to adverse environmental conditions. Moreover, a feeding deterrent effect cannot be excluded, in analogy to what was observed for sap-feeding pests.[31, 32, 50,51]

When *L. botrana* larvae were reared on kaolin-covered berries, survival and development were not affected. In the previous study on *L. botrana*, a high mortality rate was recorded 72 h after kaolin was directly sprayed on of newly-hatched larvae.[33] However, under field conditions this effect is negligible because of the very low probability of hitting larvae directly with the kaolin spray, considering the prolonged egg-hatching period and the short time spent by the newly-hatched larvae outside the berries before penetrating them. In contrast to our results, an increase in mortality was observed for larvae of *C. pomonella* and *C. rosaceana* reared on kaolin-treated leaves.[38,39] However, kaolin did not substantially influence mortality or development of *C. rosaceana* larvae when the clay was mixed with artificial diet, suggesting that its negative effect only occurs when kaolin constitutes a physical barrier to feeding.[39] Therefore, we may suppose that the effect of kaolin on survival of *C. rosaceana* and *L. botrana* larvae varies in relation to their different feeding behaviours. The larvae of the first species, feeding on leaves, are continuously subjected to the kaolin barrier throughout their development, whereas the larvae of the second species, feeding on berries, are affected by the kaolin barrier only during the short time that they consume the small amount of berry skin necessary to penetrate the berries.

4.3. Influence of kaolin and leaf removal on larval infestation in vineyards

In the study vineyards, kaolin reduced the infestation of *L. botrana* with good efficacy, but this was significantly lower than that of *B. thuringiensis*. The efficacy level of kaolin for *L. botrana* in vineyards is in accordance with reports for *C. pomonella* in apple orchards.[34–36]

Bunch-zone leaf removal was also able to reduce the infestation of *L. botrana*, as already reported.[19]

Although kaolin was less effective than *B. thuringiensis* in the *L. botrana* control, the differences in efficacy between the two products were no longer significant when combined with bunch-zone leaf removal, suggesting a more positive interaction with this cultural practice by kaolin than by *B. thuringiensis*. Moreover, kaolin could be preferable to *B. thuringiensis* because of its ability to control also cicadellids.[31,5254] Furthermore, kaolin may provide more consistent results than *B. thuringiensis*, it not being degraded by UV radiation and having a high resistance to washing off by rain.[31,55] Additionally, when associated with bunch-zone leaf removal, kaolin confers some agronomical advantages including reducing sunburn damage of exposed berries and lowering grapevine water stress, without having negative effect on grape yield and grape qualitative parameters.[56–58]

Based on our visual observations, much better kaolin coverage was achieved on leaves than on berries. This is probably due to the presence of waxy compounds on the berry surfaces that interfere with kaolin adhesion. A better coverage of bunches could be obtained using trailed sprayers because of the higher pressure and smaller drop diameter they provide compared to the hand or backpack sprayers used in this study. An increase in kaolin bunch coverage could ensure the same efficacy against *L. botrana* with a single application as currently achieved with two.

4.4. Concluding remarks

Kaolin could be of profitable for use in vineyards because of its ability to control *L. botrana* and other grapevine pests and because of its positive interaction with bunch-zone leaf removal.

Further studies should be carried out on the possible positive interaction between kaolin, which inhibits egg laying, and *B. thuringiensis,* which exerts larvicidal activity, also taking into account that kaolin could increase the persistence of *B. thuringiensis* by mitigating the effect of UV radiation, as suggested by the literature.[59]

5. References

1 Ioriatti C, Lucchi A, and Varela LG, Grape berry moths in Western European vineyards and their recent movement into the New World, in Arthropod management in vineyards: pest, approaches, and future directions, ed by Bostanian NJ, Vincent C, and Isaacs R,. Springer Science+Business Media B.V., Dordrecht, The Netherlands, pp. 339–360 (2012).

2 Martín-Vertedor D, Ferrero-Garcia JJ and Torres-Vila LM, Global warming affects phenology and voltinism of *Lobesia botrana* in Spain. *Agric For Entomol* **12**:169–176 (2010).

3 Pavan F, Floreani C, Barro P, Zandigiacomo P and Dalla Montà L, Occurrence of two different development patterns in *Lobesia botrana* (Lepidoptera: Tortricidae) larvae during the second generation. *Agric For Entomol* **14**:398–406 (2013).

4 Fermaud M and Giboulot A, Influence of *Lobesia botrana* larvae on field severity of Botrytis rot of grape berries. *Plant Dis* **76**:404–409 (1992).

5 Pavan F, Girolami V and Sacilotto G, Second generation of grape berry moths, *Lobesia botrana* (Den. & Schiff.) (Lep., Tortricidae) and *Eupoecilia ambiguella* (Hb.) (Lep., Cochylidae): spatial and frequency distributions of larvae, weight loss and economic injury level. *J Appl Entomol* **122**:361–368 (1998).

6 Pavan, F, Bigot G, Cargnus E and Zandigiacomo P, Influence of the carpophagous generations of the European grapevine moth *Lobesia botrana* on grape bunch rots. *Phytoparasitica* **42**:61–69 (2014).

7 Moschos T, Yield loss quantification and economic injury level estimation for the carpophagous generations of the European grapevine moth *Lobesia botrana* Den. et Schiff. (Lepidoptera: Tortricidae). *Int J Pest Manag* **52**:141–147 (2006).

8 Civolani S, Boselli M, Butturini A, Chicca M, Fano EA and Cassinelli S, Assessment of insecticide resistance of *Lobesia botrana* (Lepidoptera: Tortricidae) in Emilia-Romagna region. *J Econ Entomol* **107**:1245–1249 (2014).

9 Pavan F, Cargnus E, Bigot G and Zandigiacomo P, Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies. *Bull Insectol* **67**:273–280 (2014).

10 Ioriatti C, Anfora G, Tasin M, De Cristofaro A, Witzgall P and Lucchi A, Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). *J Econ Entomol* **104**:1125–1137 (2011).

11 Ifoulis AA and Savopoulou-Soultani M, Biological control of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae by using different formulations of *Bacillus thuringiensis* in 11 vine cultivar under filed conditions. *J Econ Entomol* **97**:340–343 (2004)

12 Boselli M, Scannavini M and Melandri M, Confronto fra strategie di difesa contro la tignoletta della vite. *L'Informatore Agrario* **56** (19):61–65 (2000).

13 Fermaud M, Cultivar susceptibility of grape berry clusters to larvae of *Lobesia botrana* (Lepidoptera: Tortricidae). J Econ Entomol **91**:974–980 (1998).

14 Moreau J, Benrey B and Thiéry D, Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). *Bull Entomol Res* **96**:205–212 (2006).

15 Moreau J, Rahme J, Benrey B and Thiéry D, Larval host plant origin modifies the adult oviposition preference of the female European grapevine moth *Lobesia botrana*. *Naturwissenschaften* **95**:317–324 (2008), doi: 10.1007/s00114-007-0332-1

16 Sharon R, Zahavi T, Soroker V and Harari AR, The effect of grapevine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels. *J Pest Sci* **82**:187–193 (2009).

17 Pavan F, Stefanelli G, Cargnus E and Villani A, Assessing the influence of inflorescence traits on the susceptibility of grape to vine moths. *J Appl Entomol* **133**:394–401 (2009).

18 Vartholomaiou AN, Navrozidis EI, Payne CC and Salpiggidis GA, Agronomic techniques to control *Lobesia botrana*. *Phytoparasitica* **36**:264–271 (2008).

19 Pavan F, Cargnus E, Kiaeianmoosavi S, Bigot G, Tacoli F and Zandigiacomo P, Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* and grey mould. *Bull Insectol* **69**:107–115 (2016).

20 Kiaeian Moosavi F, Cargnus E, Pavan F and Zandigiacomo P, Mortality of eggs and newly hatched larvae of *Lobesia botrana* (Lepidoptera: Tortricidae) exposed to high temperatures in the laboratory. *Environ Entomol* **46**:700–707 (2017).

21 Kiaeian Moosavi F, Cargnus E, Pavan F and Zandigiacomo P, Effect of grapevine bunch exposure to sunlight on berry surface temperature and *Lobesia botrana* (Lepidoptera: Tortricidae) egg laying, hatching and larval settlement. *Agric For Entomol* (2017). DOI:10.1111/AFE.12274

22 Puterka GJ, Glenn DM, Sekutowski DG, Unruh TR and Jones SK, Progress toward liquid formulations of particle films for insect and disease control in pear. *Environ Entomol* **29**:329–339 (2000).

23 Lo Verde G, Rizzo R, Barraco G and Lombardo A, Effects of kaolin on *Ophelimus maskelli* (Hymenoptera: Eulophidae) in laboratory and nursery experiments. *J Econ Entomol* **104**:180–187 (2011).

24 Nateghi MF, Paknejad F and Moarefi M, Effect of concentrations and time of kaolin spraying on wheat aphid. *J Biol Environ Sci* **7**:163–168 (2013).

25 Silva CAD and Ramalho FS, Kaolin spraying protects cotton plants against damages by boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). *J Pest Sci* **86**:563–569 (2013).

26 Bostanian NJ and Racette G, Particle films for managing arthropod pests of apple. *J Econ Entomol* **101**:145–150 (2008).

27 Glenn DM, Puterka G, Vanderzwet T, Byers RE and Feldhake C, Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. *J Econ Entomol* **92**:759–771 (1999).

28 Vincent C, Hallman G, Panneton B and Fleurat-Lessard F, Management of agricultural insects with physical control methods. *Annu Rev Entomol* **48**:261–281 (2003).

29 Barker JE, Fulton A, Evans KA and Powell A, The effect of kaolin particle film on *Plutella xylostella* behaviour and development. *Pest Manag Sci* **62**:498–504 (2006).

30 Lapointe SL, McKenzie CL and Hall DG, Reduced oviposition by *Diaprepes abbreviates* (Coleoptera: Curculionidae) and growth enhancement of citrus by Surround particle film. *J Econ Entomol* **99**:109–116 (2006).

31 Tacoli F, Pavan F, Cargnus E, Tilatti E, Pozzebon A and Zandigiacomo P, Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellide) in vineyards. *J Econ Entomol* **110**:1164–1178 (2017).

32 Tacoli F, Mori N, Pozzebon A, Cargnus E, Da Vià S, Zandigiacomo P, Duso C and Pavan F. Control of *Scaphoideus titanus* with natural products in organic vineyards. Insects, 8, 129 (2017). doi: 10.3390/insects8040129

33 Pease CE, López-Olguín JF, Pérez-Moreno I and Marco-Mancebón V, Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *J Econ Entomol* **109**:740–745 (2016).

34 Unruh TR, Knight AL, Upton J, Glenn DM and Puterka GJ, Particle films for suppression of the codling moth (Lepidoptera: Tortricidae) in apple and pear orchards. *J Econ Entomol* **93**:737–743 (2000).

35 Knight AL, Christianson BA, Unruh TR, Puterka G and Glenn DM, Impact of seasonal kaolin particle films on apple pest management. *Can Entomol* **133**:413–428 (2001).

36 Markó V, Blommers LHM, Bogya S and Helsen H, Kaolin particle films suppress many apple pests, disrupt natural enemies and promote woolly apple aphid. *J Appl Entomol* **132**:26–35 (2008).

37 Lalancette N, Belding RD, Shearer PW, Frecon JL and Tietjen WH, Evaluation of hydrophobic and hydrophilic kaolin particle films for peach crop, arthropod and disease management. *Pest Manag Sci* **61**:25–39 (2005).

38 Knight AL, Unruh TR, Christianson BA, Puterka GJ and Glenn DM, Effects of a kaolin-based particle film on obliquebanded leafroller (Lepidoptera: Tortricidae). *J Econ Entomol* **93**:744–749 (2000).

39 Sackett TE, Buddle CM and Vincent C, Effect of kaolin on fitness and behaviour of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) larvae. *J Econ Entomol* **98**:1648–1653 (2005).

40 Cadogan BL and Scharbach ED, Effect of kaolin-based particle film on spurce budworm (*Choristoneura fumiferana* (Lepidoptera: Torticidae)) oviposition in the laboratory. *Pest Manag Sci* **61**:1215–1219 (2005).

41 Rapagnani MR, Caffarelli V, Barlattani M and Minelli F, Descrizione di un allevamento, in laboratorio, della tignoletta dell'uva *Lobesia botrana* Den. e Schiff. (Lepidoptera – Tortricidae) su un nuovo alimento semi-sintetico. *Boll Ist Ent «G. Grandi» Univ Bologna* **44**:57–64 (1990).

42 Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U and Weber E, Growth stages of the grapevine: phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*). Codes and descriptions according to the extended BBCH scale. *Aust J Grape Wine Res* **1**:100–103 (1995).

43 Maher N and Thiéry D, A bioassay to evaluate the activity of chemical stimuli from grape berries on the oviposition of *Lobesia botrana* (Lepidoptera: Tortricidae). *Bull Entomol Res* **94**:27–33 (2004).

44 Galet P, Les Maladies et les Parasites de la Vigne. Tome II. Les parasites animaux. Paysan du Midi, Montpellier, France (1982).

45 Mantel N, Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* **50**:163–170 (1966).

46 Glenn DM and Puterka GJ, Particle films: a new technology for agriculture. *Hortic Rev* **31**:1–44 (2005).

47 Stavridis DG and Savopoulou-Soultani M, Larval performance on and oviposition preference for known and potential hosts by *Lobesia botrana* (Lepidoptera: Tortricidae). *Eur J Entomol* **95**:55–63 (1998).

48 Stelinski LL, Pelz-Stelinski KS, Liburd OE and Gut LJ, Control strategies for *Rhagoletis mendax* disrupt host-finding and ovopositional capability of its parasitic wasp, *Diachasma alloeum*. *Biol Control* **36**:91–99 (2006).

49 Ebeling W, Sorptive dusts for pest control. *Annu Rev Entomol* **16**:123–158 (1971).

50 Puterka G, Glenn DM and Pluta RC, Action of particle films on the biology and behaviour of pear psylla (Homoptera: Psyllidae). *J Econ Entomol* 98:2079–2088 (2005).

51 Sánchez-Ramos I, Pascual S, Marcotegui A, Fernández CE and Gonzáles-Núñez M, Laboratory evaluation of alternative control methods against the false tiger, *Monosteira unicostata* (Hemiptera: Tingidae). *Pest Manag Sci* **70**:454–461 (2014).

52 Wood M and McBride J, Scientists sharpen strategies to sabotage: glassy-winged sharpshooter. *Agr Res Mag* **49**:20–22 (2001).

53 Puterka GJ, Reinke M, Luvisi D, Ciomperik MA, Bartels D, Wendel L and Glenn DM, Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. *Plant Health Progress* Online, doi: 10.1094/PHP-2003-0321-01-RS (2003).

54 Tubajika KM, Civerolo EL, Puterka GJ, Hashim JM and Luvisi DA, The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. *Crop Prot* **26**:92–99 (2007).

55 Hostetter DL, Ignoffo CM, Kearby WH, Persistence of formulations of *Bacillus thuringiensis* spores and crystals on eastern red cedar foliage in Missouri. *J Kans Entomol Soc* **48**:189–193 (1975).

56 Glenn DM, Cooley N, Walker R, Clingeleffer P and Shellie K, Impact of kaolin particle film and water deficit on wine grape water use efficiency and plant water relations. *HortScience* **45**:1178–1187 (2010).

57 Coniberti A, Ferrari V, Dellacassa E, Boido E, Carrau F, Gepp V and Disegna E, Kaolin over sun-exposed fruit affects berry temperature, must composition and wine sensory attributes of Sauvignon blanc. *Eur J Agron* **50**:75–81 (2013).

58 Brillante L, Belfiore N, Gaiotti F, Lovat L, Sansone L, Poni S and Tomasi S Comparing kaolin and pinolene to improve sustainable grapevine production during drought. *PLoS ONE* **11**(6): e0156631 (2016).

59 Glenn DM, Effect of highly processed calcined kaolin residues on apple productivity and quality. *Sci. Hortic.* **201**:101–108 (2016).

Table 1. Influence of kaolin on *L. botrana* in the laboratory bioassays. For egg hatching, different letters indicate significant differences ($\alpha = 0.05$).

	Control	Kaolin-covered berries	Kaolin-covered eggs	Statistical analyses
Mean±SD of eggs per female (two-choice)	67.3±32.6	31.5±10.5	–	$G = 105.9$, df = 1, $P < 0.0001$
Mean±SD of eggs per female (no-choice)	146.7±77.5	26.1±20.5	–	$t = 5.63$, df = 26, $P < 0.0001$
% egg hatching	99.5 b	98.4 b	85.8 a	Ryan test, $P < 0.05$
% larval settlement (two-choice)	77.8	22.2	–	$G = 23.53$, df = 1, $P < 0.0001$

Table 2. Influence of kaolin-covered berries on *L. botrana* larval survival and development parameters.

Parameter	Kaolin		Control		Statistical results
	N. of individuals	% or mean±SD	N. of individuals	% or mean±SD	
Total larvae	40		40		
Dead larvae	11	27.5%	12	30.0%	Fisher's Exact test, *P* = 0.99
Male pupae		51.7%		60.7%	Fisher's Exact test, *P* = 0.82
Development time (days)					
Total	29	28.2±4.0	28	26.6 ±4.3	*t* = 1.38, df = 55, P = 0.17
Females	14	28.1±3.0	11	27.5±3.3	*t* = 0.49, df = 23, P = 0.63
Males	15	28.3±4.8	17	26.1±5.0	*t* = 1.24, df = 30, *P* = 0.22
Mandible length (mm) (*)					
Total	27	23.3±3.3	25	24.1±1.1	*t* = 1.19, df = 50, *P* = 0.24
Females	14	23.3±4.5	10	24.9±0.9	*t* = 1.11, df = 22, *P* = 0.28
Males	13	23.4±1.3	15	23.7±1.0	*t* = 0.65, df = 26, *P* = 0.52
Pupal weight (mg)					
Total	29	8.1±1.8	28	8.4±2.0	*t* = 0.66, df = 55, *P* = 0.51
Females	14	9.5±1.6	11	10.0±1.9	*t* = 0.76, df = 23, *P* = 0.45
Males	15	6.8±0.8	17	7.4±1.4	*t* = 1.45, df = 30, *P* = 0.16

(*) The length was not measured on 5 individuals because the mandibles were found to be broken.

Table 3. Field trials. Results of three-way ANOVA performed on larval nests of *L. botrana*.

Source of variation	Larval nests		
	F	df	*P*
Corrected Model	7.706	23,72	< 0.0001
Treatment	37.460	2,72	< 0.0001
Leaf removal	30.688	1,72	< 0.0001
Vineyard	5.690	3,72	0.001
Treatment*Leaf removal	3.269	2,72	0.044
Treatment*Vineyard	4.343	6,72	0.001
Vineyard*Leaf removal	4.866	3,72	0.004
Vineyard*Treatment*Leaf removal	1.227	6,72	0.303

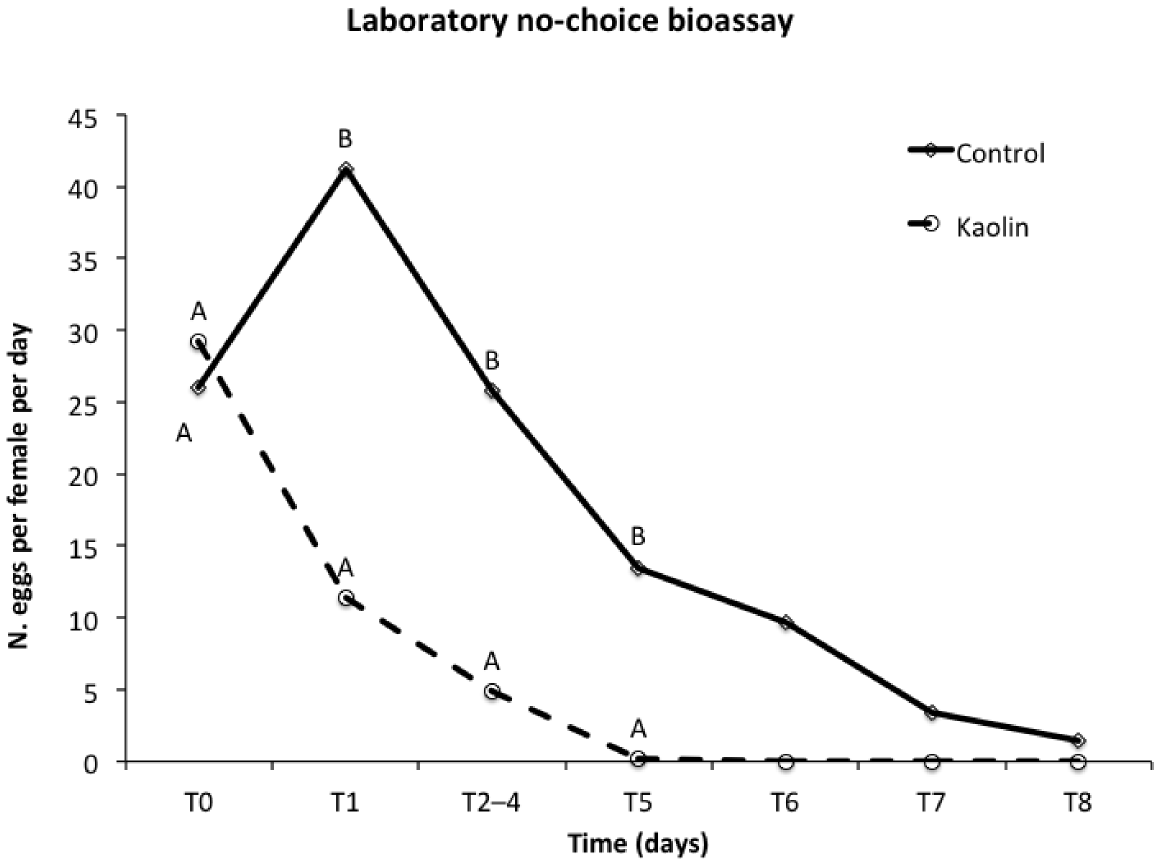

Figure 1. Eggs laid over time by *L. botrana* females placed on kaolin or not from T1. T0 eggs previously laid on glass tubes by the same females. Different capital letters between treatments on the same day indicate significant differences according to a *t*-test ($\alpha = 0.01$).

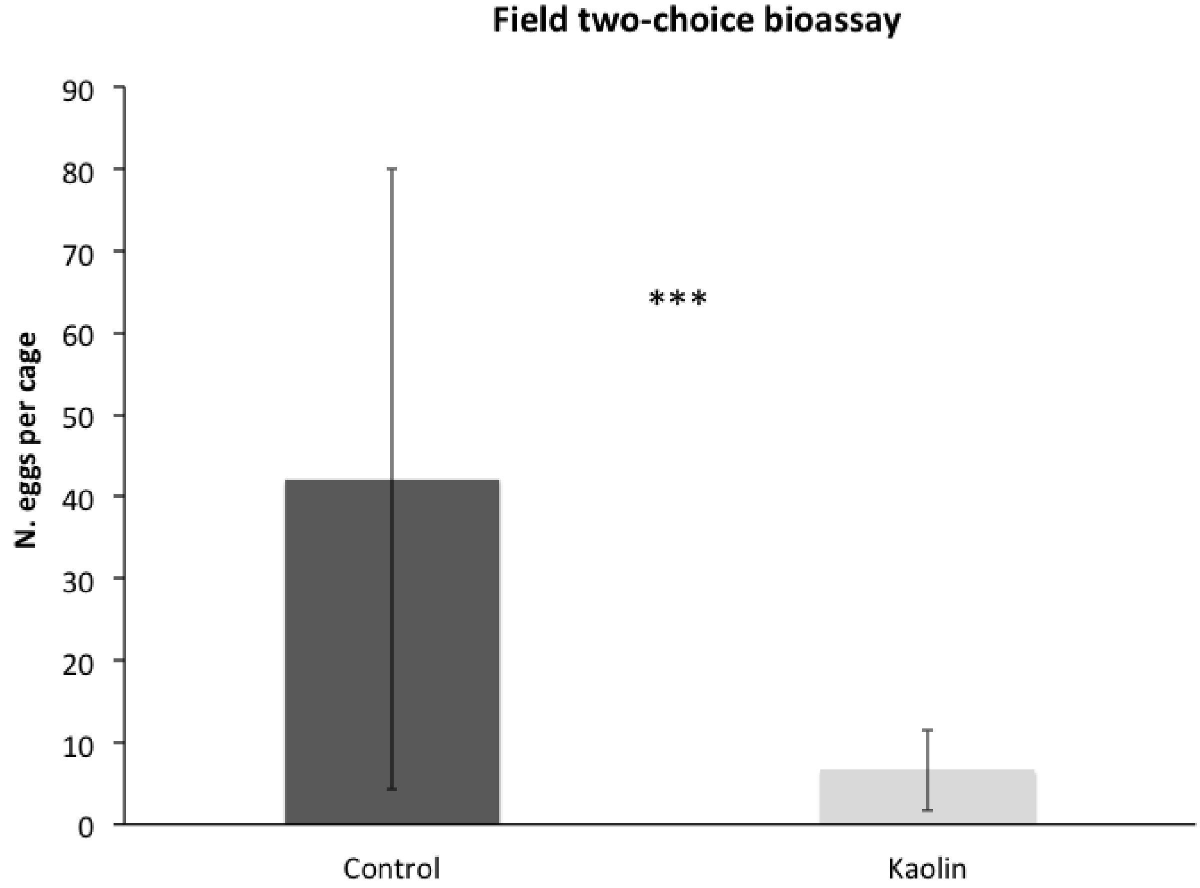

Figure 2. Mean ± standard deviation of *L. botrana* eggs laid on berries of bunches treated or not with kaolin. *** indicates significant differences according to a *G*-test ($\alpha = 0.001$).

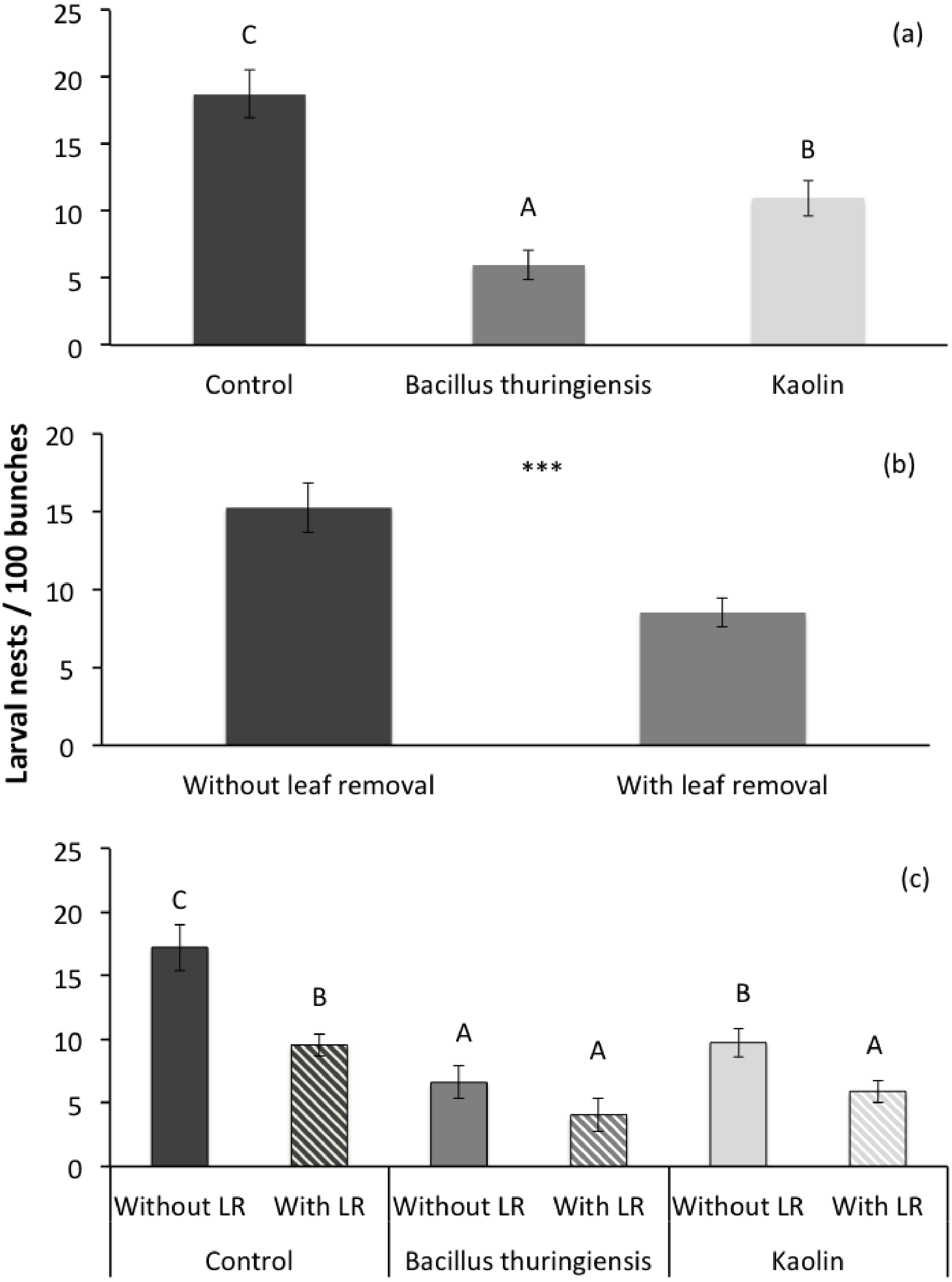

Figure 3. Field trials on the effect of different treatments (a), bunch-zone leaf removal (LR) (b) and their interaction (c) on *L. botrana* infestations (mean ± SE of larval nests). Different capital letters among treatments or LR indicate significant differences according to Tukey's test ($\alpha = 0.01$) and *** indicates significant differences according to ANOVA ($\alpha = 0.0001$).

Chapter 5 - Side effects of sulfur dust on *Lobesia botrana* (Den. & Schiff) (Lepidoptera: Tortricidae) in vineyards

Chapter 5 will be published as:
Federico Tacoli, Elena Cargnus, Francesco Pavan. Side effects of sulfur dust on *Lobesia botrana* (Den. & Schiff) (Lepidoptera: Tortricidae) in vineyards.

Abstract

The activity of sulfur dust on the European grapevine moth *Lobesia botrana* (Den. & Schiff.) was investigated in the laboratory. In 2017, two trials were carried out in as many vineyards of northeastern Italy to compare the efficacy of sulfur dust with that of kaolin and of *Bacillus thuringiensis* Berliner on *L. botrana* against both the second and third generations. In the first case, each treatment was in combination or not with bunch-zone leaf removal. In the two trials, sulfur dust was applied once at a rate of 30 kg/ha at the start of the second and third flights of *L. botrana* males, respectively. In the laboratory bioassays, sulfur dust reduced both egg laying and larval settlement and egg hatching of eggs covered by sulfur dust. In both field trials, sulfur dust caused a significant decrease in the number of *L. botrana* larval nests and of damaged berries with an efficacy not different from both *B. thuringiensis* and kaolin. Bunch-zone leaf removal did not significantly reduce *L. botrana* infestation. Based on these results, the side effect of sulfur dust against *L. botrana* could be considered in the context of IPM strategy in vineyards.

1. Introduction

The European grapevine moth, *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) is the most important carpophagous pest in European vineyards and is recently spreading to some Nearctic grape-growing areas (Ioriatti *et al.*, 2012). In north-eastern Italy, the moth completes two-three generations a year, of which the first is antophagous and the other two are carpophagous (Pavan *et al.,* 2013). Damage is mostly associated with second and third generations which cause yield and qualitative losses also favoring the spread of *Botrytis cinerea* Pers. Fr. bunch rot (Fermaud & Giboulot, 1992; Moschos, 2006; Pavan *et al*., 2014a).

