A SURVEY OF SCALE INSECTS (HEMIPTERA: COCCOIDEA) AND TENDING ANTS IN TUNISIAN VINEYARDS

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Abstract: In 2009, two mealybug species *Planococcus ficus* (Signoret) and *Planococcus citri* (Risso) and an armoured scale species, *Hemiberlesia lataniae* (Signoret) were recorded throughout Tunisian table-grape vineyards. *P. ficus* was by far the most widespread scale insect species within the investigated grape growing areas and was also recorded on fig trees. Among 31 inspected vineyards, 8 were non-infested, 16 slightly to moderately infested, and 7 highly infested by mealybugs. Therefore, the latter are principal pests in vineyards and should be sustainably managed to avoid major crop losses on grapevine. Six ant species were found in Tunisian mealybug-infested table-grape vineyards. They are *Tetramorium semilaeve* André, *Plagiolepis schmitzii* (Forel), *Tapinoma nigerrimum* (Nylander), *Crematogaster schmidti* (Mayr), *Pheidole pallidula* (Nylander) and *Pheidole teneriffana* Forel. The four latter species were reported tending mealybugs on grapevines. *T. nigerrimum* was found to be the ant most associated with the pseudococcid mealybugs. Our findings are new data for Tunisian grape-growing areas and will contribute to enhance Integrated Pest Management programs in vineyards.

Key words: mealybugs, pest status, biological control, Integrated Pest Management, *Vitis vinifera*

INTRODUCTION

In vineyards, scale insects are considered serious pests and can often be vectors of grapevine viruses (Sforza *et al.* 2003; Mahfoudhi 2009; Tsai *et al.* 2010). Throughout the world, the scale insects occurring in grape-growing areas mostly belong to the families: Diaspididae, Coccidae, Margarodidae and Pseudococcidae. Among these families, Pseudococcidae (mealybugs) are considered the most important scale insect group attacking the grapevines in several cultivation areas of the world. Pseudococcidae are found in such places as Italy (Dalla Montá *et al.* 2001; Buonocore *et al.* 2008), Portugal (Godinho and Franco 2001), Spain (Cid *et al.* 2010), the USA - especially in California (Daane *et al.* 2004) and Oregon (Skinkis *et al.* 2009), South Africa (Walton *et al.* 2009) and in New Zealand (Lo *et al.* 2009).

Biological control using species-specific encyrtid parasitoids is considered a key component of an integrated mealybug-pest management program. Such a program, for example, is used in California vineyards (Daane *et al.* 2002, 2006). However, since honeydew excreted by mealybugs is the primary food source for ants, the latter may thereby disturb the activity of natural enemies. The ants tend and prevent enemies from attacking the mealybugs on grapevine, in return for honeydew. This association creates a temporal refuge for mealybugs (Gutierrez *et al.* 2008) and decreases parasitism rates on these pests in vineyards (Daane *et al.* 2002, 2007; Tollerup *et al.* 2007). In the Cap-Bon (Tunisia) vineyards, the ant-mealybug association was often observed and probably disturbed parasitoid activity towards mealybugs (Mansour *et al.* 2009). For this reason, it is important to identify which ant species are associated with mealybugs in vineyards in order to determine the suitable control tools needed to reduce ant populations, and accordingly to optimize the effectiveness of parasitoids on mealybugs.

In Tunisia, two mealybug species, *Planococcus ficus* (Signoret) and *Planococcus citri* (Risso) were recently studied on grapevines, precisely in the Cap-Bon Region (North-East) (Mansour 2008; Mahfoudhi and Dhouibi 2009; Mansour *et al.* 2009) and in both the Borj Amri and Bousalem areas (Mahfoudhi and Dhouibi 2009). In these vineyards, *P. citri* is thought to be the dominant mealybug species (Mahfoudhi and Dhouibi 2009), but no related quantitative data was provided. Two other soft scales, *Ceroplastes rusci* L. and *Parthenolecanium* sp. were sporadically found on grapevines in Tunisia (Mahfoudhi 2009). The complete list

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of the scale insect fauna and both the predominance and the pest status of the present species, mainly of mealybugs, were until recently, unknown in the Tunisian grapevine areas, especially in Mornag, the most important grape-growing region. Additionally, both the composition and the status of mealybug-tending ant fauna are unknown throughout Tunisian vineyards and are poorly described within Mediterranean grape-growing areas. The present study was carried out during the spring-summer of 2009, in several Tunisian table-grape producing areas. The aim was to provide information about the scale insect fauna, especially about the mealybug tending ant relationship on grapevines. We also intended to implement a basic step for Integrated Pest Management (IPM) perfecting.

