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A survey of mealybugs infesting South-Brazilian wine vineyards

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ABSTRACT

Mealybugs (Hemiptera: Pseudococcidae) are important pests of the grapevine *Vitis* spp. and are responsible for direct and indirect damage to production. The main mealybug species present in wine grapevine (*Vitis vinifera* L.) in Southern Brazil were identified and their incidence evaluated. Bunch-samples ($n = 50$) from 131 vineyards located in the Serra Gaúcha Region (RS) of Brazil were analyzed at harvest, and the occurrence of mealybugs in the roots was evaluated at the time of eradication of plants for replanting. Mealybugs were reared in laboratory until adulthood for species determination. The species *Dysmicoccus brevipes* (Cockerell, 1983), *Dysmicoccus* sp., *Planococcus citri* (Risso, 1813), *Pl. minor* (Maskell, 1897), *Pseudococcus viburni* (Signoret, 1875) and *Pseudococcus* sp. were identified in bunches. *Dysmicoccus* sp., *D. umbambae* Granara de Willink, 2009, *Pl. citri* and *Pseudococcus* sp. were found in the roots. *Pl. citri* (31.4%) and *Dysmicoccus* sp. (22.7%) were the most common species found in wine grape bunches in the Serra Gaúcha Region.

Introduction

Mealybugs (Hemiptera: Pseudococcidae) colonize all vegetative organs of vine plants but are not easily found due to their cryptic habits, living under the bark, on the abaxial leaf surface, inside the bunches, occasionally on berries, and, depending on soil texture, in the roots (Godfrey et al., 2005; Becerra et al., 2006). When located, mealybugs are readily recognized by the presence of powdered wax deposited on the surface of the body and, in some species, by the presence of white waxy filaments distributed in the margins (Cox and Pearce, 1983).

Direct and indirect damage results from nymphs and adults phloem ingestion, resulting in degeneration of plants, decreased vigor, early defoliation, reduced bunch quality and decreased organoleptic characteristics of the obtained wines. They may also reduce the marketability of table grapes because of the excretion of honeydew, which covers the leaves and clusters, and serves as a substrate for sooty molt growing (Cox, 1989; Godfrey et al., 2006; Bordeu et al., 2012; Daane et al., 2012). In addition, mealybugs are important vectors of viruses, such as the grapevine leafroll-associated Virus GLRaV (Closteroviridae), which is primarily transmitted in a semi-persistent way by different mealybug species (Naidu et al., 2014). GLRaV complex viruses cause loss of quality of the produced grapes, delayed maturation, plant degeneration and death (Golino et al., 2002; Fajardo et al., 2003; Golino and Almeida, 2008). Mealybugs have been reported to be the main vehicle for GLRaV-3 dispersion in vineyards in South Africa, Australia, USA, New Zealand, South America and Europe (Charles et al., 2006).

Due to the incidence of the virus, farmers are obliged to replace vineyards earlier than expected, resulting in high costs and losses (Golino et al., 2008). In Brazil, the main management strategy has been the plantation of new vineyards (Fajardo et al., 2003). However, even with the introduction of virus-free material, field observations have shown a steady increase in the incidence of viral diseases in vineyards, probably associated with the presence of insect vectors (Fajardo et al., 2003). Several mealybug species have been identified as pests in vineyards in major producing countries (Daane et al., 2012). In Brazil, the species *Dysmicoccus brevipes* (Cockerell, 1983), *Planococcus citri* (Risso, 1813), *Pseudococcus longispinus* (Targioni Tozzetti, 1767), *Ps. maritimus* (Ehrhorn, 1900) and *Ps. viburni* (Signoret, 1875) have been reported (Silva et al., 1968; Woerner, 1983; Foldi and Soria, 1989; Kuniyuki et al., 2005; Bertin et al., 2013). However, this information was obtained

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without an inventory of the species of mealybugs present in the main grape-producing areas intended for wine production. In table grapes (*Vitis labrusca* L.), 17 species of mealybug were identified in three major grape growing regions in Brazil, where *D. brevipes*, *Pl. citri* and *Ps. viburni* were the most frequently observed (Pacheco-da-Silva et al., 2014).

Knowledge of mealybug species present in the vineyards is the basis for the establishment of integrated pest management strategies. This study aimed to survey the mealybug species associated with wine vineyards grown in the main wine grape-production area of Brazil.

Material and methods

Mealybugs were collected in grape clusters during harvest in different wineries located in Bento Gonçalves City, RS, Brazil, during 2006–2007. Grapes from 131 vineyards were analyzed. Fifty bunches per batch were sampled, and all development stages of the mealybugs (nymphs and/or females with or without ovisacs) were collected. The insects were reared to adulthood in the Laboratory of Entomology of Embrapa Grape and Wine for species determination. Pumpkins *Cucurbita maxima* Duchesne were used as the food substrate, and mealybugs were kept in plastic cages closed with voile tissue at $25 \pm 1\,^{\circ}C$, a relative humidity of $70 \pm 10\%$ and a photoperiod of 14 h.