The control of *L. botrana* is typically achieved using synthetic insecticides, but concern about environmental and sanitary problems and the occurrence of insecticide resistance to some active ingredients (Civolani *et al.*, 2014; Pavan *et al.*, 2014b) are leading to different approaches in the control of this pest.

In the context of IPM, mating disruption (Ioriatti *et al.*, 2011) and *Bacillus thuringiensis* Berliner (Ifoulis and Savopoulou-Soultani, 2004) are the main alternatives and are becoming of common use in many grape-growing areas and, mostly, in organic farms. However, although their eco-toxicological profile is positive, the adoption of these control tools is not always satisfactory for farmers. Mating disruption does not provide a good control in presence of high population densities, in small and irregularly-shaped vineyards, and in windy regions. Moreover, mating-disruption cost is higher compared to synthetic insecticides and can further increase because of its high specificity that can make necessary control measures against other grapevine pests, such as the carpophagous *Eupoecilia ambiguella* (Hb.) (Lepidoptera: Tortricidae), leafhoppers and scales. *B. thuringiensis*, on the other hand, does not usually meet the requirements of farmers due to its low persistence, that requires two applications per generation and often determines lower efficacy than synthetic insecticides (Boselli et al., 2000). Also cultivars choice (Fermaud, 1998; Moreau *et al.*, 2006; Pavan *et al.*, 2009; Sharon *et al.*, 2009) and cultural practices, such as controlled nitrogen fertilization (Vartholomaiou *et al.*, 2008), bunch-zone leaf removal (Pavan *et al.*, 2016; Kiaeian Moosavi *et al.*, 2017a, 2017b) and kaolin applications (Tacoli *et al.*, this PhD thesis), can be considered to reduce infestations and damage by *L. botrana*.

Wettable and dustable sulfur powders are largely used in viticulture due to their fungicidal activity against powdery mildew (Bencivelli, 1975) and their effectiveness in the control of grapevines phytophagous mites is well known (Touzeau, 1987; Goebel *et al.*, 2001). Some preliminary data suggest an efficacy of sulfur dust against *L. botrana* and the leafhopper *Empoasca vitis* (Göthe) (Touzeau, 1987). However, wettable sulfur was not effective against *L. botrana* and the leafhopper *Erythroneura elegantula* Osborn (Jepsen *et al.*, 2007). A study on the lepidopteran pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) reported reduction of oviposition on tomato covered with sulfur dust (Zappalà *et al.*, 2012).

In north-eastern Italy, sulfur dust is traditionally used in vineyards for the control of powdery mildew after BBCH 71 (i.e., “Fruit set” according to Lorenz *et al.*, 1995) and just before BBCH 79 (i.e., “Majority of berries touching”) growth stages to allow for long term coverage inside the bunches before it is not anymore possible. For the earliest cultivars, the start of *L. botrana* second seasonal flight occurs before BBCH 79 growth stage and therefore it can be supposed that a sulfur dust application in this time could have side effects on *L. botrana*.

The aim of this study was to investigate the activity of sulfur dust on *L. botrana* in the laboratory and to test its efficacy in field trials.

2. Materials and methods

Laboratory bioassays and field trials on the side effects of sulfur dust on *L. botrana* were carried out during 2015–2017.

2.1. Laboratory bioassays

2.1.1. *Lobesia botrana* mass rearing

L. botrana individuals used in the laboratory bioassays derived from a mass rearing of the moth conducted in a climatic chamber at 24±1°C temperature, 70±5% RH and photoperiod of 16:8 (L:D). Larvae were fed on artificial diet (Rapagnani *et al.*, 1990) and females laid eggs on polyethylene (PE) bags. The rearing originated from larvae collected in May 2013 from a Pinot Gris vineyard located in northeaster Italy (locality Corona di Mariano del Friuli, Gorizia district, 45°55'30"N, 13°29'44"E, 40 m a.s.l.) and was refreshed each year in order to prevent inbreeding effects. The grape growing area of larval collection was the same of the field trials.

2.1.2. Application of sulfur dust on berry surface

For the laboratory bioassays, unripen grapevine berries of cultivar Pinot Gris (BBCH 75) were used. The berries, removed from the bunch with about half a centimeter of petiole, were first dipped in water for one second and then dusted with sulfur dust (Zolfo ventilato Stella, Pasquale Mormino & Figlio S.R.L, Termini Imerese (PA), Italy, 98.5% pure sulfur). In this way berries were covered with a thin layer of sulfur dust.

2.1.3. Influence of sulfur dust on female egg laying

A no-choice bioassay was conducted at the same climatic conditions described for the mass rearing to evaluate whether any effect on egg laying by *L. botrana* females occurred when sulfur dust covered berries. Before the start of the bioassay, one-day-old adults from the rearing were placed in PE bags (30 cm × 15 cm) for mating. Females were collected after 48 h and individually confined for further 24 h into glass tubes (3 cm diameter × 10 cm height). Only females that had laid eggs inside the tubes were used for the bioassay. For each female, the number of eggs laid inside the tube was counted.

For the bioassay, four berries were hold on the lid of a glass Petri dish (8 cm diameter) through four rubber rings (1.5 cm external diameter × 1 cm internal diameter × 0.5 cm height) placed near the lid border at the same distance from each other. Each lid including rubber rings was covered with tulle. Then, the lid with berries was placed in a polystyrene box (16 cm × 9 cm × 8 cm) lined with black felt and closed at the top with tulle and a transparent polycarbonate cover (Maher & Thiéry 2004). Felt and tulle were used to avoid oviposition on any other surface than berries. An equal number of the boxes had berries covered with sulfur dust or sprayed with water (control). One fertile female was released into each box. Females were subdivided between the two treatments so to have in each treatment females that on average had laid a similar amount of eggs per female inside the tubes. The berries were changed daily for five days. The eggs laid by each female on the surface of sulfur-dust-covered berries and of control ones were counted. The assay was replicated 18 times for both the treatments.

2.1.4. Influence of sulfur dust on egg hatching

A bioassay was carried out at the same climatic conditions described for the mass rearing to establish the influence of sulfur dust on *L. botrana* egg-hatching by comparison of three treatments, i.e. untreated eggs on sulphur-dust-covered berries, sulphur-dust-covered eggs and control eggs. For this bioassay, berries with eggs already laid on their surface coming from the previous bioassay on female egg laying were used. In particular, part of the eggs coming from the control was treated with sulfur dust within a maximum of 24 h from egg laying. For the three treatments there were 155, 188 and 377 eggs, respectively. Berries with eggs were placed into cylindrical polystyrene boxes (5 cm diameter × 1.8 cm height) and were checked after 10 days for egg hatching.

2.1.5. Influence of sulfur dust on larval settlement

A two-choice bioassay was conducted at the same climatic conditions described for the mass rearing to assess whether any feeding-deterrent effect on *L. botrana* larvae occurred when sulfur dust covered berries. At this purpose, polystyrene rectangular boxes (9 cm length × 6 cm width ×

1.8 cm height) and black-head-stage eggs laid on PE bag were used. To guarantee air exchange and avoid excess of relative humidity inside the boxes, the lid of the boxes was drilled to create a hole (2.5 cm diameter) that was then closed with tulle fixed with hot glue. To assess larval preference, two eggs were placed on the middle of the box floor with four berries, two treated with sulfur dust and two with water (control), at the corners. Each box was checked after 18 h to see on which berries newly-hatched larvae had settled. This bioassay was replicated 40 times.

2.2. Field trials

In 2017, two field trials on the influence of sulfur dust on larval infestation of *L. botrana* were carried out in as many vineyards of north-eastern Italy. Vineyard A (Cormons, Gorizia district, 45°58’32’’N, 13°27’23’’E, 44 m a.s.l., cultivar Pinot Gris) is a 10-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.4 m and 0.7 m, respectively. Vineyard B (Cormons, Gorizia district, 45°56’32’’N, 13°27’26’’E, 44 m a.s.l., cultivar Tocai Friulano) is a 20-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of 3.0 m and 1.0 m, respectively. In both vineyards, standard organic fungicide programs were followed and no insecticide was sprayed before and after the trial applications.

In the first trial (Vineyard A) the efficacy of sulfur dust in the control of *L. botrana* was evaluated against the second generation, whereas in the second trial (Vineyard B) against the third generation. In the first trial, treatments in comparison were control, sulfur dust (see laboratory bioassays) applied at the rate of 30 kg/ha, and *B. thuringiensis* (Dipel DF, Sumitomo Chemical Agro Europe S.A.S, Saint Didier au Mont d’Or, FR, 1% W/V, Dipel DF/water). In the second trial, also kaolin (Surround WP, Tessenderlo Kerley Inc., Phoenix, Arizona, USA, 2% W/V, Surround WP/water) was tested. All products were applied using a backpack sprayer (M1200, Cifarelli s.p.a., Voghera (PV), Italy). *B. thuringiensis* and kaolin were sprayed at the rate of 1000 L/ha while for sulfur dust applications the backpack sprayer was equipped with a kit for the distribution of powders (OG.346.00, Cifarelli s.p.a., Voghera (PV), Italy). Applications were done at the occurrence of certain *L. botrana* phenological stages as expected on the basis of male flights monitored with pheromone traps and on average air temperatures. Kaolin was applied on two occasions at the beginning of male flight (31 July and 2 August in Vineyard B), sulfur dust was applied once at the beginning of egg laying (20 June in Vineyard A, 1 August in Vineyard B) and *B. thuringiensis* was applied at the beginning of egg-hatching and after a week (22 June and 03 July in Vineyard A, 4 and 11 August in Vineyard B). When sulfur dust was distributed, the grapevines were at BBCH 77 growth stage (i.e., “Berries beginning to touch”) in the first trial and at BBCH 85 growth stage (i.e., "Softening of berries") in the second trial. In both trials, a randomized block design with four replicates (rows) was adopted. Each row was divided into three plots of 28 (vineyard A) or into four plots of 30 (vineyard B) grapevines. In the first trial, in order to test the efficacy of treatments combined with bunch-zone leaf removal, plots were divided into two subplots of 14 grapevines that were subjected or not to manual removal of all leaves that covered bunches on 19 June (hereafter named with or without bunch-zone leaf removal).

The infestation of *L. botrana* was estimated at about 40 days from the beginning of the second or third seasonal males' flight. In each subplot of vineyard A and plot of vineyard B, 100 bunches were sampled from 12 (vineyard A) or 28 (vineyard B) grapevines, excluding edge plants. The number of grapevines sampled was different due to the different amount of bunches per grapevine in the two vineyards. The sampling was based on an *a priori* design (Pavan *et al*., 1998) to avoid the subjective choice of the sampled bunches. On each bunch, the number of larval nests and damaged berries was counted.

2.3. Statistical analyses

Statistical analyses were performed with Microsoft Excel 2013 for Windows (Microsoft Corporation 2013) and IBM SPSS Statistics 20 (IBM Corporation 2011). Two-sample *t*-test for the no-choice bioassay, repeated *G*-test of goodness-of-fit for the two-choice bioassay and Ryan's test for proportions comparison were used. One-way or two-way ANOVA were used to compare data in field trials. Differences among treatments were evaluated using a Tukey's post-hoc test with Bonferroni adjustment of the p-values ($\alpha = 0.05$). Data were checked for analysis assumptions and were log (x+1) transformed prior to the analyses.

3. Results

3.1. Laboratory assays

3.1.1. Influence of sulfur dust on female egg laying

The day before the beginning of the no-choice bioassay, the number (mean±SD) of eggs laid inside the tubes was not significantly different between the two groups of females used in sulfur dust (26.9±13.4 eggs/female) and control (22.6±18.3 eggs/female) ($t = 0.81$, df = 34, $P = 0.42$). During the no-choice experiments females laid significantly fewer eggs in sulfur dust (11.6±11.48 eggs/female) than control (52.72±36.84 eggs/female) ($t = 4.52$, df = 34, $P < 0.001$). On average sulfur dust reduced female fecundity by 78%.

3.1.2. Influence of sulfur dust on egg hatching

The hatching rate of eggs covered by sulfur dust (89.4%) was significantly lower than that of both control eggs (99.5%) and untreated eggs laid on sulfur-dust-covered berries (93.5%) (Ryan test, $P < 0.05$). On average, coverage of eggs with sulfur dust reduced their hatching by 10%.

3.1.3. Influence of sulfur dust on larval preference

A significantly lower number of larvae settled on sulfur-dust-treated berries (30.8%) than control ones (69.2%) ($G = 9.87$; $df = 1$, $P < 0.001$). On average, sulfur reduced larval preference for settlement by 55%.

3.2. Field trials

3.2.1. *L. botrana* male flights

The second flight of *L. botrana* males was very short and occurred from mid to late June with a peak on 19 June (Fig. 1).

The third flight of *L. botrana* males lasted one month from 28 July to 28 August and showed a peak in coincidence with the beginning of the flight (Fig. 1).

3.2.2. Efficacy of sulfur dust against *L. botrana* second generation

In the field trial against the second generation, treatment source of variation was significant, whereas the effect of bunch-zone leaf removal did not reach the significant level (Table 1; Fig. 2). Sulfur dust and *B. thuringiensis* showed significantly less larval nests ($P = 0.011$ and $P = 0.017$, respectively) and damaged berries ($P = 0.0001$ and $P = 0.001$, respectively) than control with no difference between each other ($P = 0.381$ for larval nests, $P = 0.623$ for damaged berries).

3.2.3. Efficacy of sulfur dust against *L. botrana* third generation

In the field trial against the third generation, treatment source of variation was significant (Table 1; Fig. 3). Sulfur dust, kaolin and *B. thuringiensis* showed significantly less larval nests ($P = 0.020$, $P = 0.012$ and $P = 0.008$, respectively) and damaged berries ($P = 0.018$, $P = 0.017$ and $P = 0.005$, respectively) than control with no difference among each other ($P \geq 0.941$ for larval nests, $P \geq 0.885$ for damaged berries).

4. Discussion

In the laboratory, sulfur dust reduced the egg lying of *L. botrana* in accordance with the results reported by Zappalà *et al.* (2012) for *T. absoluta*. The coverage of *L. botrana* eggs with sulfur dust only lightly reduced their hatching and then the impact of this effect in the field should be negligible also considering the prolonged period of egg laying. On the contrary, the high feeding-deterrent effect on larvae found in the laboratory could influence in an important way larval settlement under field conditions.

In the field trials, the efficacy of sulfur dust against *L. botrana* was not significantly different from those of *B. thuringiensis* and kaolin. The reduction of larval infestation was greater than that reported for *T. absoluta* (Zappalà *et al.*, 2012), against which sulfur dust was less effective than *B. thuringiensis*. This difference in effectiveness could be explained by the feeding behavior of these two lepidopteran pests and by plant morphology. In fact, while *T. absoluta* feeds on all plant, *L. botrana* feeds only on bunches which coverage can be achieved more easily than that of a whole tomato plant. Moreover, the sheltered position of bunches inside the canopy offers also protection from rain wash-off influencing sulfur-application persistence.

Preliminary field data had suggested an efficacy of sulfur dust against *L. botrana* (Touzeau, 1987), although applications occurred in advance with respect to the correct timing of insecticide applications. Based on our laboratory data, we can suppose that sulfur dust reduced moth infestation because of its inhibition of egg laying.

In the first field trial, leaf removal did not significantly reduce *L. botrana* infestation, as showed in other studies (Pavan *et al.*, 2016; Tacoli *et al.*, this PhD thesis), although a positive tendency was observed. This can be explained by the low vigor of the experimental vineyard. In fact, even after leaf removal the exposition of bunches was not visually much different between plots submitted or not to leaf removal.

Based on this study, the application of sulfur dust against powdery mildew in vineyards, just before the occurrence of BBCH 79 growth stage (i.e., Majority of berries touching each other), can be considered an added value for its positive side effects against *L. botrana* second generation and perhaps also *E. vitis*. An important factor for the success of this strategy is the timing of the sulfur powder application since it has to be done following not only plant phenology, but also monitoring the start of *L. botrana* flights with pheromone traps. In fact, according to our results, the effect of sulfur dust is mostly due to the reduction of oviposition, since little effect can be obtained on egg development and effects on larval settlement have to be confirmed in field conditions. The trial conducted against the third generation of *L. botrana* indicates that sulfur dust has the same efficacy even when it is applied after the occurrence of BBCH 79.

Sulfur dust performed not differently from *B. thuringiensis* with the benefit of requiring one application instead of two per *L. botrana* generation. However, *B. thuringiensis*, on the other hand, has probably a more favorable ecotoxicological impact on non-target arthropods due to its selectivity. The detrimental effects of sulfur dust on non-target arthropods, particularly phitoseiid mites, are well known (Hessan *et al.*, 1994; Gadino *et al.*, 2011) and therefore we suggest to limit the number of applications to avoid disruption of biological control.

5. References

Bencivelli, A. and G. Rapparini. 1975. The activity of chemicals of known and of recent formulation against grapevine powdery mildew. Informatore Fitopatologico. 25 (4): 19–21.**Boselli, M., M. Scannavini, and M. Melandri. 2000.** Confronto fra strategie di difesa contro la tignoletta della vite. L'Informatore Agrario 56 (19): 61–65.

Civolani, S., M. Boselli, A. Butturini, M. Chicca, E. A. Fano, and S. Cassinelli. 2014. Assessment of insecticide resistance of *Lobesia botrana* (Lepidoptera: Tortricidae) in Emilia-Romagna region. J. Econ. Entomol. 107: 1245–1249.

Fermaud, M., and A. Giboulot. 1992. Influence of *Lobesia botrana* larvae on field severity of Botrytis rot of grape berries. Plant. Dis. 76: 404–409.

Fermaud, M. 1998. Cultivar susceptibility of grape berry clusters to larvae of *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 91: 974–980.

Gadino, A. N., V. M. Walton, and A. J. Dreves. 2011. Impact of vineyard pesticides on a beneficial arthropod, *Typhlodromus pyri* (Acari: Phytoseiidae), in laboratory bioassays. J. Econ. Entomol. 104: 970–977.

Galet, P. 1982. Les Maladies et les Parasites de la Vigne. Vol. 2. Paysan du Midi, Montpellier, France.

Goebel, O., C. Vergnet, and J. J. Heller. 2001. Control of acarinosis and grape blister. Phytoma 537: 39–41.

Ifoulis, A. A., and M. Savopoulou-Soultani. 2004. Biological control of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae by using different formulations of *Bacillus thuringiensis* in 11 vine cultivar under filed conditions. J. Econ. Entomol. 97: 340–343.

Ioriatti, C., A. Lucchi, and L. G. Varela. 2012. Grape berry moths in Western European vineyards and their recent movement into the New World, pp. 339–360. In N.J. Bostanian, C. Vincent and R. Isaac (Eds.), Arthropod management in vineyards: pest, approaches, and future directions. Springer Science+Business Media B.V., Dordrecht, The Netherlands.

Ioriatti, C., G. Anfora, M. Tasin, A. De Cristofaro, P. Witzgall, and A. Lucchi. 2011. Chemical ecology and management of *Lobesia botrana* (Lepidoptera: Tortricidae). J. Econ. Entomol. 104: 1125–1137.

Jepsen, S. J., J. A. Rosenheim, and M. E. Bench. 2007. The effect of sulfur on biological control of the grape leafhopper, *Erythroneura elegantula*, by the egg prasitoid *Anagrus erythroneurae.* Biocontrol 52: 721–732.

Hassan, S. A., F. Bigler, H. Bogenschütz, and H. Vogt. 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS-Working Group «Pesticides and Beneficial Organisms». Biocontrol 39: 107–119.

Kiaeian Moosavi, F., E. Cargnus, F. Pavan, and P. Zandigiacomo. 2017. Mortality of eggs and newly hatched larvae of *Lobesia botrana* (Lepidoptera: Tortricidae) exposed to high temperatures in the laboratory. Environ. Entomol. 46: 700–707.

Kiaeian Moosavi, F., E. Cargnus, F. Pavan, and P. Zandigiacomo. Effects of grapevine bunch exposure to sunlight on berry surface temperature and *Lobesia botrana* (Lepidoptera: Tortricidae) egg laying, hatching and larval settlement. Agric. For. Entomol. doi: 10.1111/AFE.12274.

Maher, N., and D. Thiéry. 2004. A bioassay to evaluate the activity of chemical stimuli from grape berries on the oviposition of *Lobesia botrana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 94: 27–33.

Moreau, J., B. Benrey, and D. Thiéry. 2006. Grape variety affects larval performance and also female reproductive performance of the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). Bull. Entomol. Res. 96: 205–212.

Moschos, T. 2006. Yield loss quantification and economic injury level estimation for the carpophagous generations of the European grapevine moth *Lobesia botrana* Den. et Schiff. (Lepidoptera: Tortricidae). Int. J. Pest. Manag. 52: 141–147.

Özsemerci, F., F. Özlem Altindisli, F. Ates, A. Altindisli, and C. Takma. 2013. The effect of different leaf removal systems and fungicide combinations on phytophagous pests and predatory mite populations, and yield of organic Sultani Cekirdeksiz grape variety. IOBC-WPRS Bull. 91: 101–109.

Pavan, F., C. Floreani, P. Barro, P. Zandigiacomo, and L. Dalla Montà. 2013. Occurrence of two different development patterns in *Lobesia botrana* (Lepidoptera: Tortricidae) larvae during the second generation. Agric. For. Entomol.14: 398–406.

Pavan, F., G. Bigot, E. Cargnus, and P. Zandigiacomo. 2014a. Influence of the carpophagous generations of the European grapevine moth *Lobesia botrana* on grape bunch rots. Phytoparasitica, 42: 61–69.

Pavan, F., E. Cargnus, G. Bigot, and P. Zandigiacomo. 2014b. Residual activity of insecticides applied against *Lobesia botrana* and its influence on resistance management strategies. Bull. Insectol. 67: 273–280.

Pavan, F., E. Cargnus, S. Kiaeianmoosavi, G. Bigot, F. Tacoli, and P. Zandigiacomo. 2016. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* and grey mould. Bull. Insectol. 69: 107–115.

Pavan, F., G. Stefanelli, E. Cargnus, and A. Villani. 2009. Assessing the influence of inflorescence traits on the susceptibility of grape to vine moths. J. Appl. Entomol. 133: 394–401.

Rapagnani, M. R., V. Caffarelli, M. Barlattani, and F. Minelli. 1990. Descrizione di un allevamento, in laboratorio, della tignoletta dell'uva *Lobesia botrana* Den. E Schiff. (Lepidoptera – Tortricidae) su un nuovo alimento semi-sintetico. Bollettino dell'Istituto di Entomologia «G. Grandi» Università di Bologna. 44: 57–64.

Sharon, R., T. Zahavi, V. Soroker, and A. R. Harari. 2009. The effect of grapevine cultivars on *Lobesia botrana* (Lepidoptera: Tortricidae) population levels. J. Pest Sci. 82: 187–193.

Stravridis, D. G., and M. Savopoulou-Soultani. 1998. Larval performance on and oviposition preference for known and potential hosts by *Lobesia botrana* (Lepidoptera: Tortricidae). Eur. J. Entomol. 95: 55–63.

Touzeau, J. 1987. Les actions secondaires du soufre sur les maladies et ravageurs de la vigne, pp. 167–177. *In* Proceedings of the International Symposium elemental sulphur in agriculture, Acropolis, Nice, France, 25-27 Mars 1987.

Vartholomaiou, A. N., E. I. Navrozidis, C. C. Payne, and G. A. Salpiggidis. 2008. Agronomic techniques to control *Lobesia botrana*. Phytoparasitica. 36: 264–271.

Zappalà, L., G. Siscaro, A. Biondi, O. Mollá, J. González-Cabera, and A. Urbaneja. 2012. Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidocoris tenuis.* J. Appl. Entomol. 136: 401–409.

Table 1. Field trials. Results of two-way and one-way ANOVA performed on larval nests of *L. botrana* and damaged berries.

Source of variation	Larval nests			Damaged berries		
	F	df	*P*	*F*	df	*P*
Trial 1 – 2nd generation						
Corrected Model	5.652	5,18	0.003	4.888	5,18	0.005
Treatment	12.637	2,18	0.0001	10.480	2,18	0.001
Leaf removal	2.192	1,18	0.156	2.571	1,18	0.126
Treatment*Leaf removal	0.398	2,18	0.677	0.455	2,18	0.642
Trial 2 – 3rd generation						
Treatment	7.186	3,12	0.005	7.468	3,12	0.004

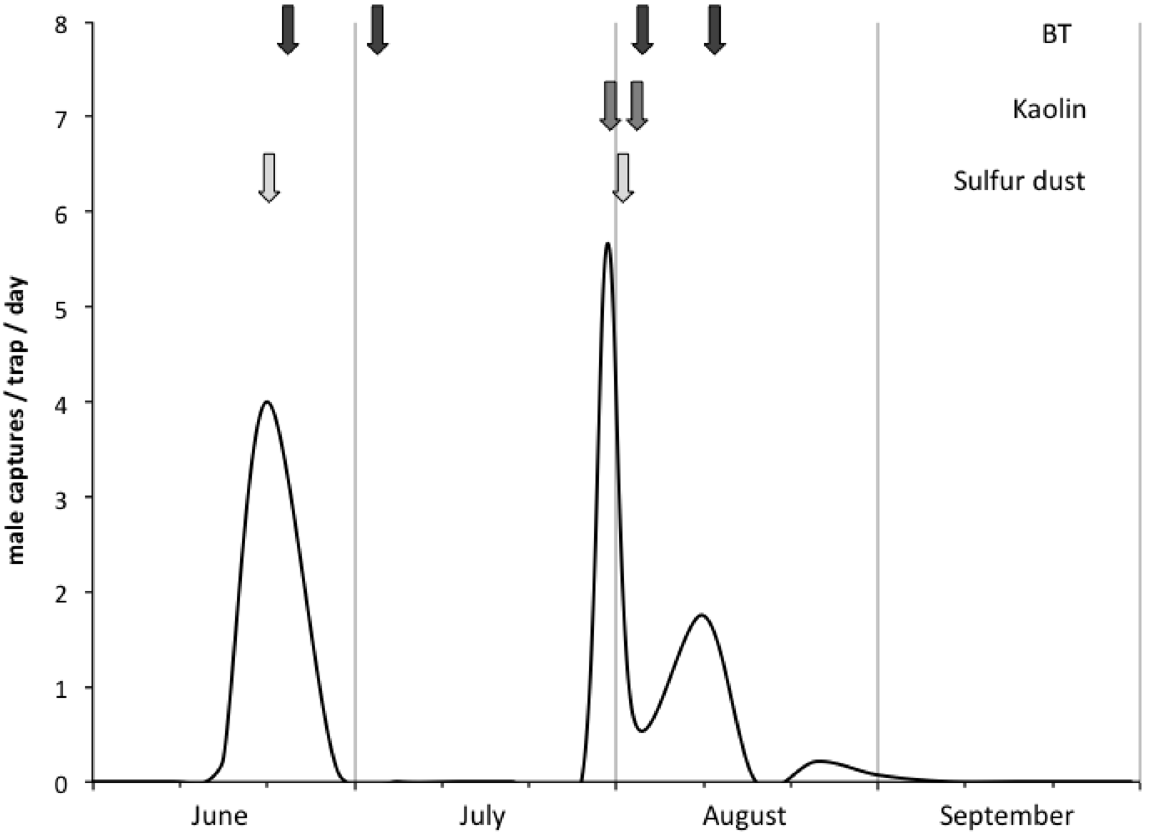

Fig. 1. Second and third flights of *L. botrana* males recorded with pheromone traps. The arrows indicate the time of application of the different products in the two trials.

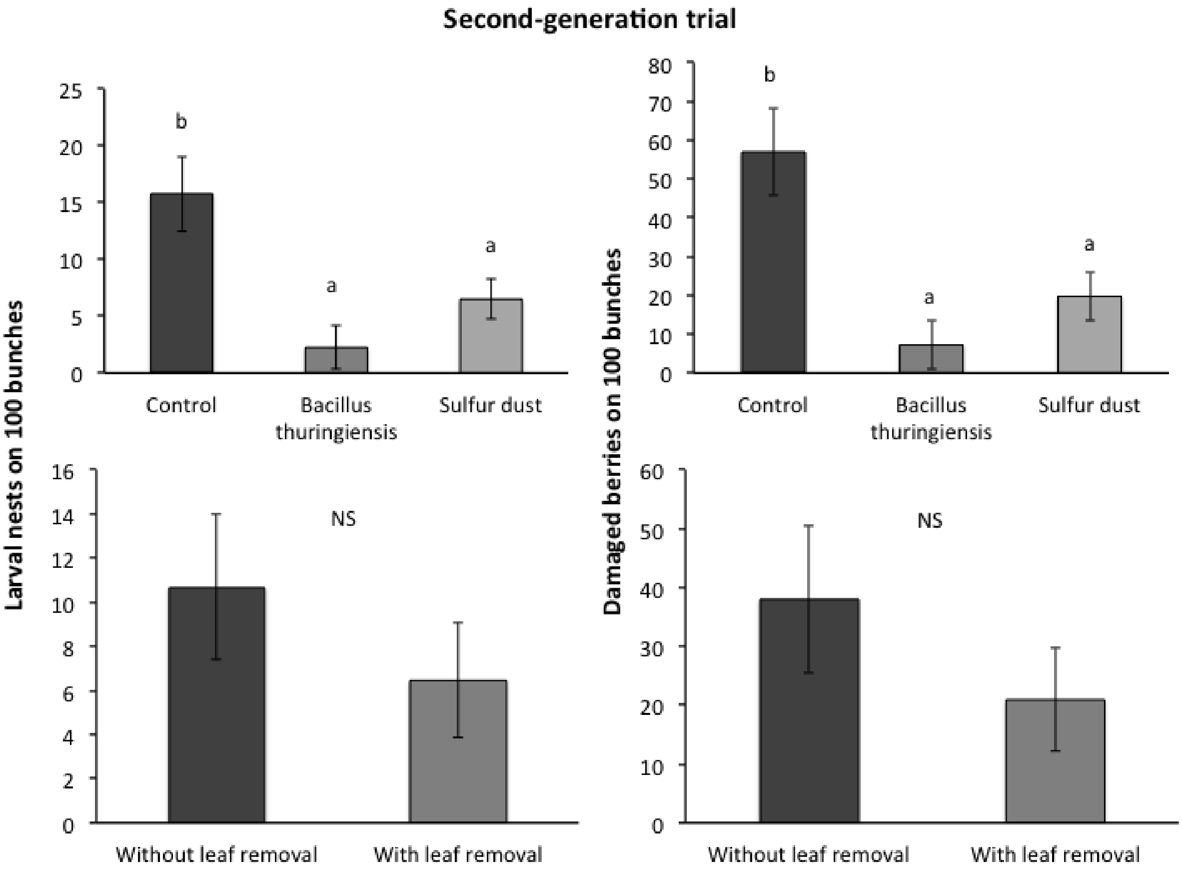

Fig. 2. Field trial on the effect of different treatments and bunch-zone leaf removal on *L. botrana* second-generation infestations (mean ± standard deviation of larval nests and damaged berries). Different letters among treatments indicate significant differences according to Tukey's post-hoc test ($\alpha = 0.05$) and NS no significant differences according to ANOVA.