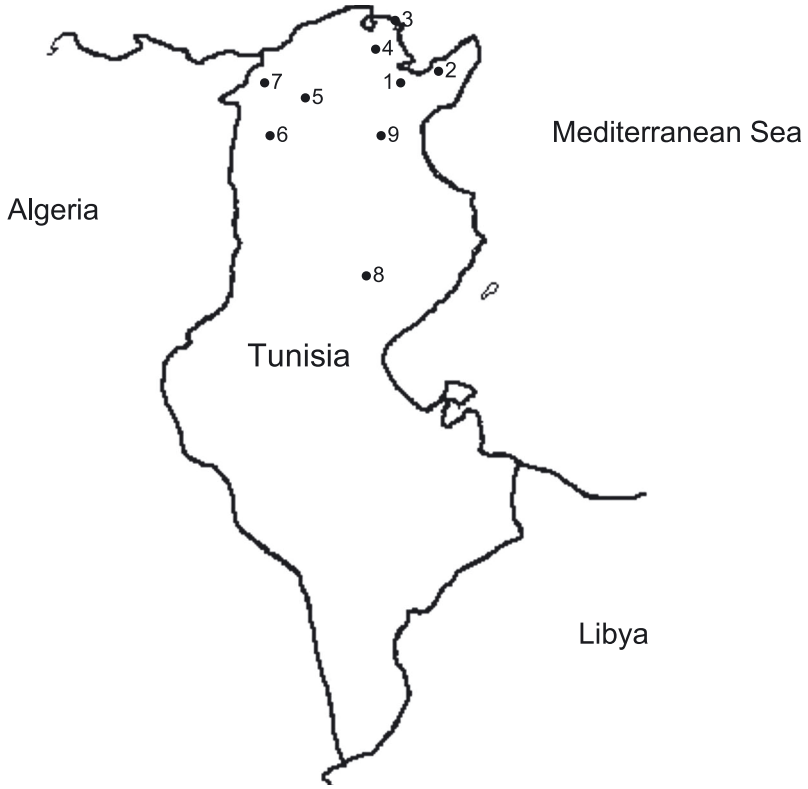

Fig. 1. Geographic distribution of the investigated table-grape growing sites in Tunisia, where: (1) Mornag; (2) Cap-Bon; (3) Rafraf; (4) Sidi Thabet; (5) Testour; (6) Le Kef; (7) Bousalem; (8) Regueb; (9) Nadhour

MATERIALS AND METHODS

Study period and selected sites

The survey of the scale insect fauna and ants, and the assessment of the mealybug pest status on grapevine (*Vitis vinifera* L.) were performed during the same period; from May to August 2009, and within the same vineyards. The study period corresponds to the most intense scale insect activity in Tunisian vineyards. This period is likely the most propitious for determining mealybug-tending ant fauna on grapevine. A total number of 31 mature table-grape vineyards belonging to 9 major and minor grape-growing sites were randomly selected, without prior knowledge of their mealybug infestation status (Fig. 1,Table 1). The investigated vineyards ranged from 3 to 10 ha and more than 80% of which were planted with the "Muscat of Italy" variety, led in pergola and drip irrigated. Vineyards received one early-June application of methidathion or chlorpyriphos-ethyl (organophosphate insecticides). These are the most commonly used insecticides for mealybug control in Tunisian vineyards, but they had limited efficacy on these insects.

