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Identification of *Planococcus ficus* and *Planococcus citri* (Hemiptera: Pseudococcidae) by PCR-RFLP of COI gene

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The vine mealybug, *Planococcus ficus* (Signoret), and the citrus mealybug, *Planococcus citri* (Risso), are currently the most economically important pseudococcids in vineyards in Italy (Dalla Montà *et al.*, 2001). *Pl. ficus* is also considered to be a key pest in many other countries (Ben-Dov, 1994). The two species cause severe damage to the host plant, and significantly reduce the crop due to the production of honeydew, which allows the growth of sooty molds, and to their potential as virus vectors. The vine mealybug, in particular, is known to transmit grapevine leafroll and corky-bark diseases (Engelbrecht & Kasdorf, 1990; Tanne *et al*., 1989).

Populations of these two sibling species may coexist apparently without mutual interference (Rotundo & Tremblay, 1982), but some differences occur on their relationships with parasitoids. For instance, *Pl. citri* is successfully controlled by *Leptomastix dactylopii* Howard (Hymenoptera Encyrtidae) but this parasitic wasp is ineffective in controlling *Pl. ficus*, whereas *Anagyrus pseudococci* (Girault) (Hymenoptera Encyrtidae) can be employed in biological control programs for both mealybugs (Abdelkhalek *et al*., 1998; Mendel *et al*., 1999). Therefore, a well-timed identification of pests is very important for choosing the most suitable biocontrol agents for IPM.

The two scale insects are similar morphologically and their taxonomic separation is based on the distribution and presence of multilocular pores and tubular ducts on the adult females and these are difficult to detect (Cox & Ben-Dov, 1986; Williams & Granara de Willink, 1992). Therefore the discrimination between these two species needs a simpler and more reliable method, such as by molecular analyses.

In this study, restriction fragment length polymorphism (RFLP) analysis of regions of mitochondrial gene cytochrome oxidase subunit I (COI) was used to distinguish populations of these two mealybugs associated with grapevine.

Analysis methods. Twenty-six samples of *Planococcus* populations were collected between 2005-2007 in different Mediterranean localities (Table 1). From each sample, ten adult females were mounted on slides for morphological analysis and the identification was confirmed by comparison with slide-mounted vouchers deposited at the Di.S.Te.F. Scale Insects Collection. In addition, a total of 128 specimens, females and immature stages, were individually subjected to DNA extraction according to the protocol described by Walsh *et al.* (1991) and modified by De Barro and Driver (1997). Total DNA extracted by mealybug populations was amplified by PCR, following Bosco *et al*. (2006), with some changes. Samples of *Pseudococcus longispinus* Targioni Tozzetti were used as control species.

The PCR reaction was conducted using the primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon *et al*., 1994). All PCR reactions were performed in 20 µl volumes with 0.85X of FailSafe™ PCR 2X premix F (Epicentre technologies), 0.5 µM of each primer 10 µM, 1.5 U of Taq polymerase 5U (Invitrogen) and 2 µl of DNA template. The cycling conditions were: 96°C for 5 min, then 35 cycles at 96°C for 45 s, 45°C for 1 min, 72°C for 1 min, followed by final cycle at 72°C for 10 min. Reactions and cycling conditions were conducted in an automated thermal cycler [GeneAmp® PCR System 2700 (Applied Biosystems)].

Some amplified COI fragments of *Pl. ficus*, *Pl. citri* and *Ps. longispinus* were sent to a sequencing service (BMR Genomics). The obtained sequences showed high similarity when compared with those of *Pl. ficus*, *Pl. citri* and *Ps. longispinus* deposited at GenBank. The sequences were then analyzed for restriction reception sites using the program NEBcutter V2.0 (Vincze *et al*., 2003). The chosen restriction enzymes were: *Bsp*PI, *Hinf*I, *Hph*I, *Mbo*II, *Ssp*I. Amplified DNA of all specimens was then cut with these five enzymes to produce multiple fragments which were used as

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