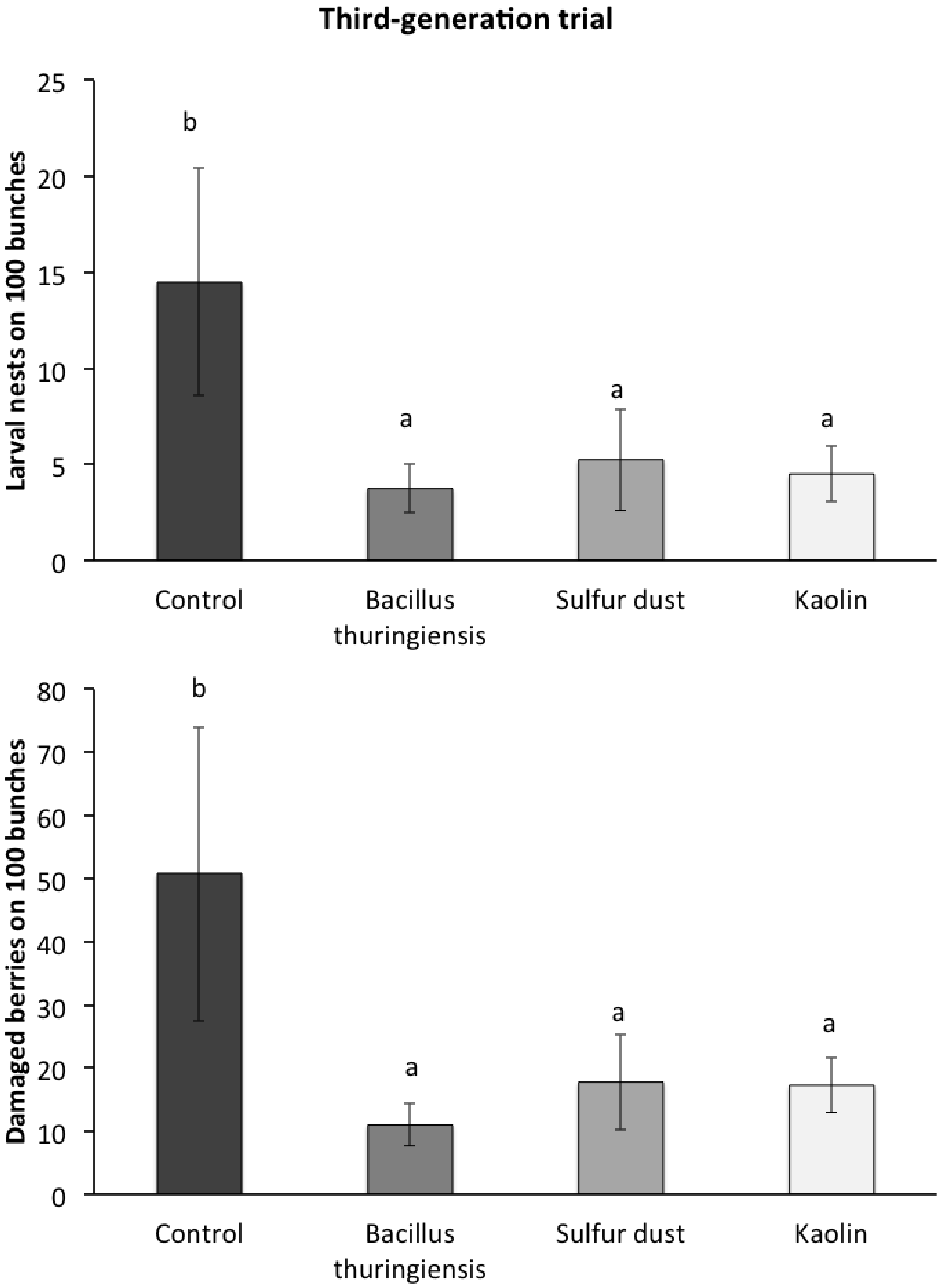

Fig. 3. Field trial on the effect of different treatments on *L. botrana* third-generation infestations (mean ± standard deviation of larval nests and damaged berries). Different letters among treatments indicate significant differences according to Tukey’s post-hoc test (α = 0.05).

Chapter 6 - Insecticidal activity of natural products to control vineyard mealybugs (Hemiptera: Pseudococcidae)

Chapter 6 is formatted according to the journal author guidelines and has been submitted to Crop Protection as:
Federico Tacoli, Vaughn A. Bell, Elena Cargnus, Francesco Pavan. Insecticidal activity of natural products to control vineyard mealybugs (Hemiptera: Pseudococcidae).

Abstract

Mealybugs are worldwide pests in vineyards due to both direct damage and spread of grapevine leafroll disease. The objective of this study was to find natural products valuable as an alternative 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. Kaolin was ineffective at controlling mealybugs in the vineyard, and in one case, leaf infestations increased while bunch infestations declined. In contrast, citrus essential oil increased mealybug mortality in the laboratory and reduced leaf (but not bunch) infestation in the vineyards.

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 et al., 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 both grapevines and ground cover plants, and completes two and possibly a partial third generation per year (V. Bell, unpublished data). *Pseudococcus longispinus* overwinters under the bark of grapevines as adult females and crawlers (first instar nymph), 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; Mansur et al., 2017). *Planococcus ficus* is the most important mealybug pest for 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 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 mealybug is their status as vectors of Grapevine leafroll-associated virus 3 (GLRaV-3), the predominant type species causing the grapevine leafroll disease (GLD) (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., 2008a; Cocco et al., 2014; Wallingford et al., 2015). However, insecticide applications can be of limited effectiveness because of both 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 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 substances are available for use against mealybugs (e.g., mineral oil or insecticidal soap) (Regulation 2008/889/EC). Biological control by release of predatory coccinellids (Coleoptera) or parasitoid wasps (Hymenoptera) (Daane et al., 2008b; Marras et al., 2016; Pozzebon et al., in press) and mating disruption (Walton et al., 2006; Cocco et al., 2014; Sharon et al., 2016) can be valid alternatives to chemical control, although the high cost of these options justifies use only in certain circumstances. 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 substances 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 acting against mealybugs (Karamaouna et al., 2013). In particular, citrus essential oil, 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 (Gleen 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, it 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 plant essential oils, terpenes, kaolin and insecticidal soap against mealybugs infesting grapevines in New Zealand and Italian vineyards. For essential oils, 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 be also be detectable against this pest group under field conditions. For kaolin, we predicted evidence of some feeding deterrence among mealybugs, analogous to that which was observed among other sap feeders (Tacoli et al., 2017), together with an inhibition of mealybug crawler migration from bark to leaves or from leaves to bunches (Gleen et al., 1999). For insecticidal soap, we predicted that could be toxic to *Pl. ficus* crawlers, analogous to that which was observed for psyllids and aphids (Tremblay et al., 2009; Hall and Richardson, 2012).

2. Materials and methods

During 2015–2017 natural insecticides were tested in the laboratory and in the field against vineyard mealybugs in New Zealand and in Italy.

2.1. Natural products tested

The natural products tested in the laboratory and in vineyards in New Zealand and Italy are listed in table 1.

The New Zealand orange oil emulsion was added with 4% citric acid and 1% sodium laureth sulphate to improve the efficacy of the product (Hollingsworth and Hamnett, 2010).

2.2. Laboratory experiments in New Zealand

Two laboratory experiments were carried out in the Southern Hemisphere early-summer in 2015. In the first experiment, the mortality of *Ps. calceolariae* first-instar nymphs was compared in two treatments: (i) nymphs on untreated leaves (control) and (ii) nymphs on kaolin treated leaves (kaolin). In the second experiment, mortality was compared over three treatments: (i) nymphs on untreated leaves (control), (ii) nymphs on citrus-essential-oil sprayed leaves (CEO-only-on-leaves) and (iii) nymphs and leaves sprayed with CEO (CEO-on-leaves-and-nymphs).

For the laboratory experiments, grapevine leaves were 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.). Mealybugs of first and second instar nymphs 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. Fifty grapevine leaves per treatment were individually placed together with one first instar *Ps. calceolariae* into transparent self-sealing plastic bags (20 × 35 cm). The products were applied with a Potter spray tower (Burkard Scientific Ltd, Uxbridge, UK) spraying 2 mL of solution per leaf at the pressure of 15 psi (103 kPa). 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).

2.3. Field trials in New Zealand

2.3.1. Years and vineyards

During the 2015-2016 Southern Hemisphere growing season, three field trials were carried out in two vineyards. 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. 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. In Vineyard A, Buprofezin (Ovation 50 WDG, Etec crop solutions, Auckland, New Zealand, 0.25% solution) was applied in late October (pre-flowering application) 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).

2.3.2. Treatments in comparison

In Vineyard A, two trials were carried out comparing the control (i.e., only the farm application of Buprofezin) with kaolin (Trial 1) and CEO (Trial 2) treatments, respectively. It was decided to run two separate trials to simplify the distribution of products. Kaolin was sprayed three times (3 and 24 December 2015 and 2 February 2016), with the first application timed before the emergence of the second generations of *Ps. calceolariae* and *Ps. longispinus*. CEO was applied once on 3 December 2015, on the basis of farmer needs, even if the second generations had not yet started. In Vineyard B, one trial was carried out comparing the control (i.e., no farm application of insecticides) with kaolin applied to the vine foliage three times (4 and 23 December 2015 and 9 February 2016). All the products were sprayed using a two rows recycling sprayer (FMR, 2300R, FMR GROUP LTD, Blenheim, New Zealand) at a rate of 1000 L/ha. In both trials, 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.

2.3.3. Pheromone trapping

In both Vineyards A and B, adult male *Ps. calceolariae* and *Ps. longispinus* were monitored during the season using species-specific pheromone-baited traps. Two traps per mealybug species were deployed in each vineyard. In both vineyards, monitoring started on 2 November 2015 (leaf development BBCH 11-19, Lorenz et al., 1995) and ended on 22 March 2016 (ripening of berries BBCH 85-89). The sticky bases were changed fortnightly and the number of mealybug males of each species was counted with the aid of a dissecting microscope. Pheromone lures for both species were replaced every six weeks. Data were expressed as number of males captured per trap per day.

2.3.4. Leaf samplings

In both Vineyards A and B, mealybug nymphs and adult females were sampled on leaves four times between flowering (BBCH 61-69) and ripening of berries (BBCH 85-89) (i.e., in Vineyard A on 30 November 2015, and 18 January, 17 February and 15 March 2016; in Vineyard B on 2 December 2015, and 13 January, 15 February and 11 March 2016). Samples consisted of 20 basal leaves per replicate, considering two leaves from each grapevine of the 10 central grapevines of both rows, for a total of 400 leaves per treatment per sampling date. On the first date, counting of mealybugs was done directly in the vineyards, while on the other three dates, the leaves were collected and mealybugs were identified based on morphological characteristic and counted in the laboratory under a dissecting microscope.

2.4. Laboratory experiments in Italy

Two laboratory experiments were carried out in Northern Hemisphere autumn 2016. In the first experiment, the mortality of *Pl. ficus* first-instar nymphs was compared in four treatments: (i) nymphs on untreated agar (control), (ii) nymphs on kaolin-sprayed agar (kaolin), (iii) nymphs on citrus-essential-oil-sprayed agar (CEO-only-on-agar) and (iv) nymphs and agar sprayed with CEO (CEO-on-agar-and-nymphs). In the second experiment, control and different concentrations of CEO (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6%) were tested, as described for CEO-on-agar-and-nymphs, to determine the lethal concentrations LC_{50} and LC_{95} and the 95% confidence intervals (CI). Glass Petri-dishes (5 cm diameter) were half filled with agar gel substrate (1.5%, 10 mL) and laboratory-reared first and second instar *Pl. ficus* were transferred from seed potatoes using a small soft painting brush. Fifty mealybugs per treatment were placed in 10 Petri dishes in groups of five individuals. The two products were applied with a Potter spray tower (Burkard Scientific Ltd, Uxbridge, UK) spraying 1.2 mL of solution per Petri dish at the pressure of 15 psi (103 kPa). The amount of solution deposited was 1.9-2.0 mg cm^{-2}, as recommended by the IOBC guideline (Hassan, 1985). To record nymph mortality, the Petri dishes were checked after 4 h and again at 24 h for the first experiment, and after 24 h for the second experiment.

2.5. Field trials in Italy

2.5.1. Years and vineyards

In 2016 and 2017 Northern Hemisphere growing seasons, two field trials looking at the control of *Pl. ficus* were carried out in Vineyards C and D, respectively. Vineyard C (Manzano, Udine district, 46°01'19"N, 13°23'17"E, 83 m a.s.l., cultivar Merlot) was a 15-year-old conventional vineyard growing using double arched Guyot training system with distances between and along rows of 2.4 m and 0.6 m, respectively. Vineyard D (Udine, 46°02'04"N, 13°13'33"E, 90 m a.s.l., cultivar Chardonnay) was a 15 year-old conventional vineyard growing using a Guyot training system with distances between and along rows of 2.4 m and 1.0 m, respectively. No insecticides were sprayed on the blocks before and during the trials.

2.5.2. Treatments in comparison

In Vineyard C, the treatments in comparison with the control were kaolin, CEO and terpenes applied to the vine canopy to control the *Pl. ficus* second generation, whereas, in Vineyard D, the treatments were kaolin, CEO and insecticidal soap applied to the vine canopy to control the *Pl. ficus* second and the third generations (Table 1). In Vineyard C, kaolin was applied post-flowering on 3 and 17 June and 8 July 2016, while CEO and terpenes were applied on 8 and 15 July 2016. In Vineyard D, kaolin applications were timed for 31 May, 15 June and 5 July 2017; CEO and insecticidal soap were applied, respectively, on 5 July and, 5 and 13 July 2017 (against the second generation), and on 9 August, and 9 and 16 August 2017 (against the third generation). Kaolin applications occurred early in the season to provide coverage before the start of the migration of the second generation of *Pl. ficus* nymphs from woody parts of the vine to leaves and bunches, while CEO, terpenes and insecticidal soap were applied during the migration of second- and third-generation crawlers. All products were applied using a back-pack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano, RE, Italy in 2016 and M1200, Cifarelli s.p.a., Voghera, PV, Italy in 2017) at a rate of 1000 L/ha. In both Vineyards C and D, a randomized block design with 10 replicates was adopted. Each replicate consisted of 10 grapevines.

2.5.3. Pheromone trapping

In both Vineyards C and D, adult male *Pl. ficus* were monitored using a single pheromone-baited trap per vineyard. In Vineyard C, only the flight of the first generation was monitored from 24 May to 28 June (from BBCH 57 to 75). In Vineyard D, the flights of all three generations were monitored from 25 May to 29 September (from BBCH 57 to 99). The sticky bases were changed weekly and the number of males was counted using a dissecting microscope in the laboratory.

Pheromone lures were replaced every six weeks. Data were expressed as number of males captured per trap per day.

2.5.4. Leaf samplings

In Vineyard C, mealybugs on leaves were sampled seven times, on 3 and 15 June, 8, 15, 22 and 29 July and 5 August 2016 (from BBCH 60 to 81). In Vineyard D, samplings occurred 10 times, on 30 May, 13 June, 5, 12, 18 and 26 July, 2, 9, 16 and 22 August 2017 (from BBCH 60 to 99). In both years on the first two visits, samples consisted of five basal leaves per replicate, chosen on five grapevines for a total of 50 leaves per treatment. These were checked in the vineyard using a 5× magnification lens to count mealybugs. On 1 July 2016 and 28 June 2017, before the beginning of the migration of the second-generation nymphs, five of the most infested plants per replicate were marked. A grapevine was considered strongly infested when colonies of mealybugs were present under the bark and many ants walked on the trunk. This procedure was adopted to avoid systematic error in the choice of sampled plants and reduce infestation variability among replicates considering the very high aggregated distribution of this pest (Duso, 1989). To ensure reliable count of the second-generation crawlers, from the third sampling date, 10 basal leaves per replicate were collected, i.e. two leaves from each marked-grapevine, for a total of 100 leaves per treatment and sampling date. The leaves were taken to the laboratory and mealybugs were identified to species level (Tranfaglia, 1976) and counted with the aid of a dissecting microscope.

2.5.5. Bunch sampling

Only in Vineyard D did we sample bunches in order to estimate the *Pl. ficus* infestation level at the end of the second generation. On 31 July 2017, we collected a bunch from each of two marked grapevines per replicate, for a total of 20 bunches per treatment. In the laboratory, bunches were dissected in order to count *Pl. ficus* mature females using a dissection microscope.

2.6. Statistical analyses

Log-rank test (Mantel, 1966) was used together with Fisher's exact and Ryan test to compare data in all laboratory experiments. Mortality data from the dose-response experiments were analyzed using the probit transformation; the natural mortality rate was considered using the iterative approach, according to Finney (1949). The concentrations which kill a given proportion z of mealybugs (LC_z) and their confidence intervals were computed according to Finney (1971).

Mixed ANOVA with Tukey's post-hoc test was used after logarithmic transformation to compare data in all the field trials, and efficacy of treatments was calculated according to

Henderson and Tilton (1955). One-way ANOVA was used to compare bunch infestation in Vineyard D on 2017.

Statistical analyses were performed with Microsoft Excel 2013 for Windows (Microsoft Corporation 2013) and IBM SPSS Statistics 20 (IBM Corporation 2011).

3. Results

3.1. Laboratory experiments in New Zealand

3.1.1. First Experiment – Effect of kaolin on *Ps. calceolariae*

Kaolin significantly increased mortality over time of *Ps. calceolariae* in comparison with the control (Log-rank test: $\chi^2 = 15.108$, $df = 1$, $P < 0.0001$) (Fig. 1). The differences were significant from 20 h to the end of the experiment.

The proportion of nymphs on the bag surface vs. leaves over time was not significantly different between the kaolin than the control (Log-rank test: $\chi^2 = 0.969$, $df = 1$, $P = 0.325$) (Fig. 1), although from 20 to 68 h differences were significant.

3.1.2. Second Experiment – Effect of CEO on *Ps. calceolariae*

The mortality over time of *Ps. calceolariae* was significantly increased by CEO treatments (Log-rank test: $\chi^2 = 36.115$, $df = 2$, $P < 0.0001$) (Fig. 1). At 8 h, the CEO-on-leaves-and-nymphs differed significantly from the control. Similarly, at 20 h the CEO-only-on-leaves showed a significantly higher mortality rate than the control and did not differ anymore from the CEO-on-leaves-and-nymphs.

The proportion of nymphs on the bag surface over-time was significantly higher in the CEO-only-on-leaves than in both the control and CEO-on-leaves-and-nymphs (Log-rank test: $\chi^2 = 8.695$, $df = 2$, $P = 0.01$) (Fig. 1). The differences were significant at 20 h from the beginning of the experiment.

3.2. Field trials in New Zealand

3.2.1. Pheromone-trap counts of male mealybug

The captures of male *Ps. calceolariae* and *Ps. longispinus* were prevalent in Vineyard A and Vineyard B, respectively. Both mealybug species were captured from November 2015 to March 2016 and showed two distinct periods of captures (i.e., in November-December 2015 and from late January 2016), coinciding with the emergence of males of the first and second generations (Fig. 2).

3.2.2. Mealybug leaf infestation

In Vineyard A, the mealybug encountered most commonly was *Ps. calceolariae.* On 30 November 2015, pre-trial sampling resulted in us finding a single mealybug on a leaf sample. Across the whole sampling period no differences were detected between the kaolin (Trial 1) and CEO (Trial 2), and the respective controls (in Trial 1, $F = 1.237$, $df = 1{,}38$, $P = 0.273$; in Trial 2, $F = 0.001$, $df = 1{,}38$, $P = 0.974$) (Fig. 3).

In Vineyard B, the mealybug encountered most commonly was *Ps. longispinus.* On 2 December 2015, pre-trial sampling resulted in us finding three mealybugs on leaves. Across the whole sampling period no differences were detected between the kaolin and the control ($F = 0.517$, $df = 1{,}38$, $P = 0.477$) (Fig. 3).

3.3. Laboratory experiments in Italy

3.3.1. First Experiment – Effect of kaolin and CEO on *Pl. ficus*

The two CEO treatments significantly increased *Pl. ficus* mortality over time when compared with either the kaolin or the control (Log-rank test: $\chi^2 = 92.226$, $df = 3$, $P < 0.0001$) (Fig. 4). After 4 h, only the number of *Pl. ficus* nymphs on the CEO-on-agar-and-nymphs differed significantly from the control. After 24 h, also the CEO-only-on-agar showed significantly higher mortality of *Pl. ficus* compared with either kaolin or the control, but still significantly lower than that of the CEO-on-agar-and-nymphs.

3.3.2. Second Experiment – LC_{50} and LC_{95} for citrus essential oil formulation against *Pl. ficus*

The CEO formulation LC_{50} was 344 mL/L (95% CI: 297 - 380 mL/L) and LC_{95} was 654 mL/L (95% CI: 572 – 827 mL/L). Goodness-of-fit test was not statistically significant ($\chi^2 = 2.07$, $df = 4$, $P = 0.72$) and therefore no heterogeneity factor was used in the calculation of confidence intervals (CI). The regression equation was $y = 0.002x - 0.2323$ ($R^2 = 0.99$).

3.4. Field trials in Italy

3.4.1. Pheromone-trap counts of mealybug males

In Vineyard C, the captures of *Pl. ficus* first generation occurred in June and showed a peak on 15 June 2016 (Fig. 5).

In Vineyard D, *Pl. ficus* males were captured from June to September 2017 and had three distinct periods of captures (early- to mid-June, mid-July to early-August, and late-August to late-September), coinciding with male emergence of the three generations (Fig. 5).

3.4.2. *Pl. ficus* leaf infestation

In Vineyard C, over the pre-trial period (3 June to 8 July 2016), no significant treatment effect was detected (*F* = 0.625, *df* = 3,36, *P* = 0.603) (Fig. 6). Over the remaining sampling period significant differences were detected among treatments (*F* = 4.438, *df* = 3,36, *P* = 0.009), with CEO being the only treatment significantly less infested than the control (*P* = 0.021). Terpene-treated vines neither differed from control vines nor those treated with CEO (*P* = 0.076). The two CEO applications showed a cumulative Henderson-Tilton efficacy of 76% (60.6% after first application, 39.2% after second application).

In Vineyard D during the pre-trial period (30 May to 5 July 2017), no significant differences were detected among treatments (*F* = 1.423, *df* = 3,36, *P* = 0.252) (Fig. 7). Over the remaining sampling period, significant differences were detected among treatments (*F* = 13.119, *df* = 3,36, *P* = 0.0001), with kaolin-treated vines more infested than all the other treatments and the control (*P* < 0.01), while CEO-treated vines were less infested than those in the control (*P* = 0.04). No significant difference was observed between CEO and insecticidal soap (*P* = 0.262). CEO showed a Henderson-Tilton efficacy of 74.7% in the application against the *Pl. ficus* of the second generation, but of only 10.3% against the third generation.

3.4.3. *Pl. ficus* bunch infestation

Although the *Pl. ficus* second-generation bunch infestation was apparently lower in the kaolin, CEO and insecticidal soap than in the control (Abbott reduction by 57%, 47% and 37%, respectively), no significant differences emerged (*F* = 1.572, *df* = 3, *P* = 0.213) (Fig. 8).

4. Discussion

4.1. Laboratory data

Citrus essential oil increased the mortality of both *Ps. calceolariae* and *Pl. ficus* when tested under laboratory conditions. On the contrary, kaolin increased the mortality only of *Ps. calceolariae*.

The efficacy of CEO has already been highlighted against both *Pl. ficus* and *Ps. longispinus* (Hollingsworth, 2005; Hollingsworth and Hamnett, 2010; Karamaouna et al., 2013), but the formulations we tested are not currently commercially available. To evaluate the toxicity of CEO against *Ps. calceolariae*, a formulation prescribed by Hollingsworth and Hamnett (2010) was used, with the results observed being similar to those found by these authors for *Ps. longispinus*. In Italy, the toxicity of CEO against *Pl. ficus* was tested using a commercial product with no label claim for mealybugs. In our experiments, direct applications of CEO on mealybug nymphs resulted in high rates of mortality, whereas when nymphs were placed in contact with substrates previously covered with CEO, the increase in mealybug mortality was both slower and lower. Since these latter applications increased also *Ps. calceolariae* movement from the leaf to the bag surface, indicating a repellent activity of CEO on *Ps. calceolariae,* we can suppose that nymphs move to avoid volatile organic compounds (VOCs) that act on insect integument and produce effects similar to the ones of the direct contact with the product (Lee et al., 2003). These CEO properties are likely to be more evident in the laboratory than under field conditions since nymphs are confined in a close environment.

4.2. Field data

In the field trials, kaolin was not able to control mealybug infestations of either *Ps. calceolarie*, or *Ps. longispinus* and *Pl. ficus*. In the sampling occurred in January after two kaolin applications in the New Zealand vineyards, lower numbers of mealybugs were found on the kaolin-treated plots compared with those in the untreated control, although the differences were not significant. In Vineyard C in the Italian field trials, kaolin-treated plots were similarly infested to those of the untreated control. In Vineyard D, mealybug infestations in the vines were substantially higher in the kaolin-treated plots compared with the untreated control. This latter result concurred with the findings of Joubert et al. (2004), where numbers of *Ps. longispinus* increased on mango plants treated with kaolin. In Vineyard D, kaolin-treated plots had the lowest bunch infestation despite the highest population levels on leaves. This could be explained by a reduced migration of mealybugs from leaves to bunches without an increase in their mortality. Indeed, insect movement impairment from kaolin is already reported in other studies (Gleen et al. 1999; Puterka et al. 2003; Sackett et al. 2005). These results indicate that kaolin is not a solution to mealybug infestations, but when applied for other purposes its potential ability of reducing the migration of *Pl. ficus* towards bunches could increase the efficacy of insecticides. Because in north-eastern Italy most of damage is caused by *Pl. ficus* bunch infestation (Duso, 1989), the timing of applications against the second generation is often problematic, particularly when low-persistent insecticides are used. Indeed, the migration of second-generation crawlers from leaves to bunches lasts for several days and when mealybugs are inside bunches targeting them with insecticides is very difficult, mostly in compact-bunch cultivars. Due to this reason, a reduction of their migration towards the bunches can favour their control.

In New Zealand field trials, CEO did not show any activity against *Ps. calceolariae*. This result may have been influenced by the foliar application being too early relative to the emergence and movement of the mealybug second generation. The choice of timing for this input was unavoidable due to a request from the vineyard owner. With the very short withholding period, characteristic of CEO (Hollingsworth, 2005), we contend this product was unable to exert any long-term influence over mealybug populations on the vines, unlike the influence demonstrated in the laboratory experiment.

The control of the second generation of *Pl. ficus* on leaves was higher in the 2017 trial after a single CEO application than in the 2016 one after two applications, because the type of backpack sprayer used in the second year produced smaller droplets providing better leaf coverage. This was the reason because in the 2017 trial the second CEO application was postponed against the third generation. However, this last application showed a scarce efficacy against the third generation on leaves, probably for the combination of two effects: the observed low efficacy of the application against the second-generation inside bunch and a supposed subsequent migration of the third-generation crawlers toward leaves. To increase the efficacy against second generation on bunches, CEO should be applied in advance before the second-generation crawlers are hidden inside already-compact bunches. Relatively to leaf infestation, kaolin appeared more effective on bunches than CEO. We can hypothesize that the preventive kaolin application, when bunches were still loose, deterred the bunch colonization by second-generation crawlers.

Terpenes and insecticidal soap were not effective in controlling mealybugs but were statistically not different from CEO, a result suggesting that the effectiveness of both compounds requires further evaluation.

4.3. Conclusions

Even if the hoped-for results were not achieved in the field trials, CEO was effective against mealybugs, particularly *Pl. ficus* both under laboratory and field conditions. Since CEO is a contact insecticide characterized by low persistence, a first application against the second generation at the beginning of the crawlers migration, followed by a second application after a week, should allow an optimal *Pl. ficus* control, preventing most of the bunch colonization. Based on the hypothesis that kaolin may prevent mealybug population migration from leaves to bunches, the combined use of kaolin and CEO could improve the efficacy of this latter. Moreover, kaolin can be profitable in vineyards since it is effective in the control of other European grapevine pests without being harmful to non-target arthropods (Tacoli et al., 2017).

5. References

Bell, V. A., R. G. E. Bonfiglioli, J. T. S. Walker, P. L. Lo, J. F. Mackay, and S. E. McGregor. 2009. *Grapevine leafroll-associated virus 3* persistence in *Vitis vinifera* remnant roots. J. Plant Pathol. 91, 515–521.

Bertin, S., V. Cavalieri, C. Graziano, and D. Bosco. 2010. Survey of mealybug (Hemiptera: Pseudococcidae) vectors of Ampelovirus and Vitivirus in vineyards of northwestern Italy. Phytoparasitica 38, 401–409.

Beltrà, A., C. Navarro-Campos, A. Calabuig, L. Escopà, F. L. Wäckers, A. Pekas, and A. Soto. 2017. Association between ants (Hymenoptera: Formicidae) and the vine mealybug (Hemiptera: Pseudococcidae) in table-grape vineyards in Eastern Spain. Pest Manag. Sci. 73, 2473–2480.

Charles, J. G. 1981. Distribution and life history of the long-tailed mealybug, *Pseudococcus longispinus* (Homoptera: Pseudococcidae) in Auckland New Zealand vineyards. N. Z. J. Zool. 8, 285–294.

Charles, J. G. 1982. Economic damage and preliminary economic thresholds for mealybugs (*Pseudococcus longispinus* T-R.) in Auckland vineyards. N. Z. J. Agric. Res. 25, 415–420.

Charles, J. G. 1993. A survey of mealybugs and their natural enemies in horticultural crops in North Island, New Zealand, with implications for biological control. Biocontrol Sci. Technol. 3, 405–418.

Charles, J. G., D. Cohen, J. T. S. Walker, S. A. Forgie, V. A. Bell, and K. C. Breen. 2006. A review of the ecology of grapevine leafroll associated virus type 3 (GLRaV-3). N. Z. Plant Protect. 59, 330–337.

Charles, J. G. 1982. Economic damage and preliminary economic thresholds for mealybugs (*Pseudococcus longispinus* T-T.) in Auckland vineyards. N. Z. J. Agric. Res. 25, 415–420.

Cid, M., S. Pereira, C. Cabaleiro, and A. Segura. 2010. Citrus mealybug (Hemiptera: Pseudococcidae) movement and population dynamics in an arbor-trained vineyard. J. Econ. Entomol. 103, 619–630.

Cocco, A., A. Lentini, and G. Serra. 2014. Mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in vineyards using reservoir pheromone dispensers. J. Insect Sci. 14, 1–8.

Cocco, A., M. P. Marras, E. Muscas, A. Mura, and A. Lentini. 2015. Variation of life-history parameters of *Planococcus ficus* (Hemiptera: Pseudococcidae) in response to grapevine nitrogen fertilization. J. Appl. Entomol. 139, 519–528.

Daane, K. M., Bentley, W. J., Millar, J. G., Walton, V. M., Cooper, M. L., Biscay, P., and G. Y. Yokota. 2008a. Integrated management of mealybugs in California vineyards. Acta Hortic. 785, 235–252.

Daane, K. M., R. P. P. Almeida, V. A. Bell, J. T. S. Walker, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaviezo. 2012. Biology and management of mealybugs in vineyards, pp. 271–307. In N. J. Bostanian, C. Vincent, and R. Isaacs (eds.), Arthropod management in vineyards: Pests, approaches, and future directions. Springer, Dordrecht Heidelberg New York London.

Duso, C. 1989. Bioecological study on *Planococcus ficus* (Sign.) in Veneto. Boll. Lab. Entomol. Agrar. Filippo Silvestri 46, 3–20.

Duso, C., R. Trentin, M. Borgo, and E. Egger. 1985. Influenza della termoregolazione estiva mediante acqua sulle popolazioni di *Planococcus ficus* Sign. su vite. Rivista di Viticoltura e di Enologia 38, 567–607.