Table 1. Details on the studied Tunisian grape-growing sites

Grape-growing site	Geographic coordinates	No. of investigated vineyards
Mornag	36°41′7.04″N, 10°17′17.01″E	8
Rafraf	37°11′50.53″N, 10° 10′43.43″E	5
Cap-Bon	36°47′24″N, 10°37′48″E	4
Testour	36°33′17.38″N, 9°26′41.49″E	2
Regueb	34°51′36″N, 9°46′48″E	3
Le Kef	36°10′45.38″N, 8°42′50.9″E	3
Bousalem	36°36′0″N, 8°41′60″E	2
Sidi Thabet	36°54′50″N, 10°2′10″E	1
Nadhour	36°7′15.65″N, 10°3′30.04″E	3

Survey of scale insect fauna

In an attempt to acquire more information about the scale insect fauna occurring on table-grapes, several field surveys were carried out in different Tunisian grape-growing sites. On each sampling date, at least 30 grapevines from different parts of each vineyard were randomly selected. The areas beneath the trunk bark, as well as along cordon, canes, leaves and clusters were carefully investigated. From each grapevine, whenever possible, adult female scale insects belonging to the same family were randomly collected. Then these females were placed in vials containing 70% ethanol until they could be identified by genus and species. Additionally, further mealybug specimens were collected from some fig (*Ficus carica* L.) trees located in the vicinity of a sampled vineyard belonging to the Mornag site. Microscope slides were prepared following the method described by Williams and Watson (1988a, b). The mealybug species were identified according to the keys of Tranfaglia and Tremblay (1982) and Williams and Granara de Willink (1992). The armoured scale species were identified based on the slide-mounted characters of the Diaspidid adult females (Balachowsky 1948; Williams and Watson 1988a; Miller and Davidson 2005).

Evaluation of mealybug pest status on grapevines

In order to assess the current pest status of mealybugs on grapevine, an infestation rate by mealybugs was evaluated. Vineyards belonging to the same site were investigated on the same date. Sampling consisted of a 5-minute visual search of mealybug mobile developmental stages (Daane *et al.* 2006; Mansour *et al.* 2010) and of a visual investigation detecting the presence/absence of honeydew and sooty mold fungi on each vine (Walton *et al.* 2006). A category assessing the degree of infestation by these pests was allotted for each grapevine. The main infestation category for all inspected grapevines (40 grapevines/vineyard) was then attributed to the vineyard. The categorized vineyards were labeled as having: no, slight to moderate, or high mealybug infestation (Table 2).

Table 2. Categories of the inspected vineyards assessed by visual estimation and a 5-minute search for mealybugs per grapevine

Category	Degree of infestation	Characteristics
A	no infestation	– absence of mealybugs – honeydew and sooty mold fungi absent on grapevine sections
B	slight to moderate infestation	– number < 50 mealybug mobile developmental stage – honeydew and sooty mold fungi present but not widely widespread on grapevine sections
C	high infestation	– number > 50 (mainly > 100) mealybug mobile developmental stage – honeydew and sooty mold fungi present and widespread on grapevine sections

Survey of ants

All vineyards, except three which were highly infested by mealybugs (two at the Mornag site and one at the Cap-Bon site), were sampled once. In each vineyard, at least 20 mealybug-infested grapevines were carefully investigated for the presence of ants. On each grapevine, a 2-minute visual search (Daane *et al.* 2002) was performed. This meant the distribution level of ants moving up and down the trunk and along the others grapevine sections (cordon, canes, leaves, clusters) was visually estimated (ants widely distributed or not). Whenever possible, specimens belonging to each reported ant genus were collected and preserved in tubes containing 70% ethanol. The presence/absence of a close mealybug-ant association on grapevines was noted for each investigated vineyard and for each related ant genus. Ant species were identified under binocular microscope according to the keys of Agosti and Collingwood (1987).

RESULTS AND DISCUSSION

Scale insect fauna and mealybug pest status

Three scale insect species, two mealybugs *P. ficus* and *P. citri*, and one armoured scale *Hemiberlesia lataniae* (Signoret) were found attacking grapevines. The Vine Mealybug (VM) *P. ficus* occurred in all inspected vineyards and was by far the most abundant scale insect species. The Citrus Mealybug (CM) and the Latania Scale *H. lataniae* were rare and were found in a single vineyard each, located in Regueb and in Takelsa (Cap-Bon), respectively (Table 3).