Farms located in Monte Belo and Bento Gonçalves, RS, Brazil were visited when the plants were uprooted at the time of vineyard renewal. Likewise, the scale insects found were kept in laboratory for subsequent identification. Permanent slides were mounted, and identification was performed according with the methodology and identification key described by Williams and Granara-de-Willink (1992). The species identifications were subsequently confirmed by Dr. MC Granara de Willink and Dr. E Prado. Vouchers for *Dysmicoccus umbambae* (Granara de Willink, 2009) are kept at Instituto y Fundación Miguel Lillo, Tucumán, Argentina, and the others species at Laboratory of Entomology of Embrapa Grape and Wine.

Results

Seven species of mealybugs were observed in wine bunches: *D. brevipes*, *Dysmicoccus* sp., *Pl. citri*, *Pl. minor* (Maskell, 1897), *Pseudococcus viburni* and *Pseudococcus* sp. Four species were observed in wine roots: *D. umbambae* Granara de Willink, 2009, *Dysmicoccus* sp., *Pl. citri* and *Pseudococcus* sp. (Fig. 1). Mealybugs were detected in 27% of the sampling sites. Only one species was found in the same cluster, always represented by few individuals. *Pl. citri* was found in 11% of sampled sites, followed by *Pl. minor*, *Dysmicoccus* sp. and *Pseudococcus* sp. found in 6% of the sampled sites each one, and in lesser incidence *D. brevipes* and *Ps. viburni* found in 3% of sampling sites.

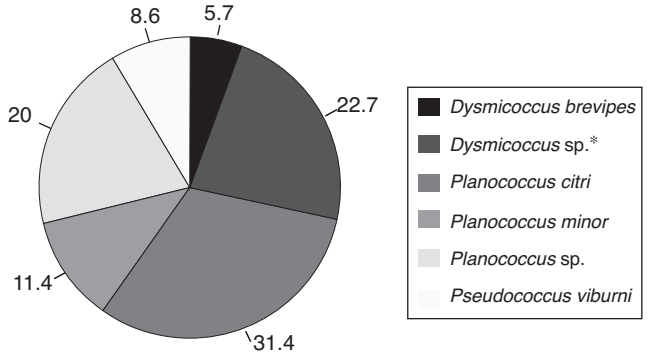

Fig. 1. Mealybug species (Hemiptera: Pseudococcidae) found in vineyards for the production of grapes for processing in the region of Bento Gonçalves (RS), Brazil and their percentage of occurrence. * Including *Dysmicoccus umbambae*.

Discussion

A high diversity of mealybug species has been observed in vine culture in Brazil (Pacheco-da-Silva et al., 2014), comparable to California (USA) (Daane et al., 2008, 2012). Mealybugs associated with grapes showed a lower diversity in several other grape-producing countries such as Argentina, Chile, Italy, New Zealand, Portugal, South Africa and Tunisia, which show an average of three species present in the culture (Borbón et al., 2004; Walton and Pringle, 2004; Mahfoudhi and Dhouibi, 2009; Walton et al., 2009; Bertin et al., 2010; Charles et al., 2010; Correa et al., 2012; Mansour et al., 2012; Maia, 2013). The high species diversity in vineyards difficult the deployment of efficient control programs due to their different life cycles and respond differently to chemical and biological management strategies.

The citrus mealybug *Pl. citri* was the predominant species found in the analyzed clusters (Fig. 1). It is a polyphagous species associated with approximately 250 plant species in 90 families (Ben-Dov et al., 2015). The citrus mealybug is commonly found in Brazil infesting different cultures. It is considered an important coffee *Coffea* sp. pest (Santa-Cecília et al., 2002, 2009) and is occasionally found in citrus *Citrus* sp. (Corrêa et al., 2005; Sousa et al., 2012) and vineyards (Morandi Filho et al., 2008; Daane et al., 2012). *Pl. citri* is also cited as a pest in vineyards in Spain, Italy and Tunisia (Mahfoudhi and Dhouibi, 2009; Cid et al., 2010; Daane et al., 2012), although it typically has not been reported in high densities in the main grape-producing countries.

In vineyards, the citrus mealybug is a vector of GLRaV-3 along with other species present in Brazil, such as *Ps. longispinus* and *Ps. viburni* (Cabaleiro and Segura, 1997; Kuniyuki et al., 2005). The dispersion of *Pl. citri* in vine plants occurs mainly during the first instar, when it moves up the trunk and branches and is rarely found in the green parts (Cid et al., 2010). Even in vineyards where symptoms of GLRaV-3 are well distributed, *Pl. citri* dispersion between plants is minimal, suggesting that this dispersion within the growing area occurs by other means, such as the wind, birds or the action of ants (Cid et al., 2010).

Planococcus minor is also an important coffee pest in Brazil (Santa-Cecília et al., 2007). In vineyards, this species was reported for the first time in South Brazil in 2008 (Morandi Filho, 2008).