Finney, D. J. 1949. The estimation of the parameters of tolerance distributions. Biometrika 36, 139–256.

Finney, D. J. 1971. Probit analysis, 3rd Edition. Cambridge University Press, Cambridge.

Glenn, D. M., G. Puterka, T. Vanderzwet, R. E. Byers, and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92, 759–771.

Hall, D. G., and M. L. Richardson. 2012. Toxicity of insecticidal soap to the Asian citrus psyllid and two of its natural enemies. J. Appl. Entomol. 137, 347–354.

Hassan, S. A. 1985. Standard method to test the side-effect of pesticides on natural enemies of insects and mites developed by IOBC/WPRS Working Group "Pesticides and beneficial Organisms". Bull. OEPP/EPPO 15, 214–255.

Henderson, C. F., and E. W. Tilton. 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48, 157–161.

Hollingsworth, R. G. 2005. Limonene, a citrus extract, for control of mealybugs and scale insects. J. Econ. Entomol. 98, 772–779.

Hollingsworth, R. G., and R. M. Hamnett. 2010. Using food-safe ingredients to optimize the efficacy of oil-in-water emulsions of essential oils for control of waxy insects. Acta Hortic. 880, 399–405.

Isman, B. M. 2000. Plant essential oils for pest and disease management. Crop Prot. 19, 603–608.

Joubert, P. H., T. Grové, M. S. De Beer, and W. P. Steyn. 2004. Evaluation of kaolin (Surround® Wp) in an IPM program on mangoes in South Africa. Acta Hortic. 645, 493–499.

Karamaouna, F., A. Kimbaris, A. Michaelakis, D. Papachristos, M. Polissiou, P. Papatsakona, and E. Tsora. 2013. Insecticidal activity of plant essential oils against the vine mealybug, *Planococcus ficus*. J. Insect Sci. 13, 142.

Lee, S., C. J. Peterson, and J. R. Coats. 2003. Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored. Prod. Res. 39, 77–85.

Lentini, A., G. Serra, S. Ortu, and G. Delrio. 2008. Seasonal abundance on distribution of *Planococcus ficus* on grape vine in Sardinia. IOBC/WPRS Bull. 36, 267–272.

Lo, P. L., and J. T. S. Walker. 2011. Soil applications of two neonicotinoid insecticides to control mealybugs (Pseudococcidae) in in vineyards. N. Z. Plant. Protect. Res. 64, 101–106.

Lorenz, D. H., K. W. Eichhorn, H. Bleiholder, R. Klose, U. Meier and E. Weber. 1995. Growth stages of the grapevine: phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*). Codes and descriptions according to the extended BBCH scale. Aust. J. Grape. Wine. Res. 1, 100–103.

Mansour, R., K. Grissa-Lebdi, M. Khemakhem, I. Chaari, I. Trabelsi, A. Sabri, and S. Marti. 2017. Pheromone-mediated mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in Tunisian vineyards: Effect on insect population dynamics. Biologia 72, 333–341.

Mantel, N. 1966. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep. 50, 163–170.

Mansour, R., K. Grissa-Lebdi, P. Suma, G. Mazzeo, and A. Russo. 2017. Key scale insects (Hemiptera: Coccoidea) of high economic importance in a Mediterranean area: host plants, bio-ecological characteristics, natural enemies and pest management strategies – a review. Plant Protect. Sci. 53, 1–14.

Marras, P. M., A. Cocco, E. Muscas, and A. Lentini. 2016. Laboratory evaluation of the suitability of vine mealybug, *Planococcus ficus*, as a host for *Leptomastix dactylopii*. Biol. Control 95, 57–65.

Muscas, E., A. Cocco, L. Mercenaro, M. Cabras, A. Lentini, C. Porqueddu, and G. Nieddu. 2017. Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate. Agr. Ecosyst. Environ. 237, 203–212.

Pease, C. E., J. F. López-Olguín, I. Pérez-Moreno, and V. Marco-Mancebón. 2016. Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). J. Econ. Entomol. 109, 740–745.

Pietersen, G., N. Spreeth, T. Oosthuizen, A. van Rensburg, M. van Rensburg, D. Lottering, N. Rossouw, and D. Tooth. 2013. Control of grapevine leafroll disease spread at a commercial wine estate in South Africa: a case study. Am. J. Enol. Vitic. 64, 296–305.

Pozzebon, A., P. Zanolli, A. Cristante, P. Tirello, R. Silvestri, G. Zanettin, A. Da Ros, and C. Duso. Biological control of the mealybug *Planococcus ficus* in vineyards of North-eastern Italy. IOBC/WPRS Bull. (in press).

Prabhaker, N., C. Gispert, and S. J. Castle. 2012. Baseline susceptibility of *Planococcus ficus* (Hemiptera: Pseudococcidae) from California to select insecticides. J. Econ. Entomol. 105: 1392–1400.

Puterka, G. J., M. Reinke, D. Luvisi, M. A. Ciomperik, D. Bartels, L. Wendel, and D. M. Glenn. 2003. Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. Online. Plant Health Progress doi: 10.1094/PHP-2003-0321-01-RS.

Sackett, T. E., C. M. Buddle, and C. Vincent. 2005. Effect of kaolin on fitness and behaviour of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) larvae. J. Econ. Entomol. 92, 1648–1653.

Sforza, R., E. Boudon-Padieu, and C. Greif. 2003. New mealybug species vectoring grapevine leafroll associated viruses-1 and-3 (GLRaV-1 and-3). Eur. J. Plant Pathol. 109, 975–981.

Sharon, R., Zahavi, T., Sokolsky, T., Sofer-Arad, C., Tomer, M., Kedoshim, R., and A. R. Harari. 2016. Mating disruption method against the vine mealybug, *Planococcus ficus*: effect of sequential treatment on infested vines. Entomol. Exp. Appl. 161, 65–69.

Tacoli, F., F. Pavan, E. Cargnus, E. Tilatti, A. Pozzebon, and P. Zandigiacomo. 2017. Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in vineyards. J. Econ. Entomol. 110, 1164–1178.

Tacoli F., N. Mori, A. Pozzebon, E. Cargnus, S. Da Vià, P. Zandigiacomo, C. Duso, and F. Pavan. 2017b. Control of *Scaphoideus titanus* with natural products in organic vineyards. Insects, 8, 129. doi: 10.3390/insects8040129

Tranfaglia, A. 1976. Studi sugli Homoptera Coccoidea IV. Su alcune cocciniglie nuove o poco conosciute per l'Italia. Bollettino Laboratorio Entomologia Agraria "Filippo Silvestri" 33, 128–143.

Tremblay, E., A. Bélanger, M. Brosseau, and G. Boivin. 2009. Toxicity effects of an insecticidal soap on the green peach aphid [Homoptera: Aphididae]. Phytoprotection 90, 35–39.

Tsai, C., A. Rowhani, D. A. Golino, K. M. Daane, and R. P. P. Almeida. 2010. Mealybug transmission of grapevine leafroll viruses: an analysis of virus-vector specificity. Phytopathology 100, 830–834.

Wallingford, A. K., Fuchs, M. F., Martinson, T., Hesler, S., and G. M. Loeb. 2015. Slowing the spread of grapevine leafroll-associated viruses in commercial vineyards with insecticide control of the vector, *Pseudococcus maritimus* (Hemiptera: Pseudococcidae). J. Insect Sci. 15, 112.

Walton, V. M., and K. L. Pringle. 1999. Effects of pesticides used on table grapes on the mealybug parasitoid *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae). South Afr. J. Enol. Vitic. 20, 31–34.

Walton, V. M., and K. L. Pringle. 2004. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A review. S. Afr. J. Enol. Vitic. 25, 54–62.

Walton, V. M., Daane, K. M., Bentley, W.J., Millar, J. G., Larsen, T. E., and R. Malakar-Kuenen. 2006. Pheromone based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. J. Econ. Entomol. 99, 1280–1290.

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

Active ingredient	Commercial product		Application rate in water	Country (Year)	Target species in the laboratory	Target species in the field
	Name	Formulation				
Kaolin	Surround WP (Tessenderlo Kerley Inc., Phoenix, Arizona, USA)	WP, 95% kaolin	3% w/v	New Zealand (2015)	*Ps. calceolariae*	*Ps. calceolariae* and *Ps. longispinus*
				Italy (2016, 2017)	*Pl. ficus*	*Pl. ficus*
Citrus essential oil CEO	Orange oil emulsion (Hawkins Watts, Auckland, New Zealand)	SL, 10% orange oil Brazilian IN105118 (Lionel Hitchen Ltd, Winchester, UK)	10% v/v	New Zealand (2015)	*Ps. calceolariae*	*Ps. calceolariae* and *Ps. longispinus*
	Prev-Am Plus (Nufarm Italia, Milano, Italy)	SL, 5.88% orange oil	0.5% v/v	Italy (2016, 2017)	*Pl. ficus*	*Pl. ficus*
Terpenes	3logy (SIPCAM, Milano, Italy)	CS, 3.2% eugenol, 6.4% geraniol, 6.4% thymol	0.4% v/v	Italy (2016)		*Pl. ficus*
Insecticidal soap	Flipper (Dow Agrosciences Italia, Milano, Italy)	SL, 47.8% potassium fatty acids	2% v/v	Italy (2017)		*Pl. ficus*

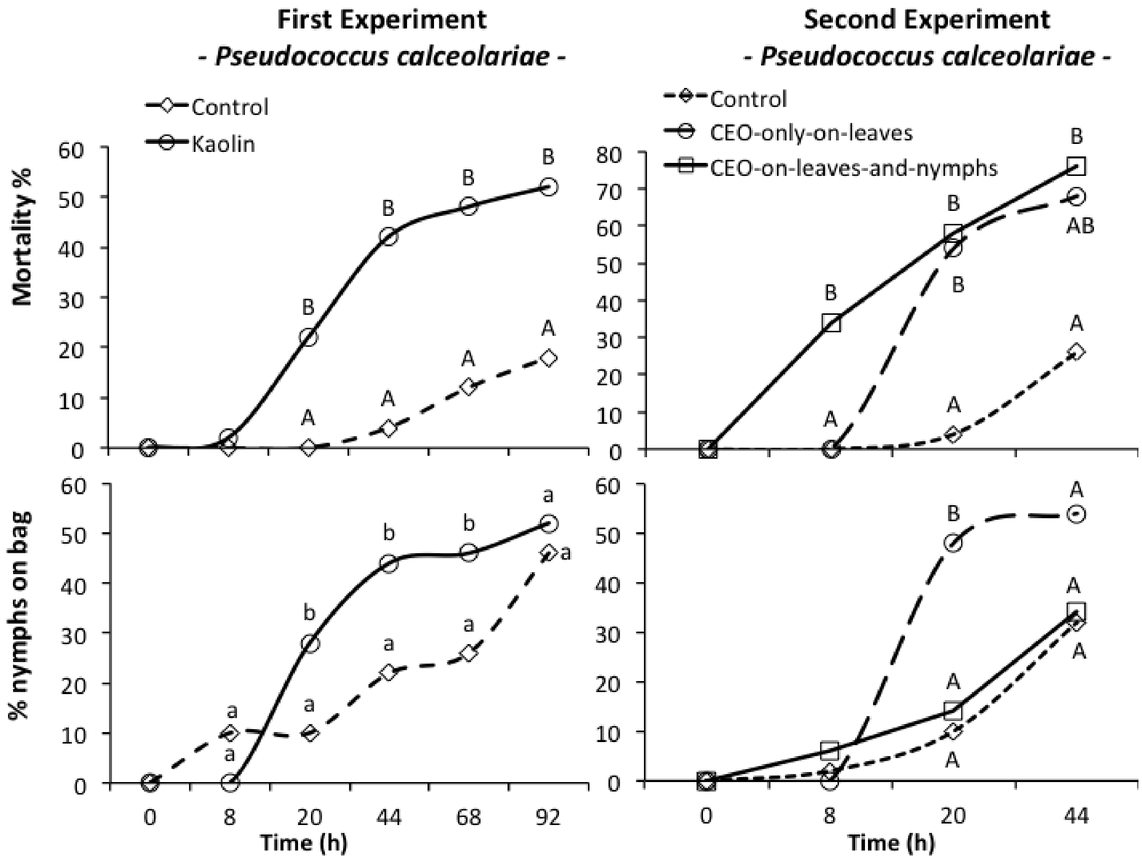

Fig. 1. *Pseudococcus calceolariae* nymph mortality and % of nymphs on bag vs. leaf surface, recorded over time in the treatments in comparison in NZ first and second laboratory experiments. Different small and capital letters among treatments on the same sampling indicate significant differences according to Ryan's test for α = 0.05 and α = 0.01, respectively.

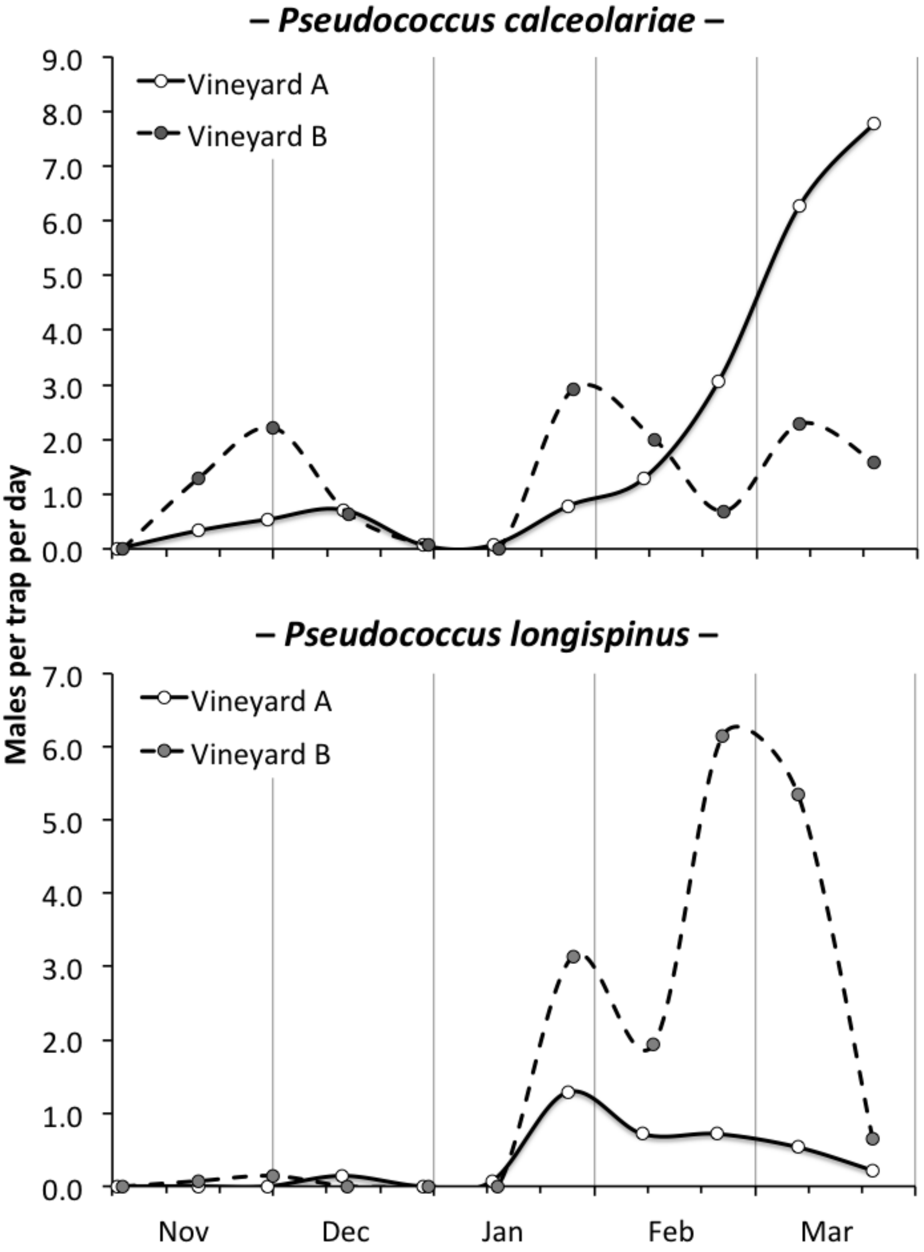

Fig. 2. Pheromone-trap captures of *Ps. calceolariae* and *Ps. longispinus* males recorded over the sampling period in vineyards A and B.

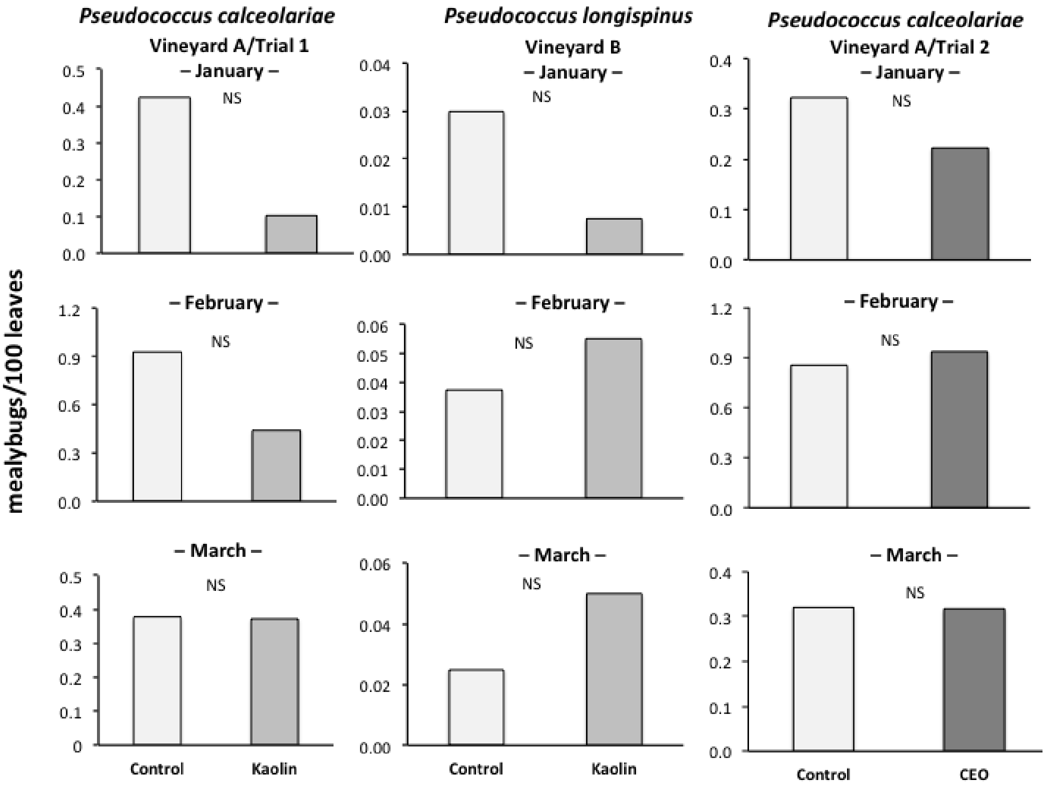

Fig. 3. Leaf infestation by mealybugs recorded over the sampling period in the treatments in comparison in the three trials conducted in the two NZ vineyards. NS = no significant differences according to Mixed ANOVA (α = 0.05).

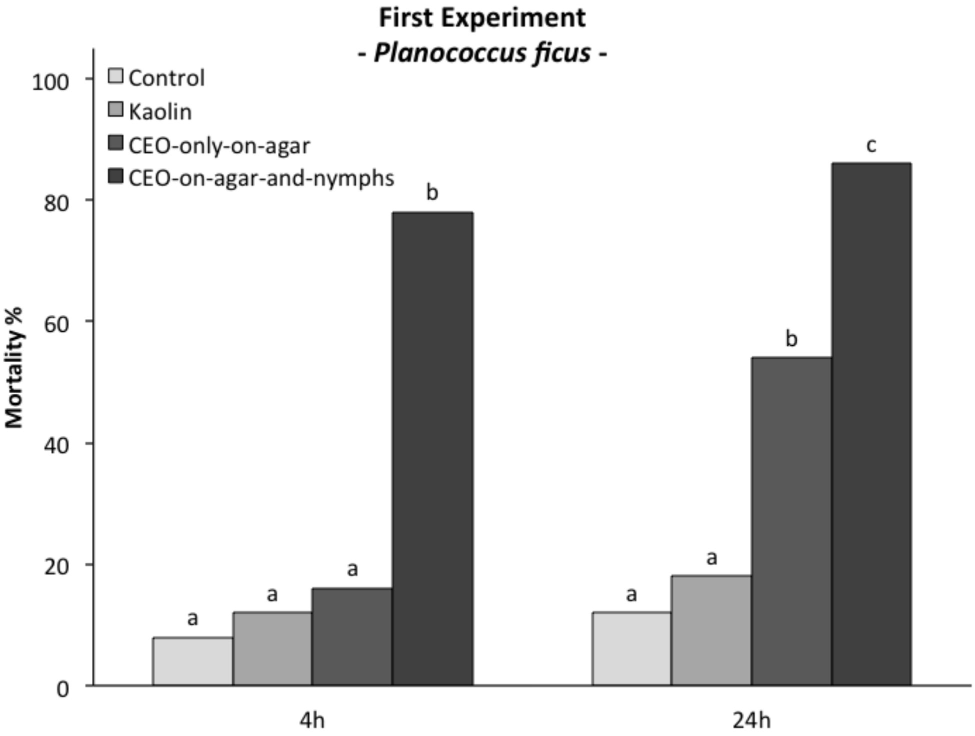

Fig. 4. *Planococcus ficus* nymphs mortality recorded in the four treatments in comparison at 4 h and 24 h from the beginning of the laboratory experiments. Different letters among treatments indicate significant differences according to Ryan's test for α = 0.01.

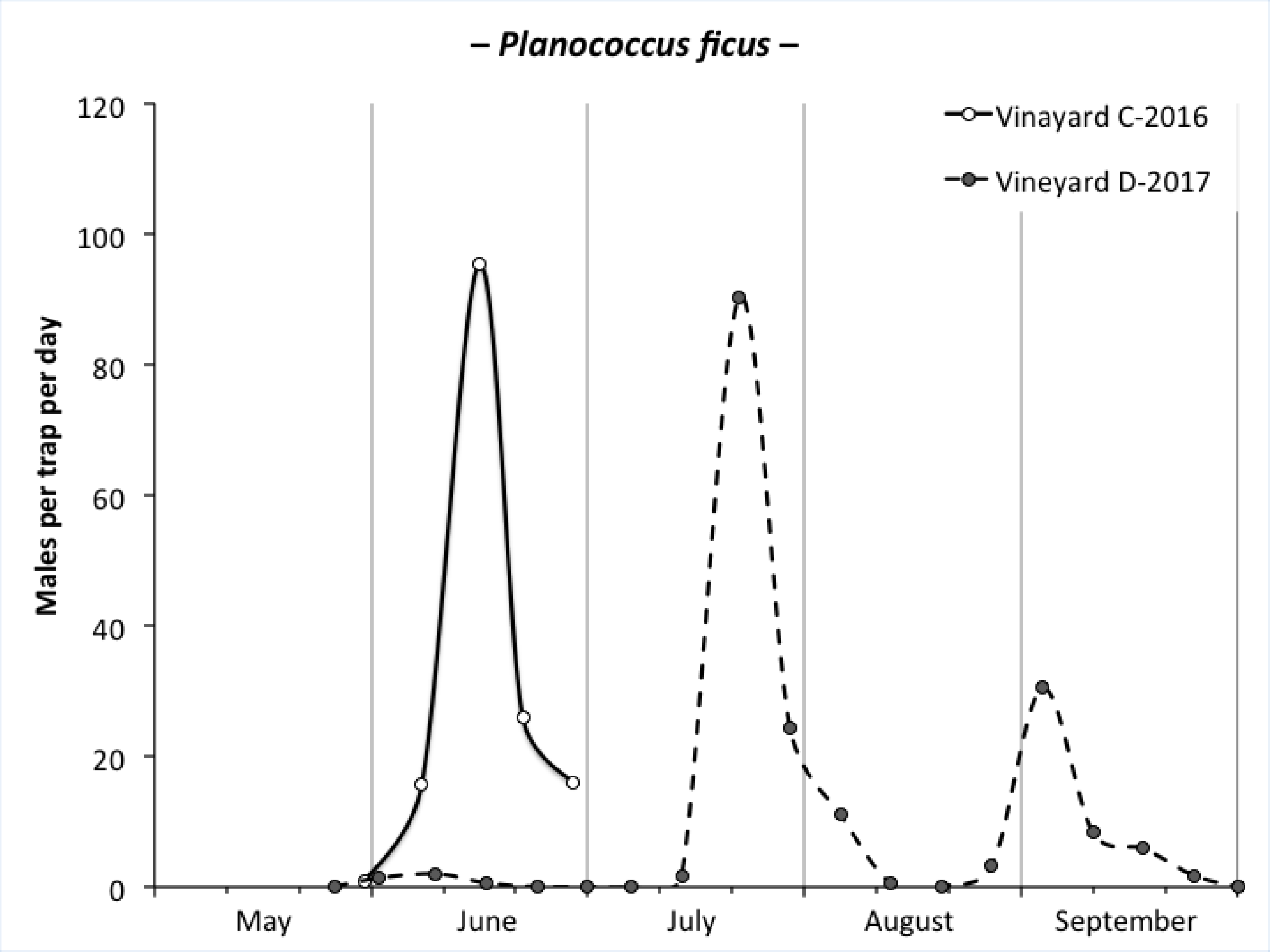

Fig. 5. Pheromone-trap captures of *Pl. ficus* males recorded over the sampling period in vineyards C and D.

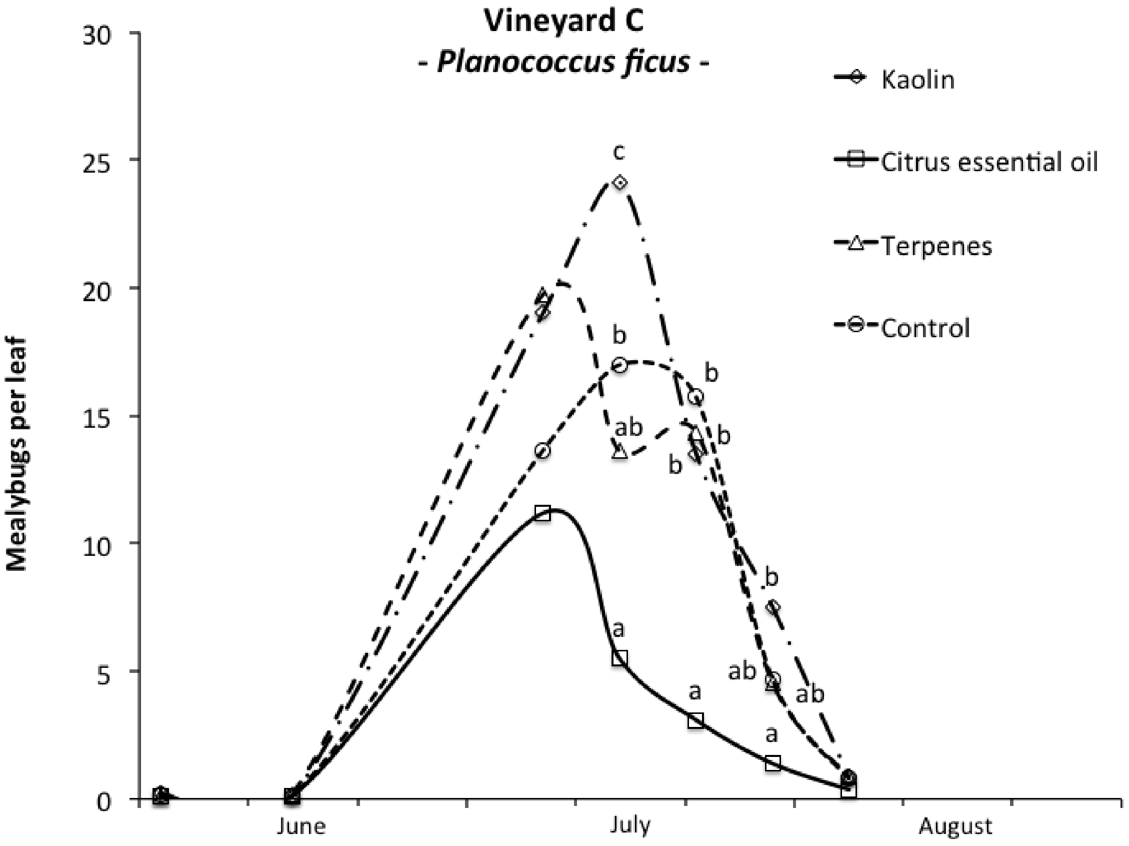

Fig. 6. Leaf infestation by *Pl. ficus* second generation recorded over the sampling period in the treatments in comparison in Vineyard C. Different letters among treatments on the same date indicate significant differences according to Tukey's post-hoc test (α = 0.05).

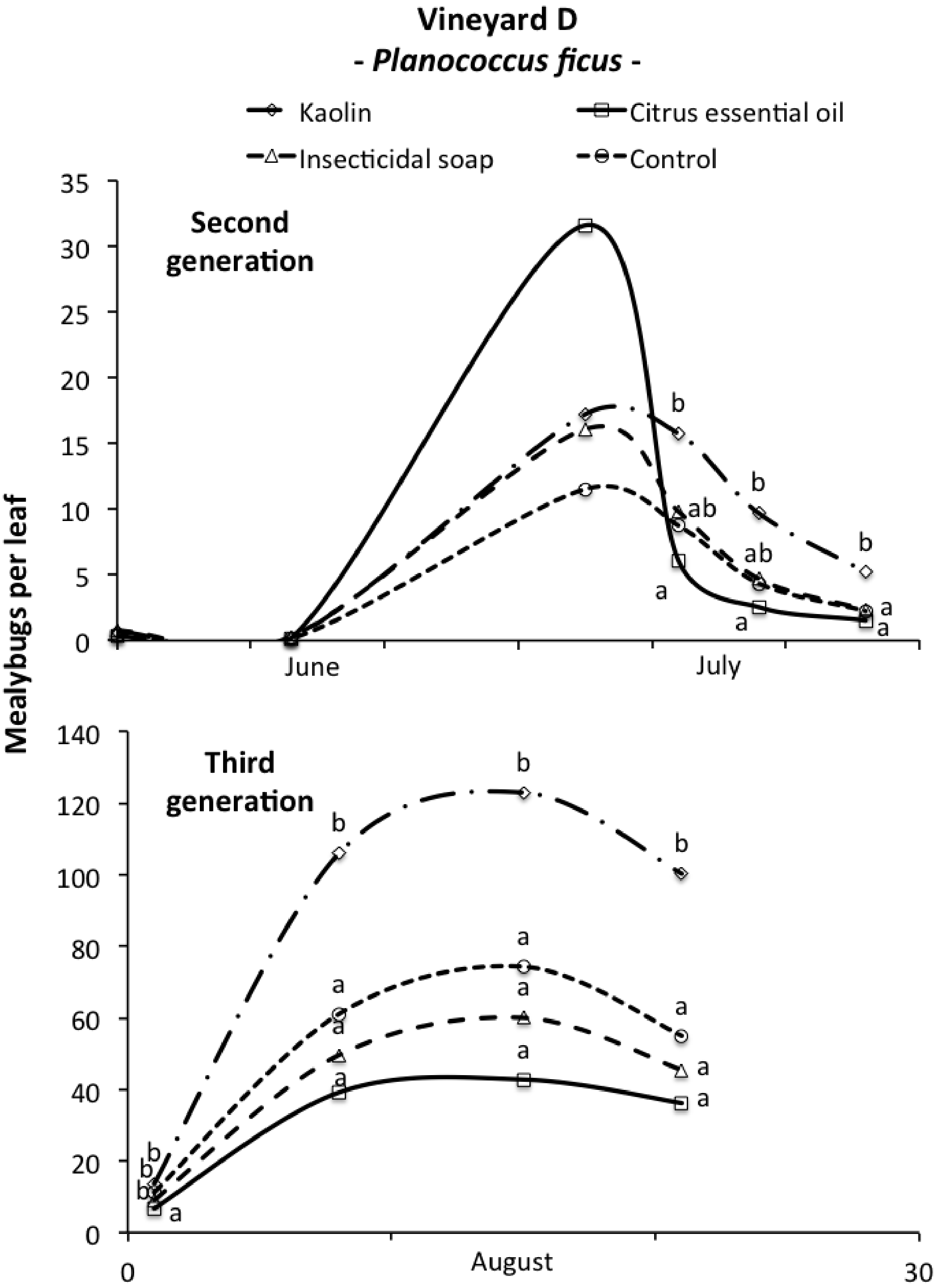

Fig. 7. Leaf infestation by *Pl. ficus* second and third generations recorded over the sampling period in the treatments in comparison in Vineyard D. Different letters among treatments on the same date indicate significant differences according to Tukey’s post-hoc test (α = 0.05).