The VM *P. ficus* was reported attacking grapevine on a worldwide scale. The presence of *P. citri* and *H. lataniae* on grapevines, on the other hand, is likely related to the occurrence of some specific ecological conditions. In Italian (Dalla Montá *et al.* 2001; Buonocore *et al.* 2008) and French (Sforza 2000) vineyards, the mealybug species *P. ficus* and *P. citri* have already been found attacking grapevines. *P. ficus* was found to be the most abundant and almost the only mealybug species occurring in the investigated Tunisian vineyards. This new data for Tunisia is similar to other results reported in Italy (Dalla Montá *et al.* 2001), in Turkey (Kaydan *et al.* 2004), in South Africa (Walton *et al.* 2009), in California (Daane *et al.* 2004, 2006) and in Argentina (Trjapitzin and Trjapitzin 1999). Whereas in Spain, the CM *P. citri* is considered the main grapevine pest in vineyards (Cid *et al.* 2010). The Diaspidid *H. lataniae* was already found on other hosts in Tunisia (Balachowsky 1948). Based on the categorization of Miller and Davidson (1990), a severe infestation (large colonies of 11–100) of this species was found attacking trunks of adjacent grapevines located in Takelsa (Cap-Bon area). Miller and Davidson (1990) consider the Latania Scale to be a serious pest in many areas of the world. Argyriou (1990) stated that this species was recorded as a pest on olives in Egypt, Turkey, Israel, California and

Table 3. Number and distribution of the identified scale insect specimens on grapevines in Tunisia

Grape-growing site	Collected specimens		
	Pseudococcidae		Diaspididae
	P. ficus	*P. citri*	*H. lataniae*
Mornag	108	–	–
Cap-Bon	39	–	10
Rafraf	34	–	–
Le Kef	33	–	–
Testour	27	–	–
Bousalem	22	–	–
Sidi Thabet	22	–	–
Regueb	19	2	–
Total	304	2	10

Table 4. Level of infestation by mealybugs in investigated vineyards

Prospected site	No. of inspected	Categories		
		A	B	C
Mornag	8	–	6	2
Cap-Bon	4	1	1	2
Bousalem	2	–	2	–
Testour	2	–	1	1
Regueb	3	–	3	–
Le Kef	3	1	1	1
Rafraf	5	3	2	–
Sidi Thabet	1	–	–	1
Nadhour	3	3		
Total	31	8	16	7

A – no infestation; B – slight to moderate infestation; C – high infestation

Chile. In addition, *H. lataniae* is considered a pest of kiwifruit in New Zealand (Hill and Holmes 2009). In our study, all mealybugs collected from fig trees were identified as *P. ficus*. The occurrence of *P. ficus* on fig trees close to a mealybug-infested vineyard provides evidence that inspections and control of mealybugs on fig trees located near grape-growing areas should also be considered and incorporated in future mealybug management programs. The aim should be to avoid possible spread of high VM populations from fig to grapevine. In contrast, *F. carica* can play an important role, as a secondary host plant for *P. ficus*, in maintaining continuous spread of VM natural enemies from fig trees to vineyards. According to Walton *et al.* (2009), in South Africa, *P. ficus* has only been collected from *V. vinifera* (Vitaceae) and *F. carica* (Moraceae).

Among the 31 inspected vineyards, 8 were non-infested, 16 slightly to moderately infested and 7 highly infested by mealybugs (*P. ficus*). This means that *P. ficus* was present in more than 70% of the investigated vineyards (Table 4). These outcomes clearly revealed that mealybugs, namely *P. ficus,* are currently principal pests in Tunisian vineyards and should be sustainably managed. In Portugal, mealybugs were considered key pests in 14.5%, occasional pests in 61.8% and potential pests in 23.6% of the cases, based on several questionnaires in major grapevine regions (Godinho and Franco 2001).