The vine mealybug *Pl. ficus* (Signoret, 1869) is also often found in vineyards in addition to *Pl. citri* and *Pl. minor* (Daane et al., 2012). *Pl. citri* and *Pl. ficus* are difficult to differentiate because they have great morphological and ecological similarity (Danzig and Gavrilov, 2010). *Pl. ficus* is distributed worldwide; it is economically important in many vine-producing regions such as California (Daane et al., 2008), South Africa (Walton and Pringle, 2005), Argentina (Borbón et al., 2004) and Uruguay (Granara de Willink et al., 1997). This species was not found in this study, although Foldi and Kozár (2006) reported this species from Veranópolis City, RS, located close to our survey region (26 km).

The pineapple mealybug, *D. brevipes* is a polyphagous species, widely distributed in South America (Granara de Willink, 2009) and it is considered an important pest in pineapple crops *Ananas comosus* (L.) Merrill. Infestations occur mainly in the roots and lower parts of the plant (Sether et al., 1998). *D. brevipes* is a transmitter of pineapple mealybug wilt-associated virus PMWaV (Sether et al., 1998), however, there are no reports of virus transmission in vine. In Brazil, *D. brevipes* is considered a key pest due to its frequency and wide distribution; nevertheless it is regarded as a secondary pest in other countries (Daane et al., 2012). Bertin et al. (2013) studied the bioecology of the species in *Vitis* spp., noting that it reproduces

by thelytokous parthenogenesis and can complete its development feeding on leaves and/or roots.

Others species of *Dysmicoccus* Ferris were frequently observed in this study. Many undescribed species of this genus have been collected in Brazil on different hosts and it appears to present a wide diversity in the region. Among these, *D. umbambae* was recently described (Granara de Willink, 2009), including specimens collected on grape roots in Monte Belo City. The presence of mealybugs on the roots increases the difficulty of handling the viruses in the vineyard, once when the plants are renewed, the roots remaining in the soil serve as source of inoculum for the virus and mealybugs (Bell et al., 2009), that are capable to develop within remaining roots up to 24 months after uprooting the plants (Walton and Pringle, 2004).

The obscure mealybug, *Ps. viburni* is also a polyphagous species and has a cosmopolitan distribution occurring in approximately 50 countries (Ben-Dov et al., 2015). Its morphology is highly variable and probably more than one species are identified as *Ps. viburni* in South America (Charles, 2011; Malausa et al., 2011; Pacheco-da-Silva et al., 2014). *P. viburni* can be found in the roots and shoots of the host plant (González, 2003b). During winter it is found in protected locations in the plant, such as bark, and migrates to the shoots during the vegetative and reproductive period, when it occasionally infests bunches (González, 2003a). In Serra Gaúcha Region, *Ps. viburni* has been found mainly in plastic covered table grapes, most likely due to increased temperatures that favor its development (Bertin, 2011).

Misidentifications of *Ps. maritimus* or *Ps. viburni* are common due to the complexity of identifying the mealybugs belonging to the *Ps. maritimus* Complex and the high number of probably undescribed species. Specimens of *Pseudococcus* that could not be assigned to a specific name corresponding to 20% of the collected specimens (Fig. 1). Polymorphism is common inside this genus and molecular data is not available for all species. There is a high diversity of specie of *Pseudococcus* in South America. At least five groups of mealybugs collected in Brazil belonging to this genus have been identified through molecular and morphological analysis as species closely related (aff. or near) and determined only to genus level (*Pseudococcus* sp.). They showed no similarity to species whose sequences are deposited in the international database (GenBank) and had differences in the morphological specific characters (Pacheco-da-Silva et al., 2014). New species belonging to the *Ps. maritimus* Complex were recently described in South America found in Chilean vineyards, *Ps. cribata* González, 2011 and *Ps. meridionalis* Prado, 2011 (Correa et al., 2011; González, 2011).

Pl. ficus and the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green, 1908) are considered primary pests in vineyards (Daane et al., 2012), but were not found in this study. *M. hirsutus* is an invasive species recently recorded in Brazil damaging different crops (Culik et al., 2013; Marsaro Júnior et al., 2013).

Ants *Linepithema micans* (Forel, 1908) were found associated to mealybugs, species previously reported in association with cochineal *Eurhizococcus brasiliensis* (Wille, 1922) in Brazil (Nondillo et al., 2013). Within the genus *Linepithema* Mayr, the species *L. humile* (Mayr, 1868) is best known associated to scale insects feeding on honeydew (Daane et al., 2007).

Although the scale insects found in clusters do not cause direct damage to grapes used for wine, the presence of different mealybug species in approximately 30% of the vineyards is considered a treat for virus infection. Cabaleiro and Segura (2006) observed that even with low populations of mealybugs at early planting, approximately 100% of the plants are infected with GLRaV-3 after 15 years.

In this study, seven species of mealybugs were found to be associated with the wine grapes. The species *Pl. citri* and *Dysmicoccus* sp. were the most frequently observed, followed by *Pseudococcus* sp., *Pl. minor, Ps. viburni* and *D. brevipes*. The species *Dysmicoccus* sp., *Dysmicoccus umbambae, Pl. citri* and *Pseudococcus* sp. were also found on roots.

Conflicts of interest

The authors declare no conflicts of interest.

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