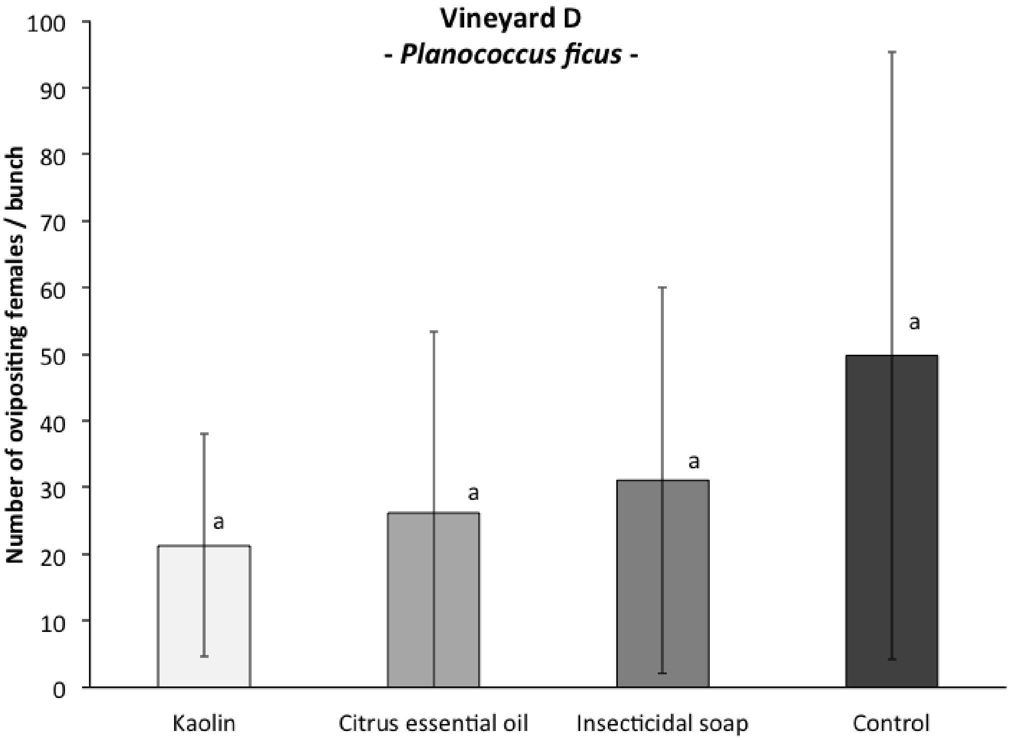

Fig. 8. Bunch infestation by *Pl. ficus* second generation recorded in the treatments in comparison in Vineyard D. Different letters among treatments indicate significant differences according to Tukey’s post-hoc test (α = 0.05).

Chapter 7 - Side effects of kaolin and bunch-zone leaf removal on predatory mite populations (Acari: Phytoseiidae) occurring in vineyards

Chapter 7 is formatted according to Journal of Economic Entomology author guidelines:
Federico Tacoli, Elena Cargnus, Alberto Pozzebon, Carlo Duso, Paola Tirello, Francesco Pavan. Side effects of kaolin and bunch-zone leaf removal on predatory mite populations (Acari: Phytoseiidae) occurring in vineyards.

Abstract

The effects of kaolin and bunch-zone leaf removal (LR) were assessed on populations of predatory mites *Kampimodromus aberrans* (Oudemans) and *Typhlodromus pyri* Scheuten in the context of four trials carried out against *Lobesia botrana* (Denis and Schiffermüller) (2015-2016) in vineyards located in north-eastern Italy. A laboratory experiment aimed at evaluating the effects of kaolin on the survival and fecundity of predatory mites, originated from the same grape-growing area, was conducted.

In field trials, kaolin was moderately harmful to populations of both predatory mite species, whereas the negative effect of LR was slight and significant only in one of the four trials. The decrease in population densities caused by kaolin was gradual and a population recovery was observed in the next spring.

In laboratory experiments, kaolin did not cause mortality to predatory mite females, whereas their fecundity was reduced by around 60%. A moderate use of kaolin and the adoption of LR did not irreversibly affect phytoseiid populations in vineyards and thus can be considered compatible with IPM strategies.

1. Introduction

Kaolin is at the basis of the particle film technology (Glenn et al. 1999), which consists in covering plants with a thin layer of this product. It acts as a physical barrier for arthropods hiding the host plant visually or chemically so deterring egg laying and feeding, with negative effects on reproduction, survival and development duration (Unruh et al. 2000, Vincent et al. 2003, Barker et al. 2006, Lapointe et al. 2006, Tacoli et al. 2017). This strategy was effective in reducing the infestation of several insect and mite pests (e.g., Knight et al. 2001, Glenn and Puterka 2005, Jaastad et al. 2005, Markó et al. 2008, Pascual et al. 2010, D'Aquino et al. 2011, Tyler-Julian et al. 2014, Marcotegui et al. 2015).

In vineyards, kaolin applications were effective in the control of hemipteran pests such as the sharpshooter *Homalodisca coagulata* (Say), vector of *Xylella fastidiosa* Wells, the causal agent of Pierce's disease (Wood and McBride 2001, Puterka et al. 2003, Barker et al. 2006, Tubajika et al. 2007), the leafhoppers *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari (Tacoli et al. 2017a) and *Scaphoideus titanus* Ball (Tacoli et al. 2017b), the grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (Sleezer et al. 2011) and the vine cicada *Psalmocharias alhageos* (Valizadeh et al. 2013). Regarding the tortricid moth *Lobesia botrana* (Denis and Schiffermüller), female oviposition rate, egg hatching, and larval survival were reduced by kaolin in the laboratory (Pease et al. 2016). In agreement with these results, kaolin significantly affected larval infestation in vineyards (F. T. et al. unpublished data).

Kaolin could be proposed as a viable alternative to synthetic insecticides in vineyards; however, the preservation of biological control agents is essential in the framework of Integrated Pest Management (IPM) and knowledge about kaolin side effects on beneficials of grapevine pests is limited. Kaolin did not negatively affect the parasitization of eggs of the grapevine leafhoppers *E. vitis* and *Z. rhamni* by *Anagrus* spp (Hymenoptera: Mymaridae) in vineyards, and *L. botrana* by *Thricogramma cacoeciae* Marchal (Hymenoptera: Thricogrammatidae) in the laboratory (Pease et al. 2016, Tacoli et al. 2017). Predatory mites belonging to the Phytoseiidae family (Acari) can control mite and insect pests of various crops (McMurtry and Croft 1997, McMurtry et al. 2013). In European vineyards, they are effective biocontrol agents of tetranychid and eriophyid mites (e.g., Pérez-Moreno and Moraza-Zorrilla 1998, Duso et al. 2012, Tixier et al. 2013).

The abundance of predatory mites in vineyards can be affected by fungicides and broad-spectrum insecticides. Several studies investigated the side effects of various pesticides on predatory mites occurring in vineyards (e.g., Duso and Pavan 1986, Duso 1994, Pozzebon et al. 2002, 2015, Baldessari et al. 2008, Göven and Güven 2008, Barić et al. 2010, Tirello et al. 2013, Auger et al. 2014). Moreover, regulatory organizations require experimental data on the selectivity of pesticides to predatory mites (and other beneficials) for the approval of new products (Candolfi et al. 1999). Negative side effects of kaolin on phytoseiid mites were observed in American and European orchards (Benedict 2005, Jaastad et al. 2006, Villanueva and Walgenbach 2010), although Bostanian and Racette (2008) did not observe such effects under laboratory conditions.

In the current study, the side effects of kaolin were evaluated on *Kampimodromus aberrans* (Oudemans) and *Typhlodromus pyri* Scheuten vineyard populations under field and laboratory conditions. These species are key biocontrol agents of phytophagous mites in vineyards and orchards and target species in the evaluation of pesticide side effects (Candolfi et al. 1999, Duso et al. 2009, Pozzebon et al. 2010, 2015, Tirello et al. 2013, Wearing 2014, Ahmad et al. 2015). Since kaolin has a positive synergy with bunch-zone leaf removal in the control of *L. botrana* (F. T. et al. unpublished data), the impact of this cultural practice on the considered predatory mite species was also evaluated. Bunch-zone leaf removal is a common cultural practice in vineyards, which consists in removing all the leaves that cover bunches, this leads to a reduction of both bunch rots and *L. botrana* infestations (Pavan et al. 2016). This practice acts by altering the grapevines canopy structure and microclimate (Kiaeian Moosavi et al. 2017), therefore could affect non-target arthropods, too.

2. Materials and methods

2.1. Field trials

The influence of kaolin and bunch-zone leaf removal on predatory mites was studied during 2015-2016 in the context of four field trials against *L. botrana* carried out in three vineyards of north-eastern Italy, so-called vineyard A (2015 and 2016), vineyard B (2015) and vineyard C (2016). Vineyard A (Cormons, Gorizia district, 45°57'51"N, 13°26'49"E, 56 m a.s.l., cultivar Pinot Gris) is a 10-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.5 m and 0.8 m, respectively. Vineyard B (Cormons, Gorizia district, 45°57'20"N, 13°26'50"E, 50 m a.s.l., cultivar Pinot Gris) is a 30-year-old organic vineyard with grapevines growing using the double arched Guyot training system with distances between and along rows of 2.8 m and 1 m, respectively. Vineyard C (Cormons, Gorizia district, 45°58'02"N, 13°31'31"E, 53 m a.s.l., cultivar Pinot Gris) is a 15-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of

2.2 m and 0.7 m, respectively. In all vineyards, standard fungicide programmes were followed and insecticides were not sprayed before and after kaolin applications.

In the four trials, kaolin (Surround WP, Tessenderlo Kerley Inc., Phoenix, Arizona, USA, 2% w/v, Surround WP/water) was sprayed at the rate of 1000 L/ha, an untreated control was included for a comparison. Kaolin was applied twice in 2015 (18 and 24 June) and three times in 2016 (10, 20, 27 June). All applications were done using a backpack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano, RE, Italy).

In all trials, a randomized block design with four replicates was adopted. Each block (row) was divided into two plots of 28 (vineyard A) or 20 (vineyard B) or 24 (vineyard C) vines. In all trials, plots were divided into two subplots of 14 or 10 or 12 vines, respectively, that were subjected or not to bunch-zone leaf removal (17 June 2015, 10 June 2016). In vineyard A, the plots submitted to kaolin applications and bunch-zone leaf removal were the same in both years.

Kaolin applications and bunch-zone leaf removal were timed on the basis of *L. botrana* second male flight Preliminary observations revealed that *K. aberrans* was the dominant species in Vineyards A and B, whereas *T. pyri* was dominant in Vineyard C.

To assess phytoseiid mite densities, five samplings were carried out in each year and vineyard (2015: 11, 22, 29 June, 06 July and 20 August; 2016: 7 and 21 June, 1 and 8 July, and 24 August). On each sampling date, 10 leaves were collected from the mid part of vine shoots in each subplot (40 leaves per subplot); they were closed in plastic bags and cool stored until being transferred to the laboratory. The leaves were checked under a dissecting microscope to assess mite numbers. At least 100 specimens per trial were slide mounted in Berlese medium and identified under 400× magnification, using current keys (Tixier et al. 2013; Cargnus and Zandigiacomo 2014).

In vineyard A, after two consecutive years (2015 and 2016) of Surround-WP applications on the same vines, overwintering phytoseiid populations were sampled (5 December 2016). Pruned two-year-old cane parts consisting in three nodes and internodes were collected from 10 grapevines per replicate (subplot without bunch-zone leaf removal) for a total of 40 cane parts per treatment. The number of predatory mites overwintering under the bark and inside the buds was counted under a dissecting microscope. At least 100 females were identified as above described (Tixier et al. 2013; Cargnus et al. 2012).

2.2. Laboratory experiments

Experiments were performed in the laboratory to evaluate the effects of kaolin on *K. aberrans* and *T. pyri* populations originating from two vineyards in which they were dominant. *K. aberrans* was collected in 2016 from vineyard A, whereas *T. pyri* was collected in 2017 from an organic vineyard located in the same area of field trials (Togliano, Udine district, 46°6'45"N, 13°24'40"E, 140 m a.s.l., cultivar Merlot). Predatory mites of each species were reared in laboratory for several generations according to Tirello et al. (2013). For both species, toxicological tests were performed using insecticide-free grapevine leaf discs (4.5 cm in diameter). Kaolin (Surround WP, 4% w/v, Surround WP/water) was applied to leaf discs with a Potter spray tower (Burkard Scientific Ltd, Uxbridge, UK) spraying 1.4 mL of suspension per leaf disc at 103 kPa (15 psi) to obtain an amount of fluid of 1.9-2.0 mg cm^{-2}, as recommended by the IOBC guidelines (Hassan, 1985). The untreated leaf discs (control) were sprayed with water following the same procedure. Leaf discs were subsequently placed on wet cotton pads and wet cotton barriers were created along their perimeter to avoid predatory mites escape. Two mated females (about 12-d old) were placed on each leaf disc. Fresh pollen was provided every 2 days as food. The experiments were conducted under controlled conditions (25 °C, 70% RH and 16L:8D photoperiod). Toxicological tests were run following Tirello et al. (2013). Female mortality was checked at 72 h from the sprayings and fecundity was assessed daily for four additional days. After 7 d, the remaining females and juvenile stages were removed and eggs were monitored until they had completely hatched in the control. A total of 50 and 38 females per treatment were assessed for *K. aberrans* and *T. pyri*, respectively.

2.3. Statistical analyses

To compare field data, One-way and Mixed ANOVA with Bonferroni adjustment, and Tukey's post-hoc test were performed after logarithmic transformation. Mortality was estimated according to Henderson and Tilton (1955).

Student's unpaired *t*-test was used to compare laboratory data. The overall effect of kaolin was expressed as $E = 100\% - (100\% - M) \times R$, where E is the coefficient for toxicity; M is the mortality percentage of females calculated following Abbott (1925); R is the ratio between the average number of hatched eggs produced by females in kaolin treatment and the average number of hatched eggs produced by females in the control treatment. According to the toxicity categories proposed by International Organization for Biological Control (IOBC) for laboratory tests the following classes were considered: Class 1, harmless ($E < 30\%$ reduction); Class 2, slightly harmful ($E = 30–79\%$ reduction); Class 3, moderately harmful ($E = 80–99\%$ reduction); and Class 4, harmful ($E > 99\%$ reduction) (Hassan et al. 1994, Boller et al. 2005). Statistical analyses were performed with Microsoft Excel 2013 for Windows (Microsoft Corporation 2013) and IBM SPSS Statistics 20 (IBM Corporation 2011).

3. Results

3.1. Field trials

3.1.1. Vineyard A

In this vineyard, *K. aberrans* was the unique species recorded and its density in the control was similar in 2015 and 2016 (Fig. 1). Phytophagous mite densities were negligible.

In both years, *K. aberrans* densities were not significantly different in the two treatments in the sampling made before the first kaolin application. As a consequence of kaolin applications, phytoseiid densities were significantly lower in the kaolin than control (2015: $F = 9.013$; df = 1,12; $P = 0.011$; 2016: $F = 80.802$; df = 1,12; $P = 0.0001$) (Fig. 1). The negative effect of kaolin at about 10 days from the last application was higher in 2016 than in 2015 (Henderson-Tilton: 69% and 49%, respectively). *K. aberrans* densities in the kaolin were significantly lower than in the control after the second kaolin application (i.e., 18 and 11 days after first application, respectively in 2015 and 2016). After two consecutive years of kaolin applications (2015 and 2016) in the same plots, the overwintering populations were not significantly different between the kaolin and the control ($F = 0.735$; df = 1; $P = 0.424$) (Fig. 2).

In both years, bunch-zone leaf removal did not influence phytoseiid densities (2015: $F = 1.029$; df = 1,12; $P = 0.33$; 2016: $F = 4.573$; df = 1,12; $P = 0.054$) (Fig. 3). The interaction between treatment and bunch-zone leaf removal effects was not significant (2015: $F = 1.120$; df = 1,12; $P = 0.311$; 2016: $F = 0.35$; df = 1,12; $P = 0.855$).

3.1.2. Vineyard B

Phytoseiid identification showed the dominance of *K. aberrans* (89%) over *Amblyseius andersoni* (Chant) (11%). Phytophagous mite densities were negligible.

In the sampling before the first kaolin application, phytoseiid densities were not significantly different between the kaolin and the control. After the two kaolin applications, phytoseiid densities were significantly lower in the kaolin than control ($F = 16.575$; df = 1,12; $P = 0.002$) (Fig. 1). The negative effect of kaolin at about 10 days from the last application was remarkable (Henderson-Tilton: 91%).

Bunch-zone leaf removal significantly reduced phytoseiid densities ($F = 11.926$; df = 1,12; $P = 0.005$) (Fig. 3). In particular, the negative effect of bunch-zone leaf removal was significant in the samplings of July and August. The interaction between treatment and bunch-zone leaf removal effects was not significant ($F = 0.499$; df = 1,12; $P = 0.493$).

3.1.3. Vineyard C

Phytoseiid specimens showed the dominance of *T. pyri* (97%) over *A. andersoni* (3%). Phytophagous mite densities were negligible.

In the sampling before the first kaolin application, *T. pyri* densities were not significantly different between the kaolin and the control. After kaolin applications, phytoseiid densities were significantly lower in the kaolin than control (F = 8.218; df = 1,12; P = 0.014) (Fig. 1). The negative effect of kaolin at about 10 days from the last kaolin application was remarkable (Henderson-Tilton: 88%).

Bunch-zone leaf removal did not significantly reduce phytoseiid densities (F = 1.577; df= 1,12; P = 0.233) (Fig. 3) and the interaction between treatment and bunch-zone leaf removal effects was not significant (F = 0.044; df = 1,12; P = 0.837).

3.2. Laboratory experiments

In the control, the fecundity rates (mean±SD) of both *K. aberrans* and *T. pyri* **(**0.78±0.23 and 0.88±0.14 eggs/female/day, respectively) were comparable with those reported by Lorenzon et al. (2012).

K. aberrans female survival was 100% in both treatments. Kaolin significantly reduced female fecundity (t = 9.27; df = 48; P < 0.0001) (Fig. 4). In terms of toxicity (IOBC classes), E value was 62.8% resulting in kaolin being moderately harmful.

T. pyri female survival was 100% in both treatments. Kaolin significantly reduced female fecundity (t = 11.21; df = 36; P < 0.0001) (Fig. 4). In terms of toxicity (IOBC classes), E value was 62.5% resulting in kaolin being moderately harmful.

4. Discussion

In field trials, kaolin affected negatively *K. aberrans* and *T. pyri* populations. For both species, significant differences occurred at least 10 days from the first kaolin application. The differences between treatments increased over time reaching their maximum after at least 20 days from the first kaolin application. These data suggest that kaolin did not exhibit knock down effects on predatory mites.

Nevertheless, the negative effect of kaolin on *K. aberrans* in vineyard A did not persist over the two seasons. The decrease in *K. aberrans* densities observed in summer 2015 after two kaolin applications was not anymore observed in the pre-trial sampling 2016. Moreover, the lower population level of *K. aberrans* observed in kaolin plots in the summer of 2016 was not confirmed in December 2016. Despite two consecutive years of kaolin applications (five applications in total), *K. aberrans* population densities in treated and the control plots were similar. We can suppose that, in late summer, phytoseiid populations redistributed through the vineyard or increased more in the kaolin than in the control plots. The first hypothesis is supported by the dispersal ability of immatures and pre-ovipositional adult females among plants, mostly through wind (Sabelis 1985, Tixier et al. 1998, Duso et al. 2010, Otto et al. 2013). The second hypothesis is supported by both the possibility of lower intraspecific competition and intraguild predation occurred in kaolin-treated plots (Duso 1989, Bengochea et al. 2013, Ahmad et al. 2015, Pozzebon et al. 2015).

The negative effects of kaolin on phytoseiid mites observed in the present study are in agreement with some contributions. Trials carried out in two subsequent years in plum and apple orchards in Norway, showed that kaolin sprayed against phytophagous mites and tortricid moths negatively affected phytoseiid mites in three out of four cases (Jaastad et al. 2006), even whether part of the effect could be associated with lower prey populations. In an apple orchard in New England (US), Benedict (2005) found significantly both higher levels of tetranychid mites and lower levels of phytoseiid population on trees treated with kaolin than on those not sprayed.

Laboratory data showed for both *K. aberrans* and *T. pyri* that kaolin reduced female fecundity. The negative effect of kaolin on egg laying is in agreement with the slow and progressive decline in phytoseiid mite densities observed in the field for both species.

Since the decrease in fecundity caused by kaolin was not associated with a decrease of female survival, the negative effect on egg laying can be attributed to the fact that kaolin-covered leaves could be an uncomfortable oviposition substrate.

In contrast with our data on *K. aberrans* and *T. pyri*, no negative effects of kaolin on the number of eggs laid by *Neoseiulus fallacis* (Garman) females has been reported (Bostanian and Racette 2008). Differently from our results, a greater mortality for *T. pyri* and *N. fallaci*s on kaolin treated leaves (25% and 12%, respectively) has been reported as a consequence of a lower *T. urticae* consumption (Benedict 2005). However, an absence of kaolin effects on survival has been showed in the study of Bostanian and Racette (2008) where *N. fallacis* females fed on *T. urticae*, too.

In vineyard A, a greater detrimental effect of kaolin on *K. aberrans* was observed in 2016 than in 2015. The reason for this difference can be attributed to the additional kaolin application done in 2016.

In vineyard B, almost tenfold lower *K. aberrans* population was observed than in vineyard A. However, in vineyard B with respect of vineyard A, a higher density of generalist predators, both spiders and insects, was recorded (E. C. et al. unpublished data).

In vineyard C, *T. pyri* population densities were lower than *K. aberrans* ones recorded in both vineyard A and B. This is in agreement with studies that report a lower density of *T. pyri* than *K. aberrans* in similar vineyard conditions (Duso 1989, 1992). In vineyard C, the populations of *T. pyri* were particularly low in summer due to the high susceptibility of this phytoseiid species to high temperatures in combination with low relative humidity and to lower availability of pollen in summer than in spring (Duso and Pasqualetto 1993, Pozzebon et al. 2005). Moreover, the absence of leaf pubescence in Pinot Gris, the cultivar of vineyard C is a further factor that can explain the very low population of *T. pyri* in this vineyard (Duso and Vettorazzo 1999, Roda et al. 2003).

A negative effect of bunch-zone leaf removal on phytoseiid populations was also observed. A less dense canopy due to bunch-zone leaf removal could determine a microclimate characterized by higher temperature and lower relative humidity known to be less favourable to phytoseiid mites (Duso and Pasqualetto 1993).

In viticulture, the use of kaolin in two or three applications per year is moderately harmful to phytoseiid populations. Moreover, in a vineyard with stable *K. aberrans* populations, these were not reduced after two consecutive years of kaolin applications. A higher selectivity could be reached when kaolin spraying is localized on the bunch zone for the control of *L. botrana* and to reduce berry sunburn. Based on these outcomes, a moderate use of kaolin and the adoption of leaf removal did not irreversibly affect phytoseiid populations in vineyards and thus can be considered compatible with IPM strategies.

5. References

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Ahmad, H., A. Pozzebon, and C. Duso. 2015. Predation on heterospecific larvae by adult females of *Kampimodromus aberrans*, *Amblyseius andersoni*, *Typhlodromus pyri* and *Phytoseius finitimus* (Acari: Phytoseiidae). Exp. Appl. Acarol. 67: 1–20.

Baldessari, M., R. Maines, and G. Angeli. 2008. Comparison of two methods for the agrochemicals side effect evaluation on Phytoseiid mites in vineyards. IOBC/WPRS Bull. 36: 237–243.

Barker, J. E., A. Fulton, K. A. Evans, and P. Glen. 2006. The effects of kaolin particle film on *Plutella xylostella* behaviour and development. P. Manag. Sci. 62: 498–504.

Barić, B., I. Pajač, and D. Bertić. 2010. Harmful and beneficial entomofauna in Croatian orchards and vineyards. IOBC/WPRS Bull. 55: 113–115.

Benedict, C. 2005. Non-target impact of kaolin on phytophagous (Acari: Tetranychidae) and predatory mites (Acari: Phytoseiidae) in an apple agro-ecosystem (MS thesis). University of Vermont, Burlington, VT.

Benghochea, P., F. Amor, R. Saelices, S. Hernando, F. Budia, A. Adán, and P. Medina. 2013. Kaolin and copper-based products applications: Ecotoxicology on four natural enemies. Chemosphere 91: 1189–1195.

Boller, E. F., H. Vogt, P. Ternes, and C. Malavolta. 2005. Working document on selectivity of pesticides. https://www.iobc-wprs.org/ip_ipm/03021_IOBC_WorkingDocumentPesticides_Explanations.pdf

Bostanian, N. J., and G. Racette. 2008. Particle films for managing arthropod pests of apple. J. Econ. Entomol. 101: 145–150.

Candolfi, M. P., F. Bakker, V. Cañez, M. Miles, C. Neumann, E. Pilling, M. Primiani, K. Romijn, R. Schmuck, S. Storck-Weyhermuller, A. Ufer, and A. Waltersdorfer. 1999. Sensitivity of non-target arthropods to plant protection products: could *Typhlodromus pyri* and *Aphidius* spp. be used as indicator species? Chemosphere 39: 1357–1370.

Cargnus, E., and P. Zandigiacomo. 2014. Taxonomic value of morphological and morphometrical characters in the immature stages of four species of *Kampimodromus* Nesbitt (Acari: Phytoseiidae) from Italy and Croatia. Zootaxa 3857: 207–229.

Cargnus, E., V. Girolami, and P. Zandigiacomo. 2012. Re-examination of specimens of three species of *Kampimodromus* Nesbitt (Acari: Phytoseiidae) from north-eastern Italy, with first report on *Kampimodromus corylosus* Kolodochka in Italy. Int. J. Acarol. 38: 583–594.

D'Aquino, S., A. Cocco, S. Ortu, and M. Schirra. 2011. Effects of kaolin-based particle film to control *Ceratitis capitata* (Diptera: Tephritidae) infestations and postharvest decay in citrus and stone fruit. Crop Prot. 30: 1079–1086.

Duso, C. 1989. Role of the predatory mites *Amblyseius aberrans* (Oud.), *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari, Phytoseiidae) in vineyards. I. The effects of single or mixed phytoseiid population releases on spider mite densities (Acari, Tetranychidae). J. Appl. Entomol. 107: 474–492.

Duso, C. 1992. Role of predatory mites *Amblyseius aberrans* (Oudemans), *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant) (Acari: Phytoseiidae) in vineyards. III. Influence of variety characteristics on the success of *A. aberrans* and *T. pyri*. J. Appl. Entomol. 114: 455–462.

Duso, C. 1994. Comparison between field and laboratory testing methods to evaluate the pesticide side-effects on the predatory mites *Amblyseius andersoni* and *Typhlodromus pyri*. IOBC/WPRS Bull. 17 (10): 7–19.

Duso, C., and F. Pavan. 1986. Il controllo delle tignole della vite (*Lobesia botrana* Den. e Schiff.; *Eupoecilia ambiguella* Hb.). 2. Considerazioni sugli effetti collaterali di insetticidi diversi. Rivista di Viticoltura e di Enologia 39: 304–312.

Duso, C., and C. Pasqualetto. 1993. Factors affecting the potential of phytoseiid mites (Acari: Phyttoseiidae) as biocontrol afents inNorth-Italian nineyards. Exp. Appl. Acarol. 17: 241–258.

Duso C., and E. Vettorazzo. 1999. Mite population dynamics on different varietes with or without phytoseiids released (Acari: Phytoseiidae). Exp. Appl. Acarol. 23: 741–763.

Duso, C., M. Fanti, A. Pozzebon, and G. Angeli. 2009. Is the paredatory mote *Kampimodromus aberrans* a candidate for the control of phytophagous mites in European apple orchards? BioControl 54: 369–382.

Duso, C., S. Kreiter, M.-S. Tixier, A. Pozzebon, and V. Malagnini. 2010. Biological control of mites in European vineyards and the impact of natural vegetation. In: Sabelis, M. W., and J. Bruin, (Eds.), Trends in Acarology, Proceedings of the 12th International Congress, Springer 399–407.

Duso, C., A. Pozzebon, S. Kreiter, M.-S. Tixier, and M. P. Candolfi. 2012. Management of phytophagous mites in European vineyards. In: Bostanian, N. J., C. Vincent, and R. Isaacs, (Eds.), Arthropod management in vineyards: pests, approaches, and future directions, Springer, New York 191–217.

Glenn, D. M., and G. J. Puterka. 2005. Particle films: a new technology for agriculture. Hortic. Rev. 31: 1–44.

Glenn, D. M., G. Puterka, T. Vanderzwet, R. E. Byers, and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92: 759–771.

Göven, M. A., and B. Güven. 2008. Side effects of pesticides used in vineyards in the Aegean region on the predatory mite *Typhlodromus perbibus* Wainstein&Arutunjan (Acari: Phytoseiidae) under laboratory conditions. IOBC/WPRS Bull. 35: 92–95.

Hassan, S. A. 1985. Standard method to test the side-effect of pesticides on natural enemies of insects and mites developed by IOBC/WPRS Working Group "Pesticides and beneficial Organisms". Bull. OEPP/EPPO 15: 214–255.

Hassan, S. A., F. Bigler, H. Bogenshutz, J. Brun, and J. N. M. Calis. 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group "Pesticides and Beneficial Organisms". Entomophaga 39: 107–119.

Henderson, C. F., and E. W. Tilton. 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48: 157–161.

Jaastad, G., D. Røen, B. Hovland, and O. Opedal. 2006. Kaolin as a possible treatment against lepidopteran larvae and mites in organic fruit production. In: Boos, M. (Ed.) Ecofruit - 12th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing: Proceedings to the Conference from 31st January to 2nd February 2006 at Weinsberg/Germany, Fördergemeinschaft Ökologischer Obstbau e.V. (FÖKO), Traubenplatz 5, D-74189 Weinsberg, pp. 31–35.

Kiaeian Moosavi, F., E. Cargnus, F. Pavan, and P. Zandigiacomo. 2017. Effects of grapevine bunch exposure to sunlight on berry surface temperature and Lobesia botrana (Lepidoptera: Tortricidae) egg laying, hatching and larval settlement. Agr. Forest. Entomol. doi: 10.1111/AFE.12274.

Knight, A. L., B. A. Christianson, T. R. Unruh, J. G. Puterka, and D. M. Glenn. 2001. Impact of seasonal kaolin particle films on apple pest management. Can. Entomol. 133: 413–428.

Lapointe, S. L., C. L. McKenzie, and D. G. Hall. 2006. Reduced oviposition by *Diaprepes abbreviate*s (Coleoptera: Curculionidae) and growth enhancement of citrus by Surround particle film. J. Econ. Entomol. 99: 109–116.

Lorenzon, M., A. Pozzebon, and C. Duso. 2012. Effects of potential food sources on biological and demographic parameters of the predatory mites *Kampimodromus aberrans*, *Typhlodromus pyri* and *Amblyseius andersoni*. Exp. Appl. Acarol. 58: 259–278.

Marcotegui, A., I. Sánchez-Ramos, S. Pascual, C. E. Fernández, G. Cobos, I. Armendáriz, A. Cobo, and M. González-Núñez. 2015. Kaolin and potassium soap with thyme essential oil to control *Monosteira unicostata* and other phytophagous arthropods of almond trees in organic orchards. J. Pest Sci. 88: 753–765.

Markó, V., L. H. M. Blommers, S. Bogya, and H. Helsen. 2008. Kaolin particle films suppress many apple pests, disrupt natural enemies and promote woolly apple aphid. J. Appl. Entomol. 132: 26–35.

McMurtry, J. A., G. J. De Moraes, and N. F. Sourasso. 2013. Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Sys. Appl. Acarol. 18: 297–320.

McMurtry, J. A., and B. A. Croft. 1997. Life-styles of phytoseiid mites and their roles in biological control. Annu. Rev. Entomol. 42: 291–321.

Otto, S., N. Mori, D. Fornasiero, A. Veres, P. Tirello, A. Pozzebon, C. Duso, and G. Zanin. 2013. Insecticide drift and its effect on *Kampimodromus aberrans* (Oudemans) in an Italian vineyard-hedgerow system. Biosyst. Eng. 116: 447–456.

Pascual, S., G. Cobos, E. Seris, and M. González-Núñez. 2010. Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove. J. Pest. Sci. 83: 121–133.

Pavan, F., E. Cargnus, F. Kiaeian Moosavi, G. Bigot, F. Tacoli, and P. Zandigiacomo. 2016. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould. Bull. Insectol. 69: 107–115.

Pease, C. E., J. F. López-Olguín, I. Pérez-Moreno, and V. Marco-Mancebón. 2016. Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). J. Econ. Entomol. 109: 740–745.

Pérez-Moreno, I., and M. L. Moraza-Zorrila. 1998. Population dynamics and hibernation shelters of *Caleptrimerus vitis* in vineyards of Rioja, Spain, with a description of a new eriophyid extraction technique (Acari: Eriophyidae). Exp. Appl. Acarol. 22: 215–226.

Pozzebon, A., C. Duso, and E. Pavanetto. 2002. Side effects of some fungicides on phytoseiid mites (Acari: Phytoseiidae) in north-Italian vineyards. Anz. Schadlingskd. J. Pest Sci. 75: 132–136.

Pozzebon, A., C. Duso, and V. Malagnini. 2005. Predatory mite population dynamics in vineyards: the role of alternative foods. AFPP – 2e Colloque Interanational sur les acariens des cultures, Montpellier, 24-25 Octobre 2005. CD Rom, pp. 1–7.

Pozzebon, A., M. Borgo, and C. Duso. 2010. The effects of fungicides on non-target mites can be mediated by plant pathogens. Chemosphere 79: 8–17.

Pozzebon, A., S. Ahmad., P. Tirello, M. Lorenzon, C. Duso. 2014. Does pollen availability mitigate the impact of pesticides on generalist predatory mites? BioControl 59: 585–596.

Pozzebon, A., P. Tirello, R, Moret, M. Pederiva, and C. Duso. 2015. A fundamental step in IPM on grapevine: evaluating the side effects of pesticides on predatory mites. Insects 6: 847–857.

Puterka, G. J., M. Reinke, D. Luvisi, M. A. Ciomperik, D. Bartels, L. Wendel, and D. M. Glenn. 2003. Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. Online. Plant Health Progress doi: 10.1094/PHP-2003-0321-01-RS.

Roda, A., J. Nyrop, and G. English-Loeb. 2003. Leaf pubescence mediates the abundance of non-prey food and the density of the predatory mite *Typhlodromus pyri*. Exp. Appl. Acarol. 29: 193–211.

Sleezer, S., D. T. Johnson, B. Lewis, F. Goggin, C. Rothrock, and M. Savin. 2011. Foliar grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), seasonal biology, predictive model and management in the Ozarks region of the United States. Acta Hortic. 904: 151–156.

Tacoli, F., F. Pavan, E. Cargnus, E. Tilatti, A. Pozzebon, and P. Zandigiacomo. 2017a. Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in Vineyards. J. Econ. Entomol. 110: 1164–1178.

Tacoli, F., N. Mori, A. Pozzebon, E. Cargnus, S. Da Vià, P. Zandigiacomo, C. Duso, and F. Pavan. 2017b. Control of *Scaphoideus titanus* with natural products in organic vineyards. Insects, 8, 129. doi: 10.3390/insects8040129

Tirello, P., A. Pozzebon, and C. Duso. 2013. The effect of insecticides on the non-target predatory mite *Kampimodromus aberrans*: Laboratory studies. Chemosphere 93: 1139–1144.

Tixier, M.-S., S. Kreiter, P. Auger, and M. Weber. 1998. Colonization of Languedoc vineyards by phytoseiid mites (Acari: Phytoseiidae): influence of wind and crop environment. Experimental and Applied Acarology 22: 523–542.

Tixier, M.-S., A. Baldassar, C. Duso, and S. Kreiter. 2013. Phytoseiidae in European grape (*Vitis vinifera* L.): bio-ecological aspects and keys to species (Acari: Mesostigmata). Zootaxa 3721: 101–142.

Tixier, M.-S., A. Arnaud, M. Douin, and S. Kreiter. 2015. Effects of agroforestry on Phytoseiidae communities (Acari: Mesostigmata) in vineyards. A synthesis of a 10-year period of observations. Acarologia. 55: 361–375.

Tubajika, K. M., E. L. Civerolo, G. J. Puterka, J. M. Hashim, and D. A. Luvisi. 2007. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. Crop Prot. 26: 92–99.

Tyler-Julian, K., J. Funderburk, G. Frantz, and C. Mellinger. 2014. Evaluation of a push-pull strategy for the management of *Frankliniella bispinosa* (Thysanoptera: Thripidae) in bell peppers. Environ. Entomol. 43: 1364–1378.

Unruh, T. R., A. L. Knight, J. Upton, D. M. Glenn, and G. J. Puterka. 2000. Particle films for suppression of the codling moth (Lepidoptera: Tortricidae) in apple and pear orchards. J. Econ. Entomol. 93: 737–743.

Valizadeh, H., Abbasipour, H., Farazmand, and H., A. Askarianzadeh. 2013. Evaluation of kaolin application on oviposition control of the vine cicada, *Psalmocharias alhageos* in vineyards (Homoptera: Cicadidae). Entomologia Generalis 34: 279–286.

Villanueva, R.T. and J. F. Walgenbach. 2010. Impact of new pesticide chemistry on acarine communities in apple orchards. In: Sabelis, M. W., and J. Bruin, (Eds.), Trends in Acarology, Proceedings of the 12th International Congress, Springer 483–487.

Vincent, C., G. Hallman, B. Panneton, and F. Fleurat-Lessard. 2003. Management of agricultural insects with physical control methods. Annu. Rev. Entomol. 48: 261–281.

Wearing, C. H. 2014. Historical tests of the toxicity of pesticides to *Typhlodromus pyri* (Acari: Phytoseiidae) and their relevance to current pest management in New Zealand apple orchards 1. Laboratory tests with eggs and larvae. Biocontrol Sci. Techn. 24: 780–809.

Wood, M., and J. McBride. 2001. Scientists sharpen strategies to sabotage: glassy-winged sharpshooter. Agricultural Research Magazine 49: 20–22.

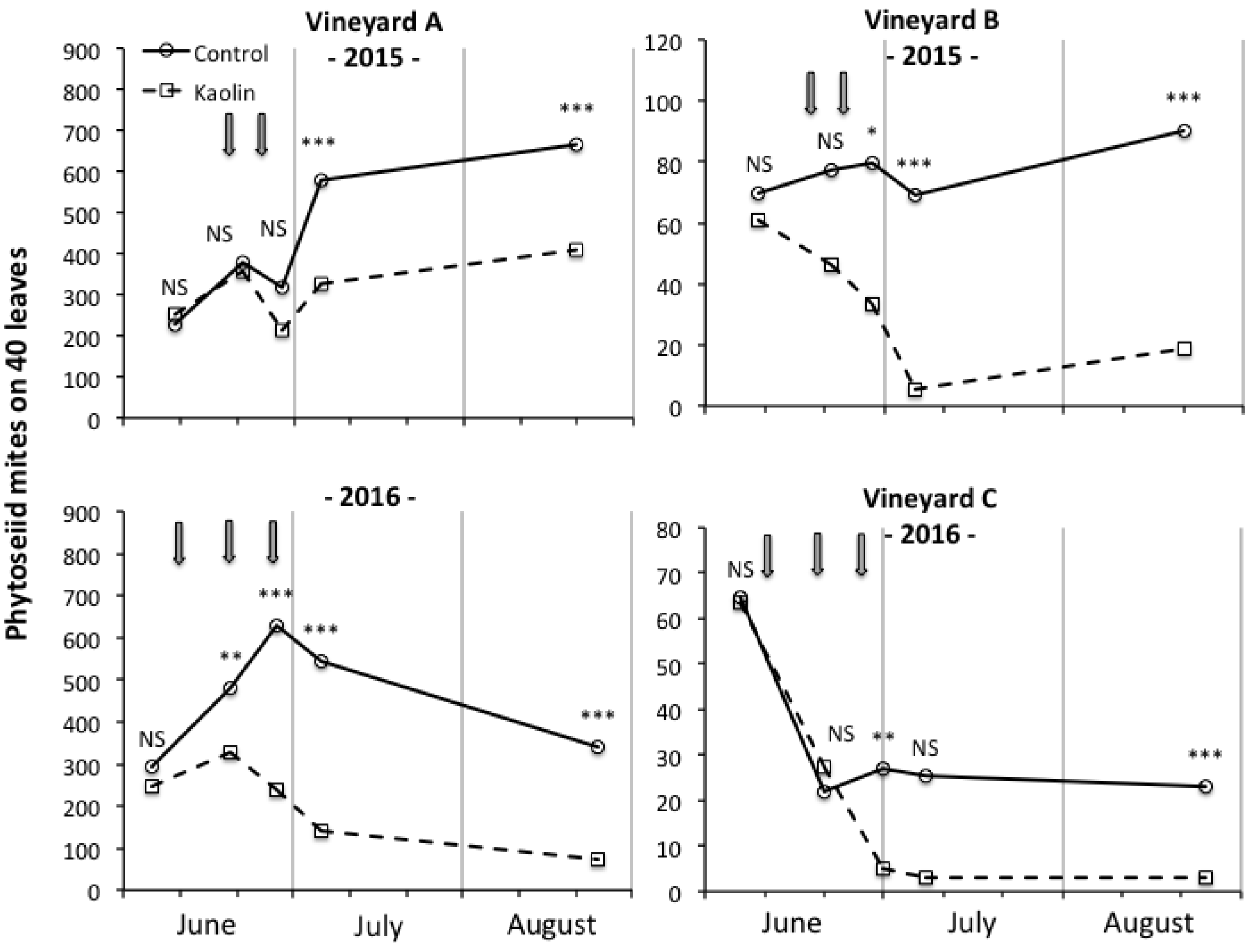

Fig. 1. Number of phytoseiid mites recorded in the kaolin and the control before the first (early June) and after two (2015) or three (2016) kaolin applications (arrows). NS, *, **, *** indicate non-significant and significant differences for α = 0.05, α = 0.01¸ α = 0.0001, respectively, between treatments according to Tukey's post-hoc test.

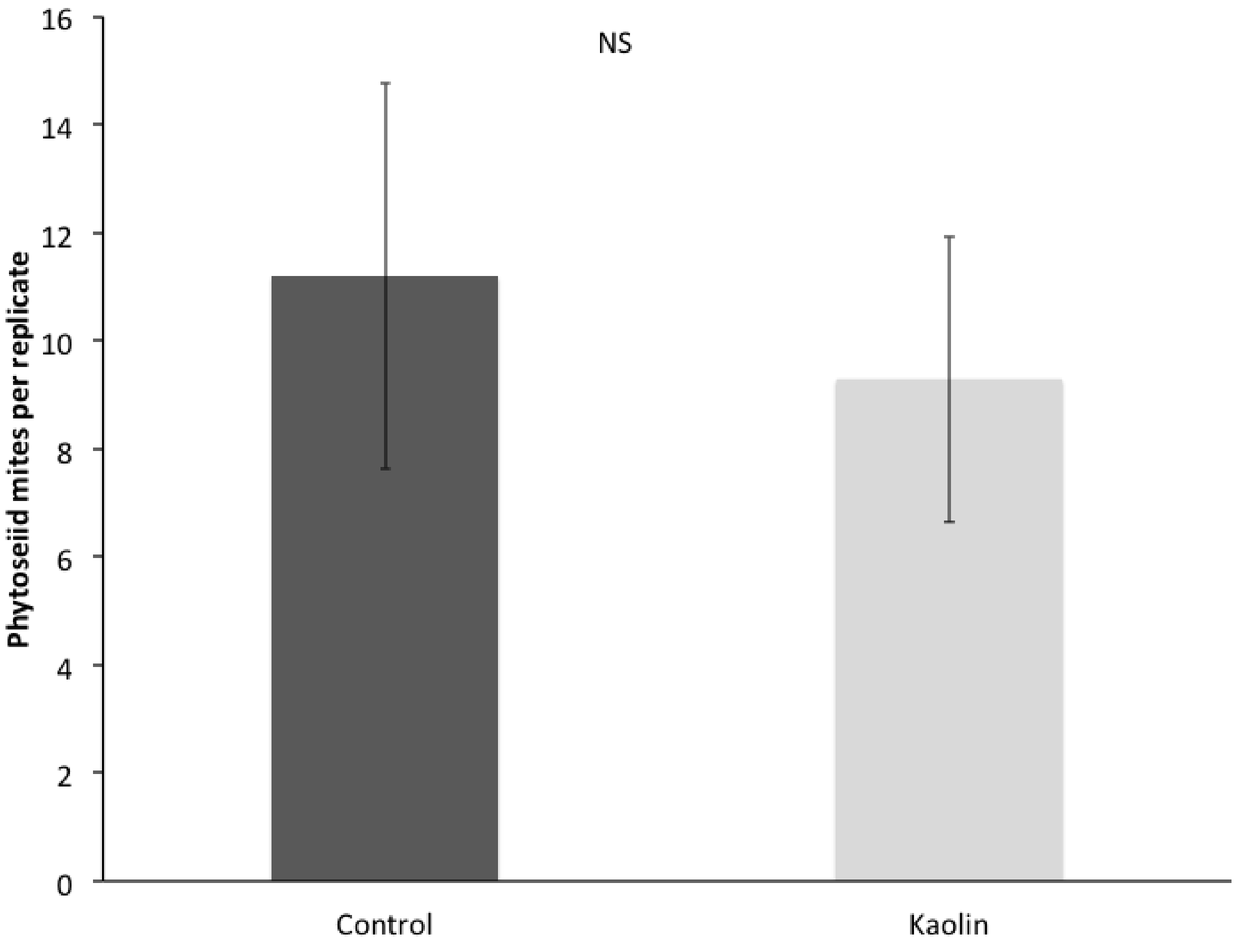

Fig. 2. Number of overwintering phytoseiid mites (mean ± standard deviation) recorded in the kaolin and the control. NS indicates non-significant differences between treatments according to one-way ANOVA.

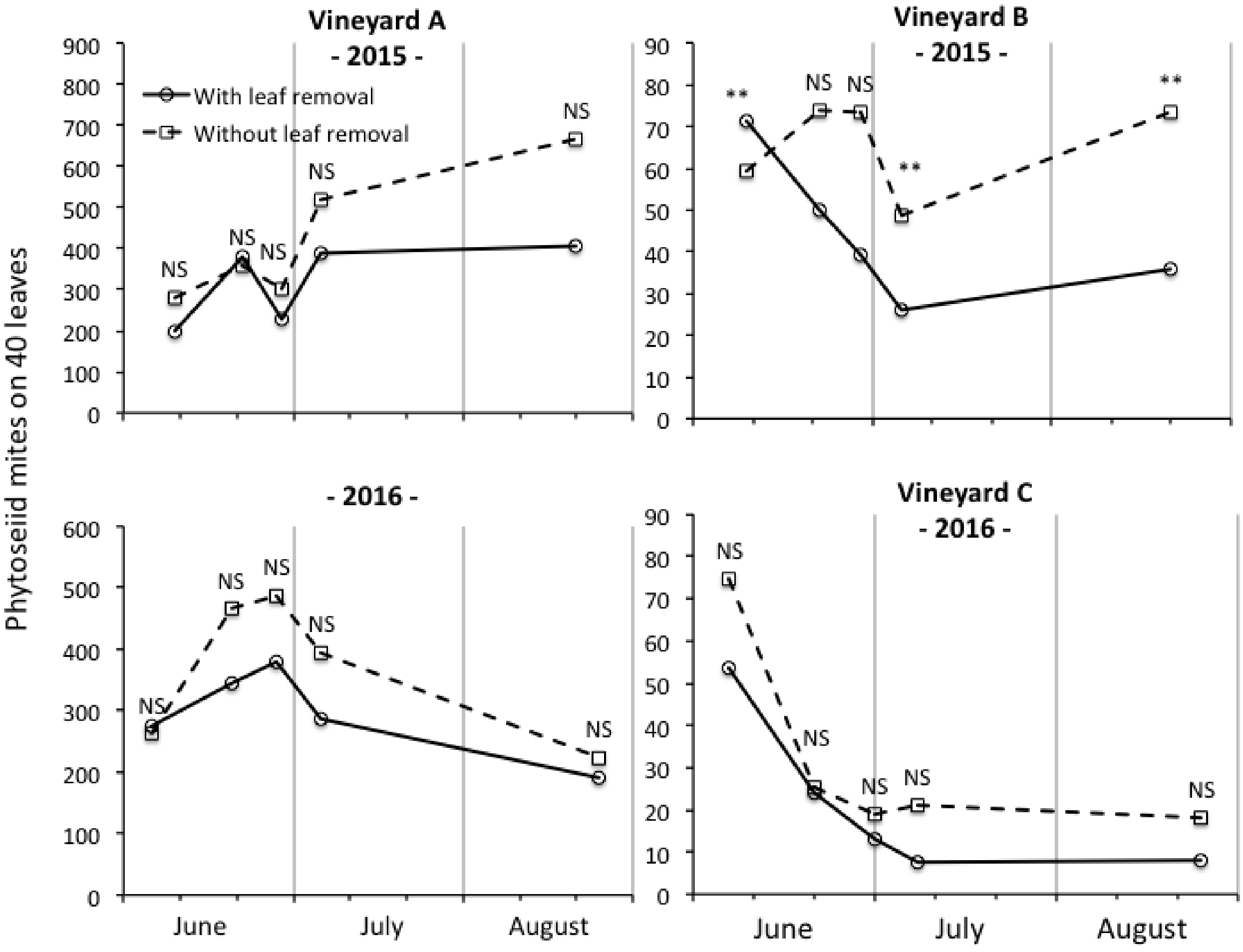

Fig. 3. Number of phytoseiid mites recorded in the sub-plots without and with leaf removal, that occurred the day after the first sampling date. NS and ** indicate, respectively, non-significant and significant differences for α = 0.01 between treatments according to Tukey's post-hoc test.

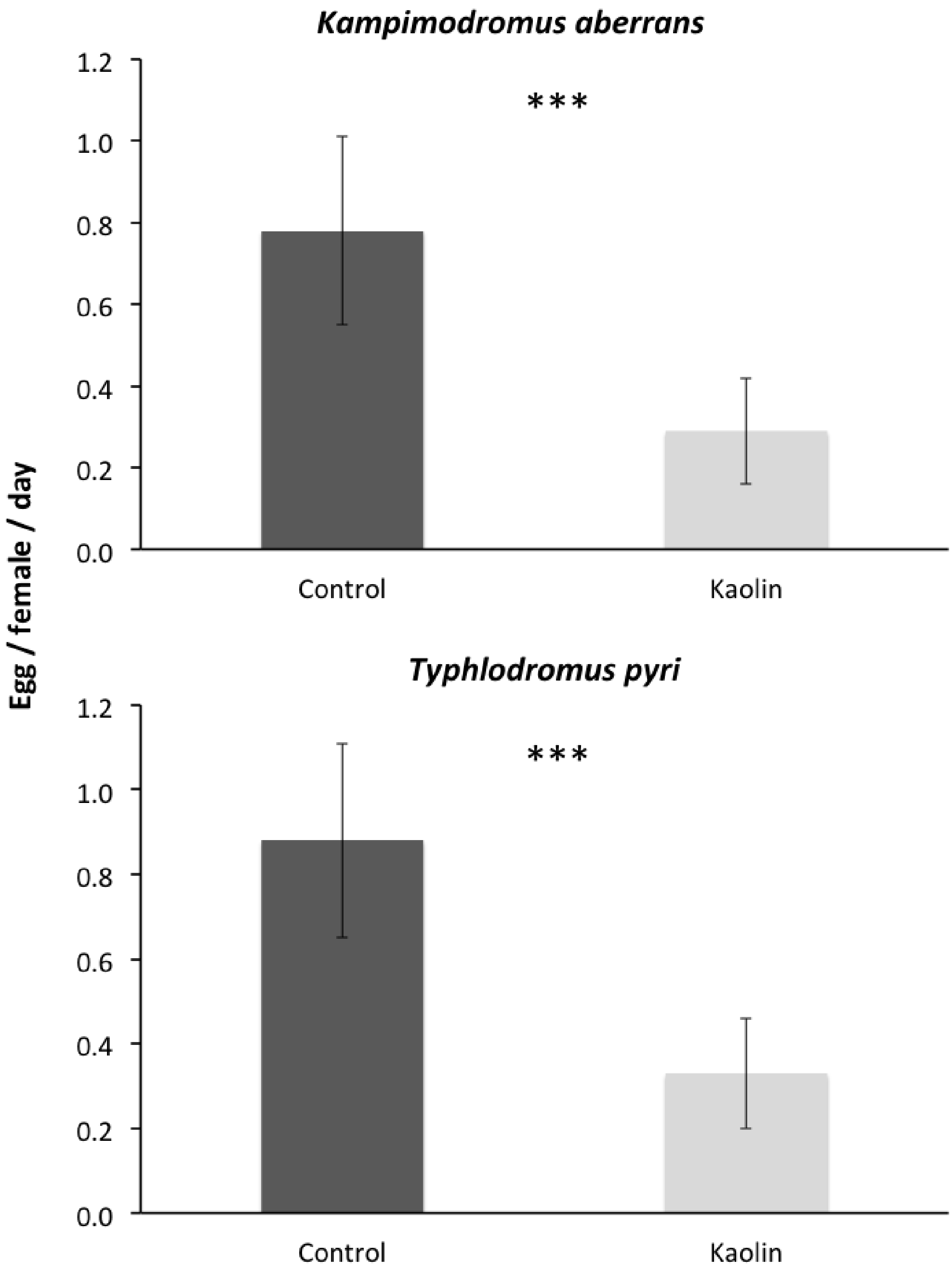

Fig. 4. Daily number of eggs (mean ± standard deviation) laid on grapevine leaf discs by a female of the two phytoseiid species in the kaolin and the control. *** indicates significant differences for $\alpha = 0.001$ between treatments according to Student's unpaired *t*-test.

Chapter 8 - Side effects of kaolin and bunch-zone leaf removal on generalist predators in vineyards

Chapter 8 will be published as:
Elena Cargnus, Federico Tacoli, Francesco Boscutti, Pietro Zandigiacomo, Francesco Pavan. Side effects of kaolin and bunch-zone leaf removal on generalist predators in vineyards.

Abstract

Kaolin and bunch-zone leaf removal (LR) resulted effective in vineyards of north-eastern Italy against leafhoppers and *Lobesia botrana*, but their side effects on non-target arthropods, including natural enemies, are still poorly studied. The impact of kaolin applications and LR was assessed in terms of species richness, functional diversity and abundance of spiders and generalist predatory insects in four trials (2015-2016) carried out in vineyards of north-eastern Italy. Kaolin and LR did not affect ecological indices for spider communities. Kaolin reduced the abundance of the hunting spiders Oxypidae and Salticidae and of the web-builders Araneidae. Among predatory insects, kaolin reduced the numbers of the anthocorid *Orius* sp. and increased those of coccinellid Scymninae. LR increased the number of the thrips *Aeolothrips* sp. These effects were not consistent in all trials. A moderate use of kaolin and the application of LR had minor impact on generalist predatory communities in vineyards and then they can be considered compatible with IPM strategies.

1. Introduction

Kaolin is a white, fine grained, not abrasive, nonexpendable, nonporous, water dispersible and inert aluminum silicate (Harben, 1995). Arthropod pest infestations and plant pathogens infections to some crops can be reduced by coating plants with kaolin. Such treated plants become visually, tactually and chemically unrecognizable to arthropod pests and their activities can be impaired due to the presence of a physical barrier and of particles that stick to their bodies (Glenn *et al.,* 1999). Kaolin can affect pest populations mostly by reducing egg laying, exerting a direct toxicity towards motile forms or inhibiting their feeding activity (Vincent *et al.,* 2003). Applications of this particle film have been found to be effective in the control of several arthropod pests in orchards and field crops (Unruh *et al.,* 2000; Knight *et al.,* 2001; Glenn and Puterka 2005; Braham *et al.,* 2007; Markó *et al.,* 2008; Pascual *et al.,* 2010; Leskey *et al.,* 2010; D'Aquino *et al.,* 2011;Lo Verde *et al.,* 2011; Marcotegui *et al.,* 2015).

In vineyards, kaolin was effective in the control of sap-feeding pests such as the sharpshooter *Homalodisca coagulata* (Say), vector of *Xylella fastidiosa* Wells, the causal agent of Pierce's disease (Wood and McBride 2001; Puterka *et al.,* 2003; Barker *et al.,* 2006; Tubajika *et al.,* 2007), the leafhoppers *Empoasca vitis* (Göthe), *Zygina rhamni* Ferrari (Tacoli *et al.,* 2017) and *Scaphoideus titanus* Ball (Tacoli *et al.,* this PhD thesis, Chapter 3), the grape phylloxera *Daktulosphaira vitifoliae* (Fitch) (Sleezer *et al.,* 2011) and the vine cicada *Psalmocharias alhageos* (Valizadeh *et al.,* 2013). Regarding carpophagous pests, kaolin was effective in the control of the tortricid moth *Lobesia botrana* (Denis and Schiffermüller) (Pease *et al.,* 2016, Tacoli *et al.,* this PhD thesis, Chapter 4) and the spotted wing drosophila *Drosophila suzukii* Matsumura (Linder *et al.,* 2017). Kaolin provided an effective management of two polyphagous scarab beetles *Macrodactylus subspinosus* (Fabricius) and *Popillia japonica* Newman, feeding on grapevine leaves and bunches (Isaac *et al.,* 2004; Maier and Williamson, 2016).

Bunch-zone leaf removal is a common cultural practice in vineyards, which consists in removing all the leaves that cover bunches contributing to control bunch rots and *L. botrana* (Pavan *et al.,* 2016).

The combination of kaolin with bunch-zone leaf removal ensures a synergic control of *L. botrana* and the reduction of berry sunburn damage (Tacoli *et al*., this PhD thesis, Chapter 4). However, kaolin and bunch-zone leaf removal can be considered viable alternatives to synthetic insecticides only if their side effects on non-target arthropods, including natural enemies, are not too deleterious. Indeed, in the context of Integrated Pest Management (IPM) strategies, the preservation of biocontrol agents is essential because of their capability in reducing pest outbreaks, damage, and so the use of pesticides.

Generalist predators can have a significant impact on pest populations (Symondson *et al.,* 2002), being sometimes more effective than a specialist at controlling pests particularly in species-rich areas (DeBach, 1946; Ehler, 1977). Hawkins *et al.* (1999) considered that most frequently a specialist can successful control an exotic pest on exotic plant, whereas control of native pests on native plants is more often influenced by communities of native generalist predators. Generalists have several features that make them important as control agents. The ability to feed on alternative preys allows generalists to be present inside a crop also when pest densities are still low and in this way they may delay or prevent pest outbreaks (Stam and Elmosa, 1990; Settle *et al.,* 1996), also bringing pest populations to extinction without a reduction in their numbers (Den Boer, 1982).

Spiders and other generalist predatory insects have the ability to reduce the populations of various pests in several agro-ecosystems (Riechert and Lockley, 1984; Marc *et al.,* 1999; Nyffeler and Sunderland, 2003). Spiders can constitute an abundant and diversified community also in vineyards. In particular, the occurrence of surrounding groves and ground cover in the inter-rows can play a very important role in the spider establishment and assemblage in vineyards, where spiders can represent up to 98% of the arthropod predators (Costello and Daane, 1997, 1998; Nobre and Meierosse, 2000; Daane and Costello, 2005; Bolduc *et al.,* 2006; Isaia *et al.,* 2006, 2007; D'Alberto *et al.,* 2012; Caprio *et al.,* 2015; Franin *et al.,* 2016). This community can undergo rapid changes in response to shifts in the vegetation structure (Asselin and Baudry, 1989), cultural practices and chemical treatments (Marc *et al.,* 1999).

In field trials on the effects of kaolin against arthropod pests, it was found that this natural product can affect differently natural enemies. In some studies reduction in the abundance of predators [spiders (Araneae), phytoseiid mites (Acari), Heteroptera, Coleoptera Coccinellidae] and Hymenoptera parasitoids was reported for apple (Markó *et al.,* 2008, 2010; Villanueva and Walgenbach, 2010), olive (González-Núñez *et al.,* 2008; Pascual *et al.,* 2010; Scalercio *et al.,* 2010) and almond (Sánchez-Ramos *et al.,* 2017). On contrary, in other studies no negative effects of kaolin were reported on the same groups of beneficials for apple (Sackett *et al.,* 2007), citrus (Smaili *et al.,* 2014), olive (Adán *et al.,* 2007; Porcel *et al.,* 2011; Gharbi and Ben Abdallah, 2016a), peach (Karagounis *et al.,* 2006), pecan (Lombardini *et al.,* 2005), blueberry (Stelinski *et al.,* 2006) and cotton (Santos *et al.,* 2013; Araujo *et al.,* 2015).

In laboratory experiments, negative effects of kaolin were reported for the spider *Araniella cucurbitina* (Clerck) (Araneidae) (Benhadi-Martin *et al.,* 2016) and for the hymenopteran parasitoid *Aprostocetus vaquitarum* (Eulophidae) (Ulmer *et al.,* 2006). On contrary, this product showed only low negative effects on certain predators, *Anthocoris nemoralis* (Fabricius) (Anthocoridae), *Chrysoperla carnea* (Stephens) (Chrysopidae) and *Chilocorus nigritus* (F.) (Coccinellidae), and some parasitoids, *Chelonus inanitus* (L.) (Braconidae) and *Scutellista cyanea* Motschulsky (Pteromalidae) (Porcel *et al.,* 2011; Bengochea *et al.,* 2010, 2013, 2014).

Considering natural enemies inhabiting vineyards, the effects of kaolin on non-target arthropods are still poorly understood. Two consecutive kaolin applications did not affect the parasitization of *E. vitis* and *Z. rhamni* eggs by *Anagrus* sp. (Tacoli *et al.,* 2017). More recently, kaolin was recognised as moderately harmful to the phytoseiid mites *Typhlodromus pyri* Scheuten and *Kampimodromus aberrans* (Oudemans) in vineyards and the result was confirmed later in laboratory experiments (Tacoli *et al.,* this PhD thesis, Chapter 7).

In the current study, we investigated the effects of kaolin and of bunch-zone leaf removal on spiders and a number of generalist predatory insects in vineyards. Bunch-zone leaf removal could negatively affect natural enemies since it changes the structure and microclimate of grapevine canopy.

2. Materials and methods

2.1. Field experiments

The influence of kaolin and bunch-zone leaf removal on spiders and generalist predatory insects populations was studied during 2015-2016 in four field trials carried out in three vineyards of north-eastern Italy, named A (2015 and 2016), B (2015) and C (2016), respectively. Vineyard A (Cormons, Gorizia district, 45°57'51"N, 13°26'49"E, 56 m a.s.l., cultivar Pinot Gris) is a 10-year-old conventional vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.5 m and 0.8 m, respectively. Vineyard B (Cormons, Gorizia district, 45°57'20"N, 13°26'50"E, 50 m a.s.l., cultivar Pinot Gris) is a 30-year-old organic vineyard with grapevines growing using the double arched Guyot training system with distances between and along rows of 2.8 m and 1 m, respectively. Vineyard C (Cormons, Gorizia district, 45°58'02"N, 13°31'31"E, 53 m a.s.l., cultivar Pinot Gris) is a 15-year-old organic vineyard with grapevines growing using the Guyot training system with distances between and along rows of 2.2 m and 0.7 m, respectively. In all vineyards, standard fungicide programs were followed and no insecticide was sprayed before and after the kaolin applications.

In the four trials, kaolin (Surround WP, Tessenderlo Kerley Inc., Phoenix, Arizona, USA, 2% suspension), sprayed at the rate of 1000 L/ha, was compared with the control. Kaolin was applied twice in 2015 (18 and 24 June) and three times in 2016 (10, 20, 27 June). All applications were done using a backpack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano, RE, Italy).

In all trials, a randomized block design with four replicates was adopted. Each block (row) was divided into two plots of 28 (vineyard A) or 20 (vineyard B) or 24 (vineyard C) grapevines. In all trials, plots were divided into two subplots of 14 or 10 or 12 grapevines, respectively, that were submitted or not to bunch-zone leaf removal (17 June 2015, 10 June 2016). In vineyard A, the plots sprayed with kaolin were the same in the two years.

Timing of kaolin applications and bunch-zone leaf removal was determined by the occurrence of the second seasonal flight of *L. botrana* that was the target pest of the trials (Tacoli *et al.,* 2017, this PhD thesis, Chapter 4).

In all vineyards and both years, spider populations on the grapevine canopy were sampled by a modified beating-tray method (hereafter named "beating tray") over the trial period (11, 22, 29 June, 06 July in 2015 and 7, 21 June, 1, 8 July in 2016). On each sampling date, the trunk of 10 grapevines per subplot was shaken five times with movements parallel to the ground while a funnel (65 × 45 cm) was placed under the canopy to collect spiders. All the specimens were then conserved in plastic tubes with 70° ethanol. Only in 2015 in the vineyards A and B, spiders and predatory insects were sampled using yellow sticky traps deployed from 4 June (2-wk before the first kaolin application) to 18 August (about two months after the second kaolin application) and replaced weekly. Only in the sampling before the first kaolin application the traps remained in the vineyards for two weeks (i.e., from 4 June to 18 June). One trap (20 × 10 cm) per subplot (with or without leaf removal), smeared with glue (Temo-O-Cid®, Kollant Srl, Vigonovo, VE, Italy), was hung on the horizontal wires of the grapevine trellis at about 1.5 m from the ground level so as to be inside the canopy, but not covered by leaves.

The specimens were identified in the laboratory. Spiders and five groups of predatory insects both reliable bioindicators and well represented in the grape-growing area of this study [i.e., *C. carnea* (Neuroptera: Chrysopidae), Coleoptera Coccinellidae Scymninae, Coccinellidae excluded Scymninae, *Aeolothrips* sp. (Thysanoptera: Aeolothripidae) and *Orius* sp. (Heteroptera: Anthocoridae)] were counted and identified to different taxonomic levels under a dissection microscope using the current keys (Mound *et al.,* 1976; Tamanini, 1988; Roberts, 1995; Bellman, 2011; Nentwig *et al.,* 2017). Spider families were grouped on the basis of their capture strategy in web-builders and hunters. All spiders collected by beating tray were grouped at family level, and only the adults were identified to genus or species. Spiders captured by yellow sticky traps were grouped in web-builders and hunters, and this latter also at family level.

2.2. Statistical analyses

The effects of kaolin and bunch-zone leaf removal on the spiders collected by beating tray in the four vineyards were examined in terms of species richness, using the Shannon's diversity index (H'), and functional diversity (FD).

Two indices were calculated using functional trait information: functional richness (i.e., width of a niche space filled with species) and functional dispersion (i.e., mean of individual species to the centroid of all species in the community) (Villéger *et al.,* 2008; Laliberté and Legendre, 2010). It was not possible to calculate functional evenness (i.e., the evenness of the distribution of abundance in niche space) due to the low number of species or absence of quantitative traits. Functional diversity indices were calculated using the FD package (Laliberté and Legendre, 2010; Laliberté *et al.,* 2014) that implements a distance-based approach that allows handling both continuous and categorical variables. The values calculated for each index, the number of spiders collected by beating tray and the number of spiders and insects captured with yellow sticky traps, were compared using a Mixed ANOVA with Bonferroni correction and Tukey's post-hoc test used after data logarithmic transformation. All statistical analyses were performed with IBM SPSS Statistics 20 (IBM Corporation 2011) and R software (R Core Team 2017).

3. Results

3.1. Vineyard A 2015

Spiders collected by beating tray belonged to seven families, with hunters more represented than web-builders (76% vs 24%) (Table 1). On total spiders, the hunters Thomisidae (44%) and Oxyopidae (19%), and the web-builders Araneidae (17%) were the most abundant families. At genus and species levels, *Xysticus* sp. and *Oxyopes* spp. were the most numerous spiders. Two kaolin applications and bunch-zone leaf removal did not significantly affect spider communities in terms of species richness, functional richness and functional dispersion (Table 2). Kaolin and bunch-zone leaf removal did not significantly affect spiders either considering their total number or that of each capture strategy group. At family level, only the populations of Oxyopidae (*Oxyopes* spp.) were significantly lower in kaolin than in control plots.

Among predators captured by yellow sticky traps, *Aeolothrips* sp. was the most abundant accounting for 71% (N. 1045), other insect predators represented only 13% (N. 186) and spiders accounted for 16% (N. 226). Within the spider community, hunters (59%) were more numerous than web-builders (41%). Thomisidae was the main family of hunters (51%). Among insects, two kaolin applications significantly affect only Coccinellidae Scymninae that showed higher populations in the kaolin than in the control plots. On contrary, *Orius* sp. showed lower captures in the kaolin than in the control plots, but not at significant level. *Aeolothrips* sp. showed significantly higher captures in plots submitted to bunch-zone leaf removal than in control. Spiders were not significantly affected by either two kaolin applications or bunch-zone leaf removal. With reference to spider families, Thomisidae showed higher captures in plots submitted to bunch-zone leaf removal than in control plots, but not at significant level (Table 4).

3.2. Vineyard B

In 2015, spiders collected by beating tray belonged to eight families, showing a similar amount of hunters and web-builders (52% vs 49%) (Table 1). On total spiders, the hunters Thomisidae (36%) and Oxyopidae (12%), and the web-builders Araneidae (20%) and Linyphiidae (13%) were the most abundant families. At genus and species levels, *Xysticus* sp., *Oxyopes* spp., *Mangora acalypha* and *Neriene* sp. were the most numerous spiders. Two kaolin applications and bunch-zone leaf removal did not significantly affect spider communities in terms of species richness, functional richness and functional dispersion (Table 2). Kaolin and bunch-zone leaf removal did not significantly affect spiders either considering their total number or that of each capture strategy group and family.

Among predators captured by yellow sticky traps, *Aeolothrips* sp. was the most abundant, accounting for 59% (N. 1185), other insect predators represented 25% (N. 496) and spiders accounted for 16% (N. 321). Within the spider community, hunters (60%) were more numerous than web-builders (40%). Thomisidae was the main family of hunters (52%). Among insects, two kaolin applications affected *Orius* sp. that showed lower captures in the kaolin than in the control plots. Insects were not influenced by bunch-zone leaf removal. Spiders were not significantly affected by either two kaolin applications or bunch-zone leaf removal (Table 4).

3.3. Vineyard A 2016

Spiders collected by beating tray belonged to nine families, with hunters more numerous than web-builders (72% vs 28%) (Table 1). On total spiders, the hunters Thomisidae (45%) and Salticidae (18%), and the web-builders Araneidae (17%) were the most abundant families. At genus and species levels, *Runcinia grammica* and *Xysticus* sp., were the most numerous spiders. Three kaolin applications and bunch-zone leaf removal did not significantly affect spider communities in terms of species richness, functional richness and functional dispersion (Table 3). Kaolin and bunch-zone leaf removal did not significantly affect spiders either considering their total number or that of each capture strategy group. At family level, the populations of Oxyopidae (*Oxyopes* spp.) and Araneidae were significantly lower in the kaolin than in the control plots.

3.4. Vineyard C

In 2016, spiders collected by beating tray belonged to seven families, with hunters more numerous than web-builders (58% vs 42%) (Table 1). On total spiders, the hunters Salticidae (25%) and Oxyopidae (22%), and the web-builders Araneidae (28%) were the most abundant families. At genus and species levels, *Oxyopes* spp. and *Nuctenea* sp. were the most numerous spiders. Three kaolin applications and bunch-zone leaf removal did not significantly affect spider communities in terms of species richness, functional richness and functional dispersion (Table 3). Kaolin and bunch-zone leaf removal did not significantly affect spiders considering either their total number or that of each capture strategy group and family.

4. Discussion

4.1. Spiders community in vineyards and influence of kaolin

Spiders collected by beating tray belonged to nine families and 29 different taxa. Twofold abundant populations of spiders were recorded in vineyard B than in the other vineyards. However, the highest diversity in number of families was observed in 2016 in vineyard A (9 out 9), even if seven families were well represented also in the other three vineyards. No negative effects were found on species richness and functional diversity.

Comparing the two sampling methods adopted in 2015, more spiders were captured by yellow sticky traps than beating tray. More hunters than web-builders were counted in all vineyards with both sampling methods. These findings are in accordance with other studies on spider assemblages in vineyards (Isaia *et al.,* 2006, 2007; Trivellone *et al.,* 2013; Caprio *et al.,* 2015).

Among hunters, the more abundant families were in decreasing order Thomisidae, Oxyopidae and Salticidae. Thomisidae were reported in vineyards (Nobre and Meierrose, 2000; Costello and Daane, 2003) and their role in *L. botrana* adult predation was considered (Addante *et al*., 2003). Oxyopidae and in particular *Oxyopes* spp. were found in all our vineyards. The species *O. lineatus*, that has been reported feeding on Cicadellidae (Huseynov, 2007), was observed feeding on *E. vitis* adults during our samplings. Salticidae together with Miturgidae (Clubionidae) are known to be predators of *L. botrana* larvae, pupae (Marchesini and Dalla Montà, 1994) and adults

(Addante *et al.,* 2007, 2008). In our visits, both Salticidae (e.g., *Salticus* sp.) and Miturgidae (e.g., *Cheiracanthium* sp.) were found. *Salticus* sp. spiders have been also reported feeding on *E. vitis* nymphs in vineyards, but their contribution to leafhopper control has not been considered important (Sentenac, 2004). In accordance with these outcomes, spiders are considered as the primary generalist predators of leafhoppers of *Erythroneura* species in American vineyards (Costello and Daane 1995, 1999), even if the plant vigour or other factors, that favour *Anagrus* sp. egg parasitoids, seem to be more important than spider abundance (Costello and Daane 2003, Hanna *et al.,* 2003, Wilson *et al.,* 2015).

Among web-builders, Araneidae were the most abundant family and were frequently observed in vineyards in other studies (Isaia *et al*., 2006, 2007; Addante *et al*., 2008). Inside this praying-strategy group, Theridiidae are known to be predators of *L. botrana* larvae, pupae (Marchesini and Dalla Montà, 1994) and adults (Addante *et al.,* 2007, 2008).

In the four trials, two (2015) and three (2016) kaolin applications did not alter the spider predatory communities in terms of abundance with three exceptions. Abundance reduction was observed for the hunter spiders Oxyopidae and Salticidae, and for the web-builders Araneidae. Kaolin interfered more negatively on hunter than web-builder spiders. According to our results, hunter spiders (Thomisidae, Philodromidae and Salticidae) were found to be more vulnerable to kaolin than web-builder spiders (Dictynidae) in apple orchards (Sackett *et al.,* 2007; Markó *et al.,* 2010). It is probable that kaolin can interfere more negatively on hunters because they are more mobile than web-builders and more exposed to the kaolin residue during their hunting activity. In particular, spiders can be exposed to kaolin directly during its application because it can form a film of particles on the spider body or later by ingesting the kaolin when feeding on sprayed prey or plant surfaces. Recently, the effect of kaolin was investigated on a web-builder species under laboratory condition (Benhadi-Marín *et al.,* 2016). In this last study, a higher mortality of *A. cucurbitina* in the kaolin treatment was explained as the consequence of the ingestion of great amounts of silk covered with kaolin, since it is reported that Araneidae often repair or rebuild their webs by ingesting them (Marc *et al*., 1999). This feeding behaviour can explain why in our study Araneidae appeared to be more sensible to kaolin than the other web-builder spiders collected. However, spiders of this family were found to be negatively affected only in one out of four study vineyards after three kaolin applications as well as in one apple orchard after four kaolin applications (Sackett *et al.,* 2007).

In our study, the relatively low negative effects of kaolin on spiders can be explained by the small number of applications. In literature, contradictory results about the effect of a high number of kaolin applications on spiders are reported. In apple orchards, after 12 kaolin applications the spider densities were significantly reduced (Markó *et al.,* 2010), and at more than four weeks from the last application the abundance of spiders was still significantly lower in the kaolin-treated plots (Markó *et al.,* 2008). Whereas, in a cotton field, spider densities were not reduced after several weekly kaolin applications (Santos *et al.,* 2013). Indeed, Sackett *et al.* (2007) stated that, being spiders less mobile than other predatory arthropods, a possible negative effect of kaolin would only be observed in the long term. In agreement with this statement, in a pecan orchard, after seven and six kaolin applications on the first and second year, respectively, spiders became fewer on kaolin treated pecan in the second year (Lombardini *et al.,* 2005). In our study, in vineyard A, in which kaolin was applied for two consecutive years on the same plots, only in the second year we observed a reduction in the abundance of Araneidae in kaolin treated plots, even though the species diversity of spiders increased in comparison to the first year.

4.2. Insect generalist predators in vineyards and kaolin effect

Among the five generalist predatory insect groups considered in the samplings with yellow sticky traps *Aeolothrips* sp. was the most abundant followed in decreasing order by Scymninae, other Coccinellidae, *Orius* sp. and *C. carnea*. *Aeolothrips* sp. are predator of other phytophagous thrips in vineyards (Trdan *et al*., 2005). The presence of Scymininae in vineyards is often associated with mealybug infestation (Daane *et al*., 2012). *C. carnea* is reported as predator of *L. botrana* larvae (Marchesini and Dalla Montà, 1994). The generalist pirate bugs *Orius* sp. are effective control agents of tetranychid and eriphyid mites in vineyards (Duso and Girolami, 1982).

In the two trials of 2015, kaolin applications did not alter the considered predatory insect abundance with two exceptions: *Orius* sp. decreased and Scymininae increased. With the same number of kaolin applications, a negative effect on *Orius* sp. was reported also in olive orchards (Pascual *et al.,* 2010). For another minute pirate bug, *Anthocoris nemoralis* (F.), negative effects of kaolin were found under laboratory and field conditions (Pascual *et al.,* 2010; Bengochea *et al.,* 2013), but in other studies no harmful effects of kaolin were seen in laboratory (Gharbi and Ben Abdallah, 2016b). The higher densities of Scymninae observed in the kaolin than in the control plots of vineyard A could be explained by a higher abundance of mealybugs, since Scymninae are associated with mealybugs and kaolin can increase their leaf infestation (Joubert *et al*., 2004; Tacoli *et al.,* this phD thesis, Chapter 6).

In our study, kaolin did not affect other Coccinellidae in agreement with other studies (Lombardini, 2005; Karagounis *et al*., 2006; Santos *et al.,* 2013), even if negative effects have been also reported (Sackett *et al.,* 2007; Pascual *et al.* 2010). In our study, also *C. carnea* was not negatively influenced by kaolin, but the data reported in literature not always confirmed the absence of negative effects (Porcel *et al.,* 2011; Bengochea *et al.,* 2014; Gharbi and Ben Abdallah, 2016b).

Kaolin did not negatively affect *Aeolothrips* sp., probably also for the presence of plant diversity in the inter-rows where they spend most of their lives in flowers feeding on small arthropods and pollen.

4.3. Effect of bunch-zone leaf removal on generalist predators

Bunch-zone leaf removal did not affect the spider and predatory insect communities in terms of species richness, functional diversity and abundance, with the exception of *Aeolothrips* sp. which were higher in subplots submitted to bunch-zone leaf removal. This can be explained because Thysanoptera are more captured on yellow sticky traps exposed to sunlight (Strapazzon *et al*., 1990), as occurred on vines submitted to leaf removal.

4.4. Conclusion remarks

In vineyards, a moderate use of kaolin and the application of LR had minor impact on spider and generalist predatory insect communities and then their use appears compatible with IPM strategies. The negative effects are negligible also after two years of consecutive application in the same plots. In any case, considering the positive effects on the control of grapevine key pests, the overall outcome of both kaolin and bunch-zone leaf removal is positive since in the context of IPM their adoption allows for the reduction of insecticide applications.

5. References

Adán, A., T. González, R. Bastante, F. Budia, P. Medina, P. del Estal, and E. Viñuela. 2007. Effects of several insecticides on *Psyttalia concolor* (Szèpligeti) (Hymenoptera: Braconidae) under extended-laboratory conditions. Boletín de Sanidad Vegetal, Plagas. 33: 391–397.

Addante, R., T. Moleas, and G. Ranieri. 2003. Preliminary investigations on the interaction between spiders (Araneae) and grapevine moth (*Lobesia botrana* (Denis et Schiffermüller)) populations in Apulian vineyards. IOBC/WPRS Bull. 26 (8): 111–115.

Addante, R., G. Ranieri, C. Pesarini, and T. Moleas T. 2007. Indagine sulle relazioni tra ragni e *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera Tortricidae) in vigneti pugliesi. Atti XXI del Congresso Nazionale Italiano di Entomologia, Campobasso 11-16 giugno 2007: 214.

Addante, R., S. Di Gioia, C. Calculli, and A. Pollice. 2008. The impact of spiders (Araneae) on *Lobesia botrana* (Denis & Schiffermüller) population density. IOBC/WPRS Bull. 36: 221–231.

Araujo, G. P. de, F. R. de Azevedo, F. A. de Albuquerque, C. A. M. dos Santos, E da S. Moura, and D. R. Nere. 2015. Natural products to agro-ecological pest management and their natural enemies of cotton plant intercropped with maize, cowpea and sesame. Agro-@mbiente On-line. 9 (2): 194–201.

Asselin, A., and J. Baudry. 1989. Les Aranèides dans un espace agricole en mutation. Acta Ecolo. Applic. 10: 143–156.

Barker, J. E., A. Fulton, K. A. Evans, and G. Powell. 2006. The effects of kaolin particle film on *Plutella xylostella* behaviour and development. Pest Manag. Sci. 62: 498–504.

Bellmann, H. 2011. Guida ai ragni d'Europa. Muzzio editore, Roma, 427 pp.

Bengochea, P., S. Hernando, R. Saelices, Á. Adán, F. Budia, M. González-Núñez, E. Viñuela, and P. Medina. 2010. Side effects of kaolin on natural enemies found on olive crops. IOBC/WPRS Bull. 55: 61–67.

Bengochea, P., F. Amor, R. Saelices, S. Hernando, F. Budia, Á. Adán, and P. Medina. 2013. Kaolin and copper-based products applications: Ecotoxicology on four natural enemies. Chemosphere 91: 1189–1195.

Bengochea, P., R. Saelices, F. Amor, Á. Adán, F. Budia, P. del Estal, E. Viñuela, and P. Medina. 2014. Non-target effects of kaolin and coppers applied on olive trees for the predatory lacewing *Chrysoperla carnea*. Biocontrol Sci. Technol. 24: 625–640.

Benhadi-Marín, J., J. A. Pereira, S. A. P. Santos. 2016. Effects of kaolin particle films on the life span of an orb-weaver spider. Chemosphere 144: 918–924.

Bolduc, E., C. M. Buddle, N. J. Bostanian, C. Vincent. 2005. Ground-dwelling spider fauna (Araneae) of two vineyards in southern Quebec. Environ. Entomol. 34: 635–645.

Bostanian, N. J., and G. Racette. 2008. Particle films for managing arthropod pests of apple. J. Econ. Entomol. 101: 145–150.

Braham, M., E. Pasqualini, and N. Ncira. 2007. Efficacy of kaolin, spinosad and malathion against *Ceratitis capitata* in *Citrus* orchards. Bull. Insectol. 60: 39–47.

Caprio, E., B. Nervo, M. Isaia, G. Allegro, and A. Rolando. 2015. Organic versus conventional systems in viticulture: Comparative effects on spiders and carabids in vineyards and adjacent forests. Agr. Syst. 136: 61–69.

Costello, M. J., and K. M. Daane. 1995. Spider (Araneae) species composition and seasonal abundance in San Joaquin Valley grape vineyards. Environ. Entomol. 24: 823–831.

Costello, M. J., and K. M. Daane. 1997. Comparison of sampling methods used to estimate spider (Araneae) species abundance and composition in grape vineyards. Environ. Entomol. 26: 142–149.

Costello, M. J., and K. M. Daane. 1998. Influence of ground cover on spider populations in a table grape vineyard. Ecol. Entomol. 23 1: 33–40.

Costello, M. J., and K. M. Daane. 1999. Abundance of spiders and insect predators on grapes in central California. Journal of Arachnology 27: 531–538.

Costello, M. J., and K. M. Daane. 2003. Spider and leafhopper (*Erythroneura* spp.) response to vineyard ground cover. Environ. Entomol. 32: 1085–1098.

D'Alberto, C. F., A. A. Hoffmann, L. J. Thomson. 2012. Limited benefits of non-crop vegetation on spiders in Australian vineyards: Regional or crop differences? BioControl 57: 541–552.

D'Aquino, S., A. Cocco, S. Ortu, and M. Schirra. 2011. Effects of kaolin-based particle film to control *Ceratitis capitata* (Diptera: Tephritidae) infestations and postharvest decay in citrus and stone fruit. Crop Prot. 30:1079-1086.

Daane, K. M., and M. J. Costello. 2005. Day vs. night sampling for spiders in grape vineyards. The Journal of Arachnology 33: 25–32.

Daane, K. M., R. P. P. Almeida, V. A. Bell, J. T. S. Walker, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaviezo. 2012. Biology and management of mealybugs in vineyards, pp. 271–307. In Bostanian, N. J., C. Vincent, and R. Isaacs (eds.), Arthropod management in vineyards: Pests, approaches, and future directions. Springer, Dordrecht Heidelberg New York London.

DeBach, P. 1946. An insecticidal check method for measuring the efficacy of entomophagous insects. J. Econ. Entomol. 39: 695–697.

Den Boer, P. J. 1982. Facts, hypotheses and models on the part played by food in the dynamics of carabid populations. *In* Feeding behaviour and accessibility of food for carabid beetles, Eur. Carabidol., 5th, Stara Brda Pilska. Warsaw Agric. Univ. Press, Warsaw, pp. 81-96.

Duso, C., and V. Girolami. 1982. Ruolo degli Antocoridi nel controllo del *Panonychus ulmi* Koch nei vigneti. Boll. Ist. Entomol. Univ. Bologna, 37: 157–169.

Ehler, L. E. 1977. Natural enemies of cabbage looper on cotton in the San Joaquin valley. Hilgardia. 45: 73–106.

Franin, K., B. Baric, and G. Kustera. 2016. The role of ecological infrastructure on beneficial arthropods in vineyards. Spanish Journal of Agricultural Research 14 (1): e0303.

Gharbi, N., and S. Ben Abdallah. 2016a. Effectiveness of kaolin treatment for the control of the olive fruit fly *Bactrocera oleae* in Tunisian groves. Tunisian Journal of Plant Protection. 11 (1): 73–81.

Gharbi, N., and S. Ben Abdallah. 2016b. Laboratory evaluation of side-effects of kaolin on two predator species found on olive groves. Tunisian Journal of Plant Protection. 11 (1): 83–90.

Glenn, D. M., and G. J. Puterka. 2005. Particle films: a new technology for agriculture. Hortic. Rev. 31: 1–44.

Glenn, D. M., G. Puterka, T. Vanderzwet, R. E. Byers, and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92: 759–771.

González-Núñez, M., S. Pascual, E. Seris, J. R. Esteban-Durán, P. Medina, F. Budia, Á. Adán, E. Viñuela, H. Vogt, J. P. Jansen, E. Vinuela, and P. Medina. 2008. Effects of different control measures against the olive fruit fly (*Bactrocera oleae* (Gmelin)) on beneficial arthropod fauna. Methodology and first results of field assay. IOBC/WPRS Bull. 35: 26–31.

Hanna, R., F. G. Zalom, and W. J. Roltsch. 2003. Relative impact of spider predation and cover crop on population dynamics of *Erythroneura variabilis* in a raisin grape vineyard. Entomol. Exp. Appl. 107: 177–191.

Harben, P. W. 1995. The industrial minerals handbook: a guide to markets, specifications & prices. Arby Industrial Minerals Division Metal Bulletin. PLC, London.

Huseynov, E. F. 2007. Natural prey of the lynx spider *Oxyopes lineatus* (Araneae: Oxyopidae). Entomol. Fennica 18: 144–148.

Isaacs, R., R. J. Mercader, and J. C. Wise. 2004. Reduced-risk insecticides for protection of grapevines against the rose chafer, *Macrodactylus subspinosus* (Coleoptera: Scarabaeidae). J. Appl. Entomol. 128: 371–376.

Isaia, M., F. Bona, and G. Badino. 2006. Influence of landscape diversity and agricultural practices on spider assemblage in Italian vineyards of Langa Astigiana (Northwest Italy). Environ. Entomol. 35: 297–307.

Isaia, M., F. Bona, and G. Badino. 2007. Spiders in the landscape of Italian vineyards: langa astigiana. In: European Landscapes and Lifestyles: The Mediterranean and Beyond. pp. 265-275.

Joubert, P. H., T. Grové, M. S. De Beer, and W. P. Steyn. 2004. Evaluation of kaolin (Surround® Wp) in an IPM program on mangoes in South Africa. Acta Hortic. 645: 493–499.

Karagounis, C., A. K. Kourdoumbalos, J. T. Margaritopoulos, G. D. Nanos, and J. A. Tsitsipis. 2006. Organic farming- compatible insecticides against the aphid *Myzus persicae* (Sulzer) in peach orchards. J. Appl. Entomol. 130: 150–154.

Knight, A. L., B. A. Christianson, T. R. Unruh, J. G. Puterka, and D. M. Glenn. 2001. Impact of seasonal kaolin particle films on apple pest management. Can. Entomol. 133: 413–428.

Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91: 299–305.

Laliberté, E., P. Legendre, and B. Shipley. 2014. FD: Measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0-12.

Leskey, T. C., S. E. Wright, D. M. Glenn, D., and G. J. Puterka. 2010. Effect of Surround WP on behavior and mortality of apple maggot (Diptera: Tephritidae). J. Econ. Entomol. 103: 394–401.

Linder, C., N. Staehli, M. Leumann, W. Siegfried, and P. Kehrli. 2017. Physical barriers against *Drosophila suzukii* in viticulture. IOBS/WPRS Bull (in press).

Lombardini, L., M. K. Harris, and D. M. Glenn. 2005. Effects of particle film application on leaf gas exchange, water relations, nut yield, and insect populations in mature pecan trees. HortScience 40(5): 1376–1380.

Lo Verde, G., V. Caleca, and V. Lo Verde. 2011. The use of kaolin to control *Ceratitis capitata* in organic citrus groves. Bull. Insectol. 64: 229–273.

Maier, R. M., and R. C. Williamson. 2016. Evaluation of kaolin clay as an alternative management tactic for Japanese beetle feeding damage in grape vineyards. J Hortic. 3: 184 (6 pp.).

Marc, P., A. Canard, and F. Ysnel. 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agric. Ecosyst. Environ. 74: 229–273.

Marchesini, E., L. Dalla Montà. 1994. Observations on natural enemies of *Lobesia botrana* (Den. & Schiff.) (Lepidoptera Tortricidae) in Venetian vineyards. Bollettino di Zoologia agraria e di Bachicoltura, Serie II, 26, 2, 201-230.

Marcotegui, A., I. Sánchez-Ramos, S. Pascual, C. E. Fernández, G. Cobos, I. Armendáriz, A. Cobo, and M. González-Núñez. 2015. Kaolin and potassium soap with thyme essential oil to control *Monosteira unicostata* and other phytophagous arthropods of almond trees in organic orchards. J. Pest Sci. 88: 753–765.

Markó, V., L. H. M. Blommers, S. Bogya, and H. Helsen. 2008. Kaolin particle films suppress many apple pests, disrupt natural enemies and promote woolly apple aphid. J. Appl. Entomol. 132: 26–35.

Markó, V., S. Bogya, E. Kondorosy, and L. H. M. Blommers. 2010. Side effects of kaolin particle films on apple orchard bug, beetle and spider communities. Int. J. Pest Manage. 56: 189–199.

Mound, L. A., G. D. Morison, B. R. Pitin, and J. M. Palmer. 1976. Thysanoptera. Handbooks for the Identification of British Insects. Vol. I (11): 1-79.

Nentwig, W., T. Blick, D. Gloor, A. Hänggi, and C. Kropf. 2017. Spiders of Europe. www.araneae.unibe.ch. (last access date: 24 October 2017).

Nobre, T., and C. Meierrose. 2000. The species composition, within-plant distribution, and possible predatory role of spiders (Araneae) in a vineyard in southern Portugal, In: Gajdos, P., and S. Pekar (eds.), Proceedings of the 18th European Colloquium on Arachnology. Ekologia, Bratislava. 12–17 July 1999. pp. 193–200.

Pascual, S., G. Cobos, E. Seris, and M. González-Núñez. 2010. Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove. J. Pest. Sci. 83: 121–133.

Pavan, F., E. Cargnus, F. Kiaeian Moosavi, G. Bigot, F. Tacoli, and P. Zandigiacomo. 2016. Bunch-zone leaf removal of grapevines to prevent damage by *Lobesia botrana* (Lepidoptera Tortricidae) and grey mould. Bull Insectol. 69: 107–115.

Pease, C. E., J. F. López-Olguín, I. Pérez-Moreno, and V. Marco-Mancebón. 2016. Effects of kaolin on *Lobesia botrana* (Lepidoptera: Tortircidae) and its compatibility with the natural enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). J. Econ. Entomol. 109: 740–745.

Porcel, M., B. Cotes, and M. Campos. 2011. Biological and behavioral effects of kaolin particle film on larvae and adults of *Chrysoperla carnea* (Neuroptera: Chrysopidae). Biol. Control 59: 98–105.

Puterka, G. J., M. Reinke, D. Luvisi, M. A. Ciomperik, D. Bartels, L. Wendel, and D. M. Glenn. 2003. Particle film, Surround WP, effects on glassy-winged sharpshooter behavior and its utility as a barrier to sharpshooter infestations in grape. Online. Plant Health Progress doi: 10.1094/PHP-2003-0321-01-RS.

Reichert, S., T. Lockley. 1984. Spiders as biological control agents. Ann. Rev. Entomol. 29: 299–320.

Roberts, M. J. 1995. Spiders of Britain and northern Europe. Harper Collins Publishers, London, 383 pp.

Sackett, T. E., C. M. Buddle, and C. Vincent. 2007. Effects of kaolin on the composition of generalist predator assemblages and parasitism of *Choristoneura rosaceana* (Lep., Tortricidae) in apple orchards. J. Appl. Entomol. 131: 478–485.

Sánchez-Ramos, I., A. Marcotegui, S. Pascual, C. E. Fernández, G. Cobos, and M. González-Núñez. 2017. Compatibility of organic farming treatments against Monosteira unicostata with non-target arthropod fauna of almond trees canopy. Spanish Journal of Agricultural Research 15(2): e1004 (10 pp.).

Santos, R. L. dos, R. C. dos S. Neves, F. Colares, and J. B. Torres. 2013. Parasitoids of boll weevil *Anthonomus grandis* and resident predators in kaolin-treated cotton. Semina: Ciências agrárias. 34 (6): 3463–3474**.**

Scalercio, S., T. Belfiore, M. E. Noce, V. Vizzari, N. Iannotta, R. K. Prange, and S. D. Bishop. 2010. Impact of kaolin and *Beauveria bassiana* treatments against olive fly on the non-target arthropods of the olive ecosystem. Acta Hortic. 873: 329–336.

Settle, W. H., H. Ariawan, E. T. Astuti, W. Cahyana, A. L. Hakim, D. Hindayana, and A. S.

Sentenac, G. 2004. Les antagonistes naturels d'*Empoasca vitis* Göth en Bourgogne. Etude de faisabilité d'une lutte biologique par augmentation. Mondiaviti, Bordeaux, 1 et 2 décembre 2004: 25-37.

Sleezer, S., D. T. Johnson, B. Lewis, F. Goggin, C. Rothrock, and M. Savin. 2011. Foliar grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), seasonal biology, predictive model and management in the Ozarks region of the United States. Acta Hortic. 904: 151–156.

Smaili, M. C., L. E. Ghadraoui, F. Gaboun, R. Benkirane, and A. Blenzar. 2014. Impact of some alternative methods to chemical control in controlling aphids (Hemiptera: Sternorrhyncha) and their side effects on natural enemies on young Moroccan citrus groves. Phytoparasitica 42: 421–436.

Stam, P. A., and H. Elmosa. 1990. The role of predator and parasites in controlling populations of *Earias insulana*, *Heliothis armigera* and *Bemisia tabaci* on cotton in the Syrian Arab Republic. Entomophaga 35: 315–327.

Stelinski, L. L., K. S. Pelz-Stelinski, O. E. Liburd, and L. J. Gut. 2006. Control strategies for *Rhagoletis mendax* disrupt host-finding and ovipositional capability of its parasitic wasp, *Diachasma alloeum*. Biol. Control 36: 91–99.

Strapazzon, A., F. Pavan, and K. Cristoferi. 1990. Monitoraggio dei fitomizi della vite con trappole cromotropiche: risposta di *Drepanothrips reuteri* Uzel al colore giallo ed alle dimensioni delle trappole. Frustula entomologica 11: 9–18.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561–594

Tacoli, F., F. Pavan, E. Cargnus, E. Tilatti, A. Pozzebon, and P. Zandigiacomo. 2017a. Efficacy and mode of action of kaolin in the control of *Empoasca vitis* and *Zygina rhamni* (Hemiptera: Cicadellidae) in vineyards. J. Econ. Entomol. 110: 1164–1178.

Tamanini, L. 1988. Tabelle per la determinazione dei più comuni Eterotteri italiani. Heteroptera. Memorie della Società Entomologica Italiana, Genova. 67 (2): 359–471.

Trdan, S., L. Andjus. E. Raspudić, and M. Kač. (2005). Distribution of *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae) and its potential prey Thysanoptera species on different cultivated host plants. J.Pest Sci. 78: 217-226.

Tubajika, K. M., E. L. Civerolo, G. J. Puterka, J. M. Hashim, and D. A. Luvisi. 2007. The effects of kaolin, harpin, and imidacloprid on development of Pierce's disease in grape. Crop Prot. 26: 92–99.

Ulmer B. J., Lapointe S. L., Peña J. E., Duncan R. E. 2006. Toxicity of pesticides used in citrus to *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae), an egg parasitoid of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Fla. Entomol. 89: 10–19.

Unruh, T. R., A. L. Knight, J. Upton, D. M. Glenn, and G. J. Puterka. 2000. Particle films for suppression of the codling moth (Lepidoptera: Tortricidae) in apple and pear orchards. J. Econ. Entomol. 93: 737–743.

Valizadeh, H., Abbasipour, H., Farazmand, H., A. Askarianzadeh. 2013. Evaluation of kaolin application on oviposition control of the vine cicada, *Psalmocharias alhageos* in vineyards (Homoptera: Cicadidae). Entomologia Generalis 34: 279–286.

Villanueva, R.T. and J. F. Walgenbach. 2010. Impact of new pesticide chemistry on acarine communities in apple orchards. In: Sabelis, M. W., and J. Bruin, (Eds.), Trends in Acarology, Proceedings of the 12th International Congress, Springer 483–487.

Villéger, S., N. W. H. Mason, and D. Mouillot. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89: 2290–2301.

Vincent, C., G. Hallman, B. Panneton, F. Fleurat-Lessard. 2003. Management of agricultural insects with physical control method. Ann. Rev. Entomol. 48: 261–81.

Wilson, H., A. F. Miles, K. M. Daane, and M. A. Altieri. 2015. Vineyard proximity to riparian habitat influences Western grape leafhopper (*Erythroneura elegantula* Osborn) populations. Agric. Ecosyst. Environ. 211: 43–50.

Wood, M., and J. McBride. 2001. Scientists sharpen strategies to sabotage: glassy-winged sharpshooter. Agricultural Research Magazine 49 (11): 20–22.

Table 1. Spiders collected by beating tray in four vineyards along 2015-2016 in plots submitted or not to kaolin and bunch-zone leaf removal.

Taxon and prey capture method	**Vineyard A 2015**				**Vineyard B 2015**				**Vineyard A 2016**				**Vineyard C 2016**				**N.**
	Kaolin		**Leaf removal**		**Kaolin**		**Leaf removal**		**Kaolin**		**Leaf removal**		**Kaolin**		**Leaf removal**		**Tot**
	Control	**K**	**No**	**Yes**	**Control**	**K**	**No**	**Yes**	**Control**	**K**	**No**	**Yes**	**Control**	**K**	**No**	**Yes**	
N. total spiders	40	30	35	35	79	81	83	77	35	30	26	39	35	25	31	29	355
Web-builders																	
Araneidae (Web-orb)																	
Araneus sp. A juvenile	1	2	2	1	3	-	3	-	-	-	-	-	-	-	-	-	6
Araneus sp. B	-	-	-	-	1	1	2	-	2	1	1	2	-	3	1	2	8
Mangora acalypha (Walckenaer)	-	1	-	1	10	3	5	8	3	-	1	2	3	-	1	2	20
Nuctenea sp.	6	2	5	3	-	6	2	4	4	1	2	3	7	4	6	5	30
Zilla diodia (Walckenaer)	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1
Araneidae sp. C	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	1
Araneidae sp. D	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1
Araneidae juvenile	-	-	-	-	4	1	3	2	-	-	-	-	-	-	-	-	5
Linyphiidae (Web-sheet)																	
Erigoninae, sp. A	-	-	-	-	-	-	-	-	1	2	2	1	4	4	4	4	11
Erigoninae, sp. B	-	-	-	-	2	-	2	-	-	1	1	-	-	-	-	-	3
Neriene sp.	1	-	1	-	5	13	8	10	1	1	-	2	-	-	-	-	21
Pityohyphantes phrygianus (C.L. Koch)	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Theridiidae (Web-tangled)																	
Theridion sp.	1	2	1	2	3	6	5	4	-	1	-	1	-	-	-	-	13

Hunters																	
Thomisidae																	
Runcinia grammica (C.L. Koch)	-	-	-	-	1	4	2	3	5	8	4	9	-	1	-	1	19
Runcinia sp.	1	-	-	1	2	1	2	1	-	-	-	-	-	-	-	-	4
Synema globosum (Fabricius)	2	1	1	2	-	-	-	-	-	1	-	1	1	-	1	-	5
Thomisus sp.	3	-	2	1	1	3	3	1	-	1	1	-	-	-	-	-	8
Xysticus sp.	7	13	12	8	15	20	17	18	4	8	4	8	2	1	-	3	70
Thomisidae sp. A	1	3	-	4	4	2	1	5	-	-	-	-	-	-	-	-	10
Sparassidae																	
Micrommata virescens (Clerck)	-	-	-	-	4	-	4	-	1	1	1	1	-	-	-	-	6
Salticidae																	
Pseudeuophrys erratica (Walckenaer)	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	1
Pseudicius encarpatus (Walckenaer)	-	-	-	-					6	2	5	3	4	2	3	3	14
Salticus scenicus (Clerck)	-	-	-	-	1	-	1	-	-	-	-	-	3	2	4	1	6
Salticidae juvenile	3	2	4	1	5	4	4	5	4	-	2	2	1	2	3	-	21
Oxyopidae																	
Oxyopes lineatus Latreille	1	-	1	-	2	4	5	1	-	-	-	-	-	-	-	-	7
Oxyopes sp. (cfr. *heterophthalmus*)	11	1	3	9	9	4	3	10	2	-	1	1	8	5	7	6	40
Gnaphosidae																	
Dressodes sp.	-	-	-	-	4	8	7	5	1	2	1	2	-	1	1	-	16
Gnaphosidae sp. A	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	4
Miturgidae																	
Cheiracanthium sp.	-	-	-	-	-	-	-	-	1	-	-	1	1	-	-	1	2

Table 2. Spiders collected by beating tray in vineyard A and vineyard B in 2015 (mean±standard deviation). The results of Mixed ANOVA for kaolin and bunch-zone leaf removal factors are reported. When the number of spiders collected over the whole sampling period was less than 10, no statistical analyses was performed. Results on Shannon's diversity index (species richness) and of functional diversity (functional richness and dispersion) are reported.

	Kaolin			**Leaf-removal**		
	Control	**Kaolin**	**ANOVA**	**Without**	**With**	**ANOVA**
Vineyard A 2015						
Species Richness	0.93 ± 0.55	0.73 ± 0.63	F = 0.871; df = 1,12; P = 0.371	0.79 ± 0.64	0.88 ± 0.54	F = 0.181; df = 1,12; P = 0.678
Functional Richness	2.75 ± 1.60	2.13 ± 1.33	F = 2.099; df = 1,12; P = 0.175	2.50 ± 1.38	2.37 ± 1.56	F = 0.070; df = 1,12; P = 0.794
Functional Dispersion	0.20 ± 0.15	0.19 ± 0.17	F = 0.034; df = 1,12; P = 0.856	0.20 ± 0.15	0.19 ± 0.16	F = 0.006; df = 1,12; P = 0.936
Araneae	5.0 ± 2.7	3.8 ± 1.8	F = 2.308; df = 1,12; P = 0.155	4.4 ± 2.7	4.4 ± 2.0	F = 0.000; df = 1,12; P = 1.000
Web-builders	1.1 ± 1.2	1.0 ± 0.8	F = 0.034; df = 1,12; P = 0.857	1.3 ± 1.0	0.9 ± 1.0	F = 0.303; df = 1,12; P = 0.592
Araneidae	0.8 ± 1.2	0.4 ± 0.7	F = 0.509; df = 1,12; P = 0.489	0.6 ± 1.2	0.5 ± 0.8	F = 0.057; df = 1,12; P = 0.816
Theridiidae	0.3 ± 0.5	0.5 ± 0.5	-	0.4 ± 0.5	0.4 ± 0.5	-
Linyphiidae	0.1 ± 0.4	0.1 ± 0.4	-	0.3 ± 0.5	0.0 ± 0.0	-
Hunters	3.9 ± 2.0	2.8 ± 1.7	F = 3.522; df = 1,12; P = 0.085	3.1 ± 1.9	3.5 ± 2.0	F = 0.391; df = 1,12; P = 0.543
Thomisidae	1.8 ± 1.7	2.1 ± 1.7	F = 0.370; df = 1,12; P = 0.554	1.9 ± 1.8	2.0 ± 1.6	F = 0.041; df = 1,12; P = 0.843
Oxyopidae	1.5 ± 1.2	0.1 ± 0.4	F = 6.153; df = 1,12; P = 0.029	0.5 ± 0.8	1.1 ± 1.4	F = 1.271; df = 1,12; P = 0.282
Salticidae	0.4 ± 0.5	0.3 ± 0.7	-	0.5 ± 0.8	0.1 ± 0.4	-
Gnaphosidae	0.3 ± 0.5	0.3 ± 0.5	-	0.3 ± 0.5	0.3 ± 0.5	-
Vineyard B 2015						
Species Richness	1.60 ± 0.34	1.38 ± 0.37	F = 1.765; df = 1,12; P = 0.210	1.57 ± 0.33	1.41 ± 0.40	F = 0.893; df = 1,12; P = 0.364
Functional Richness	4.00 ± 1.60	4.25 ± 1.28	F = 0.169; df = 1,12; P = 0.688	4.37 ± 1.40	3.87 ± 1.45	F = 0.709; df = 1,12; P = 0.417
Functional Dispersion	0.28 ± 0.05	0.30 ± 0.06	F = 0.428; df = 1,12; P = 0.526	0.31 ± 0.04	0.27 ± 0.06	F = 1.82; df = 1,12; P = 0.203
Araneae	9.9 ± 4.2	10.1 ± 5.0	F = 0.029; df = 1,12; P = 0.868	10.4 ± 3.8	9.6 ± 5.2	F = 0.261; df = 1,12; P = 0.619
Web-builders	3.8 ± 2.8	3.9 ± 3.0	F = 0.014; df = 1,12; P = 0.907	4.1 ± 2.9	3.5 ± 2.8	F = 0.352; df = 1,12; P = 0.564
Araneidae	2.1 ± 1.6	1.5 ± 1.3	F = 0.591; df = 1,12; P = 0.457	1.9 ± 1.4	1.8 ± 1.6	F = 0.024; df = 1,12; P = 0.880
Linyphiidae	0.9 ± 0.8	1.6 ± 2.7	F = 0.591; df = 1,12; P = 0.457	1.3 ± 2.1	1.3 ± 2.1	F = 0.000; df = 1,12; P = 1.000
Theridiidae	0.8 ± 1.0	0.8 ± 1.2	F = 0.000; df = 1,12; P = 1.000	1.0 ± 1.3	0.5 ± 0.8	F = 0.667; df = 1,12; P = 0.430
Hunters	6.1 ± 3.5	6.3 ± 4.0	F = 0.009; df = 1,12; P = 0.926	6.3 ± 3.2	6.1 ± 4.2	F = 0.009; df = 1,12; P = 0.926
Thomisidae	2.9 ± 1.2	3.8 ± 3.0	F = 0.452; df = 1,12; P = 0.514	3.1 ± 2.0	3.5 ± 2.7	F = 0.083; df = 1,12; P = 0.778
Oxyopidae	1.4 ± 1.6	1.0 ± 0.8	F = 0.252; df = 1,12; P = 0.625	1.0 ± 0.9	1.4 ± 1.5	F = 0.252; df = 1,12; P = 0.625
Gnaphosidae	0.5 ± 0.9	1.0 ± 0.9	F = 0.558; df = 1,12; P = 0.469	0.9 ± 0.8	0.6 ± 1.1	F = 0.140; df = 1,12; P = 0.715
Salticidae	0.9 ± 1.1	0.5 ± 0.5	F = 0.325; df = 1,12; P = 0.579	0.8 ± 1.0	0.6 ± 0.7	F = 0.036; df = 1,12; P = 0.852
Sparassidae	0.5 ± 1.1	0.0 ± 0.0	-	0.5 ± 1.1	0.0 ± 0.0	-

Table 3. Spiders collected by beating tray in vineyard A and vineyard C in 2016 (mean±standard deviation). The results of Mixed ANOVA for kaolin and bunch-zone leaf removal factors are reported. When the number of spiders collected over the whole sampling period was less than 10, no statistical analyses was performed. Results on Shannon's diversity index (species richness) and of functional diversity (functional richness and dispersion) are reported.

	Kaolin			**Leaf-removal**		
	Control	**Kaolin**	**ANOVA**	**Without**	**With**	**ANOVA**
Vineyard A 2016						
Species Richness	1.11 ± 0.55	0.81 ± 0.42	F = 1.494; df = 1,12; P = 0.247	0.76 ± 0.54	1.15 ± 0.39	F = 2.720; df = 1,12; P = 0.127
Functional Richness	2.75 ± 1.03	2.00 ± 0.92	F = 2.333; df = 1,12; P = 0.155	2.13 ± 1.13	2.63 ± 0.92	F = 0.949; df = 1,12; P = 0.351
Functional Dispersion	0.23 ± 0.10	0.21 ± 0.13	F = 0.145; df = 1,12; P = 0.709	0.20 ± 0.13	0.23 ± 0.10	F = 0.321; df = 1,12; P = 0.582
Araneae	4.4 ± 2.0	3.8 ± 3.0	F = 0.311; df = 1,12; P = 0.587	3.3 ± 2.2	4.9 ± 2.6	F = 2.104; df = 1,12; P = 0.173
Web-builders	1.4 ± 1.2	0.9 ± 1.0	F = 0.889; df = 1,12; P = 0.364	0.9 ± 1.1	1.4 ± 1.1	F = 0.889; df = 1,12; P = 0.364
Araneidae	1.1 ± 0.8	0.3 ± 0.5	F = 7.000; df = 1,12; P = 0.021	0.5 ± 0.8	0.9 ± 0.8	F = 1.286; df = 1,12; P = 0.279
Linyphiidae	0.3 ± 0.5	0.5 ± 0.8	-	0.4 ± 0.5	0.4 ± 0.7	-
Theridiidae	0.0 ± 0.0	0.1 ± 0.4	-	0.0 ± 0.0	0.1 ± 0.4	-
Hunters	3.0 ± 2.1	2.9 ± 3.4	F = 0.014; df = 1,12; P = 0.908	2.4 ± 2.1	3.5 ± 3.3	F = 1.120; df = 1,12; P = 0.311
Thomisidae	1.1 ± 1.6	2.3 ± 3.0	F = 2.505; df = 1,12; P = 0.139	1.1 ± 1.2	2.3 ± 3.1	F = 2.505; df = 1,12; P = 0.139
Salticidae	1.3 ± 0.9	0.3 ± 0.5	F = 5.647; df = 1,12; P = 0.035	0.9 ± 1.1	0.6 ± 0.5	F = 0.063; df = 1,12; P = 0.563
Oxyopidae	0.3 ± 0.5	0.0 ± 0.0	-	0.1 ± 0.4	0.1 ± 0.4	-
Gnaphosidae	0.1 ± 0.4	0.3 ± 0.5	-	0.1 ± 0.4	0.3 ± 0.5	-
Sparassidae	0.1 ± 0.4	0.1 ± 0.4	-	0.1 ± 0.4	0.1 ± 0.4	-
Miturgidae	0.1 ± 0.4	0.0 ± 0.0	-	0.0 ± 0.0	0.1 ± 0.4	-
Vineyard C 2016						
Species Richness	0.96 ± 0.50	0.85 ± 0.57	F = 0.189; df = 1,12; P = 0.672	1.12 ± 0.23	0.69 ± 0.65	F = 3.416; df = 1,12; P = 0.09
Functional Richness	2.50 ± 0.93	2.50 ± 1.51	F = 0.000; df = 1,12; P = 1.000	2.88 ± 0.83	2.13 ± 1.46	F = 1.800; df = 1,12; P = 0.207
Functional Dispersion	0.21 ± 0.10	0.22 ± 0.14	F = 0.006; df = 1,12; P = 0.939	0.26 ± 0.05	0.17 ± 0.15	F = 2.568; df = 1,12; P = 0.137
Araneae	4.3 ± 2.0	3.1 ± 1.9	F = 2.793; df =1,12; P = 0.121	3.9 ± 0.8	3.5 ± 2.7	F = 0.310; df = 1,12; P = 0.588
Web-builders	1.8 ± 2.0	1.4 ± 1.1	F = 0.474; df = 1,12; P = 0.504	1.5 ± 1.2	1.6 ± 1.9	F = 0.053; df = 1,12; P = 0.822
Araneidae	1.3 ± 1.0	0.9 ± 0.8	F = 0.692; df = 1,12; P = 0.422	1.0 ± 0.8	1.1 ± 1.1	F = 0.077; df = 1,12; P = 0.786
Linyphiidae	0.5 ± 1.1	0.5 ± 0.5	-	0.5 ± 0.5	0.5 ± 1.1	-
Hunters	2.5 ± 1.4	1.8 ± 1.4	F = 1,862; df = 1,12; P = 0.197	2.4 ± 1.2	1.9 ± 1.6	F = 0.828; df = 1,12; P = 0.381
Salticidae	1.0 ± 1.3	0.8 ± 1.0	F = 0.176; df = 1,12; P = 0.682	1.3 ± 1.2	0.5 ± 1.1	F = 1,588; df = 1,12; P = 0.232
Oxyopidae	1.0 ± 0.9	0.6 ± 0.7	F = 1.080; df = 1,12; P = 0.319	0.9 ± 0.8	0.8 ± 0.9	F = 0.120; df = 1,12; P = 0.735
Thomisidae	0.4 ± 0.7	0.3 ± 0.5	-	0.1 ± 0.4	0.5 ± 0.8	-
Gnaphosidae	0.0 ± 0.0	0.1 ± 0.4	-	0.1 ± 0.4	0.0 ± 0.0	-
Miturgidae	0.1 ± 0.4	0.0 ± 0.0	-	0.0 ± 0.0	0.1 ± 0.4	-

Table 4. Number of individuals belonging to the different taxa found on yellow sticky traps over the sampling period 2015 in vineyard A and vineyard B (mean±standard deviation). The results of Mixed ANOVA for kaolin and bunch-zone leaf removal factors are reported.

	Kaolin			**Leaf-removal**		
	Control	**Kaolin**	**ANOVA**	**Without**	**With**	**ANOVA**
Vineyard A 2015						
Araneae	6.3 ± 3.0	5.0 ± 2.1	F = 1.257; df = 1,12; P = 0.284	5.0 ± 2.1	6.4 ± 3.0	F = 1,457; df = 1,12; P = 0.251
Web-builders	2.7 ± 1.6	2.0 ± 1.4	F = 1.317; df = 1,12; P = 0.274	1.9 ±1.3	2.8 ± 1.7	F = 2.252; df = 1,12; P = 0.159
Hunters	3.7 ± 2.8	3.0 ± 1.7	F = 0.719; df = 1,12; P = 0.413	3.1 ± 2.1	3.6 ± 2.4	F = 0.515; df = 1,12; P = 0.487
Thomisidae	2.0 ± 2.1	1.5 ± 1.7	F = 1.181; df = 1,12; P = 0.298	1.3 ± 1.5	2.2 ± 2.1	F = 3.827; df = 1,12; P = 0.074
Salticidae	1.4 ± 1.4	0.8 ± 1.0	F = 1.435; df = 1,12; P = 0.254	1.4 ± 1.5	0.8 ± 1.0	F = 1.435; df = 1,12; P = 0.254
Gnaphosidae	0.2 ± 0.5	0.1 ± 0.3	F = 0.176; df = 1,12; P = 0.682	0.1 ± 0.2	0.2 ± 0.5	F = 1.588; df = 1,12; P = 0.232
Oxyopidae	0.2 ± 0.4	0.4 ± 0.6	F = 0.574; df = 1,12; P = 0.463	0.2 ±0.4	0.4 ± 0.6	F = 0.574; df = 1,12; P = 0.463
Insecta						
Aeolothrips sp.	23.9 ± 12.3	28.4 ± 27.9	F = 0.444; df = 1,12; P = 0.518	15.2 ± 9.3	37.1 ± 24.5	F = 10.230; df = 1,12; P = 0.008
Scymninae	1.3 ± 1.7	2.6 ± 2.5	F = 8.380; df = 1,12; P = 0.013	1.6 ± 1.4	2.3 ± 2.8	F = 2.430; df = 1,12; P = 0.145
Coccinellidae (excl. Scymninae)	1.6 ± 1.5	1.1 ± 1.3	F = 1.827; df = 1,12; P = 0.201	1.1 ± 1.2	1.6 ± 1.5	F = 2.729; df = 1,12; P = 0.124
Orius sp.	1.1 ± 1.2	0.4 ± 0.6	F = 3.596; df = 1,12; P = 0.082	1.1 ± 1.1	0.5 ± 0.9	F = 1.723; df = 1,12; P = 0.214
C. carnea	0.9 ± 0.9	0.4 ± 0.8	F = 3.000; df = 1,12; P = 0.109	0.7 ± 0.9	0.6 ± 0.8	F = 0.120; df = 1,12; P = 0.735
Vineyard B 2015						
Araneae	7.8 ± 5.8	8.3 ± 4.5	F = 0.104; df = 1,12; P = 0.753	7.7 ± 5.1	8.4 ± 5.3	F = 0.193; df = 1,12; P = 0.668
Web-builders	2.9 ± 2.5	3.5 ± 2.0	F = 0.497; df = 1,12; P = 0.494	3.1 ± 2.2	3.3 ± 2.4	F = 0.073; df = 1,12; P = 0.791
Hunters	4.9 ± 3.8	4.8 ± 3.8	F = 0.008; df = 1,12; P = 0.931	4.6 ± 3.8	5.1 ± 3.8	F = 0.194; df = 1,12; P = 0.668
Thomisidae	2.5 ± 2.6	2.6 ± 2.9	F = 0.011; df = 1,12; P = 0.918	2.4 ± 2.4	2.7 ± 3.1	F = 0.099; df = 1,12; P = 0.758
Oxyopidae	0.8 ± 1.3	1.0 ± 1.3	F = 0.289; df = 1,12; P = 0.601	1.0 ± 1.3	0.9 ± 1.4	F = 0.072; df = 1,12; P = 0.793
Salticidae	1.2 ± 1.2	1.0 ± 1.3	F = 0.211; df = 1,12; P = 0.655	0.9 ± 1.3	1.3 ± 1.3	F = 0.842; df = 1,12; P = 0.377
Gnaphosidae	0.4 ± 0.6	0.2 ± 0.4	F = 1.714; df = 1,12; P = 0.215	0.3 ± 0.5	0.3 ± 0.6	F = 0.000; df = 1,12; P = 1.000
Insecta						
Aeolothrips sp.	22.3 ± 11.6	37.0 ± 20.9	F = 2.020; df = 1,12; P = 0.181	25.8 ± 16.5	33.5 ± 19.6	F = 0.543; df = 1,12; P = 0.475
Scymninae	5.9 ± 6.9	9.5 ± 8.7	F = 2.422; df = 1,12; P = 0.146	6.5 ± 7.6	8.9 ± 8.3	F = 1.061; df = 1,12; P = 0.323
Coccinellidae (excl. Scymninae)	2.7 ± 2.7	3.4 ± 2.6	F = 0.610; df = 1,12; P = 0.450	2.9 ± 2.9	3.2 ± 2.4	F = 0.090; df = 1,12; P = 0.769
C. carnea	1.1 ± 1.6	0.8 ± 1.2	F = 0.316; df = 1,12; P = 0.584	0.8 ± 1.2	1.1 ± 1.6	F = 0.620; df = 1,12; P = 0.446
Orius sp.	1.2 ± 1.7	0.4 ± 0.6	F = 5.070; df = 1,12; P = 0.044	0.9 ± 1.7	0.7 ± 0.9	F = 0.158; df = 1,3; P = 0.698

Chapter 9 - General conclusions

Based on the studies of the present PhD thesis (2015-2017), natural products and cultural practices can be a valid alternative to synthetic insecticides for a sustainable management of grapevine pests.

In organic vineyards, the applications of kaolin against *E. vitis* and *Z. rhamni* (Chapter 2) and that of citrus essential oil against *P. ficus* (Chapter 6) allow an effective control of these pests that otherwise could not be obtained. Because kaolin was as effective as pyrethrins in the management of *S. titanus,* it can be used by organic growers as an alternative or in addition to pyrethrins (Chapter 3).

In the control of *L. botrana*, kaolin (Chapter 4), sulphur dust (Chapter 5) and bunch-zone leaf removal (Chapters 4 and 5) are tools that combined can have the same efficacy of *B. thuringiensis* and can be integrated with other strategies such as mating disruption to obtain optimal control of this pest.

For kaolin and bunch-zone leaf removal, a minor impact on predatory phytoseiid mites (Chapter 7) and generalist predatory insects and spiders was observed (Chapter 8), even after their adoption for two consecutive years.

Since two-three applications of kaolin were able to control both grapevine leafhoppers and *L. botrana*, application timings that allow multi-target activities would be desirable. In the field trials of this PhD thesis, kaolin was applied two-three times from start of the second flight of *L. botrana* for the management of *E. vitis*, *Z. rhamni* and *L. botrana*, or three times, i.e. at the appearance of the first, second and third instar nymphs, for *S. titanus control*. In 2016, these application timings corresponded to the second and third decades of June for the *E. vitis* and *L. botrana* control and to last decade of May–first decade of June for the *S. titanus* control. Therefore, the applications against *S. titanus* were timed much earlier than those against the other two pests. Since, unpublished data collected in 2017 showed a lower efficacy of kaolin against *S. titanus* when applied at the appearance of the third instar nymphs, it is not possible to postpone the kaolin applications against this leafhopper. In the studies of this PhD thesis, the effects of kaolin against *E. vitis*, *Z. rhamni* and *L. borana,* when timed well before the start of *L. botrana* second flight, was not evaluated. However, based on *E. vitis* phenology and the high residual activity of kaolin against this leafhopper, we can suppose that the kaolin applications, timed against *S. titanus*, effectively control *E. vitis* first generation and prevent high population levels during the subsequent generation. On the contrary, for *L. botrana*, an effective control of the second generation by kaolin sprayings timed against *S. titanus* is unlikely because the kaolin coating of berries on which females will lay eggs is not possible, being berries still absent or too small at application timings. For this reason, it is hard to implement a strategy based on kaolin that considers only two-three applications for the simultaneous management of both *S. titanus* and *L. botrana*. On the other hand, an increased number of kaolin applications is not suggestable due to possible negative side effects on natural enemies. Therefore, growers should time kaolin applications on the basis of the relative population levels of the considered pests. A strategy that should be evaluated consists in performing two early kaolin applications for the control of *S. titanus*, at the appearance of first and second instar nymphs respectively, and to reserve a third application localized on the bunch zone at the beginning of the second flight of *L. botrana*.

The use of kaolin and bunch-zone leaf removal should be encouraged even due to their multiple positive effects compared to synthetic insecticides. Kaolin is able to reduce infestations of grapevine leafhoppers and *L. botrana* as well as to reduce sunburn damage of exposed berries and grapevines water stress with minor effects on grapes quality, while bunch-zone leaf removal is able to reduce *L. botrana* infestation, bunch rots and to improve the quality of grapes.