Mealybug-tending ants

Six ant species (Hymenoptera: Formicidae), belonging to 3 sub-families, were found on grapevines in 19 mealybug-infested vineyards. These are: the Dolichoderinae *Tapinoma nigerrimum* (Nylander), the Myrmicinae *Pheidole pallidula* (Nylander), *Pheidole teneriffana* Forel, *Crematogaster schmidti* (Mayr) and *Tetramorium semilaeve* André, and the Formicinae *Plagiolepis schmitzii* (Forel). Four species, *T. nigerrimum*, *C. schmidti*, *Ph. pallidula* and *Ph. teneriffana* were observed in related vineyards tending mealybugs on grapevines. *T. nigerrimum* was found in almost all inspected vineyards, except in Sidi Thabet and in Rafraf. *T. semilaeve* and *C. schmidti* occurred in three grape growing sites, whereas *Ph. pallidula*, *Ph. teneriffana* and *P. schmitzii* were recorded on grapevines of only one grape growing site each (Table 5).

In South African vineyards, *Crematogaster* sp. and *Pheidole* sp. were recorded tending the vine mealybug *P. ficus* (Addison and Samways 2000). *T. nigerrimum* was not previously observed in close association with *P. ficus* in other vineyards around the world. It was, however, recorded tending *P. citri* in Portuguese citrus orchards in which *Linepithema humile* (Mayr) was found to be the ant species most frequently associated with the CM (Zina 2008). Moreover, Di Martino (1957) indicated that in Italian citrus orchards, *T. nigerrimum* feed on honeydew excreted by scale insects and accordingly protect these pests

Table 5. Ant species recorded in Tunisian vineyards

Site	Identified ant species					
	**T. nigerrimum*	*T. semilaeve*	**C. schmidti*	**Ph. pallidula*	**Ph. teneriffana*	*P. schmitzii*
Mornag	++	+	–	–	–	–
Cap-Bon	++	–	+	+	+	–
Bousalem	++	–	–	–	–	–
Testour	+	+	–	–	–	–
Le Kef	++	–	–	–	–	–
Sidi Thabet	–	+	+	–	–	+
Regueb	+	–	–	–	–	–
Rafraf	–	–	+	–	–	–

*species observed tending mealybugs; – species absent on grapevines; + species present but not widely distributed on grapevines; ++ species present and widely distributed on grapevines

from their natural enemies by disrupting their parasitic and/or predatory activity. *T. nigerrimum* was reported as the primary ant species (present in more than 80% of the mealybug-infested vineyards) associated with mealybugs in Tunisian vineyards. This finding leads us to believe, that the occurrence of mealybugs in Tunisian table-grape growing areas might be closely associated with the presence of this ant species. Ants have long been associated with the interference of biological control of scale insects. Therefore, a study on the interactions of *T. nigerriimum* with both *P. ficus* and their most common parasitoid(s) would be very useful to understand whether or not this ant species could affect the parasitization potential on *P. ficus*. A recent laboratory experiment study revealed the two ant species *Crematogaster peringueyi* Emery and *L. humile,* caused significant mortality of both parasitoid *Anagyrus* sp. and *Coccidoxenoides perminutus* (Timberlake). This shows that ants should be controlled prior to release of parasitoids to suppress populations of ant-tended Hemiptera in vineyards (Mgocheki and Addison 2009).

CONCLUSION

1. In 2009, among 31 inspected Tunisian table-grape vineyards, 8 were non-infested, 16 were slightly to moderately infested, and 7 were highly infested by mealybugs.
2. Among scale insects the most common and abundant was *P. ficus*. *P. citri* and *H. lataniae* were rarely recorded.
3. Four ant species were associated with mealybugs, the most common was *T. nigerrimum*. The least observed were *P. schmitzii*, *Ph. pallidula* and *Ph. teneriffana*.

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POLISH SUMMARY

BADANIA NAD TARCZNIKAMI (HEMIPTERA: COCCOIDEA) ORAZ ICH RELACJAMI Z MRÓWKAMI W TUNEZYJSKICH WINNICACH

W 2009 roku, w tunezyjskich winnicach wina deserowego stwierdzono występowanie dwóch gatunków wełnowca: *Planococcus ficus* (Signoret) i *Planococcus Citri* (Risso) oraz tarcznika *Himberlesia lataniae* (Signoret). Gatunek *P. ficus* okazał się dalece bardziej rozprzestrzenionym gatunkiem owadów tarcznikowatych na terenach objętych uprawą winorośli, a jego obecność stwierdzano również na drzewach figowych. Spośród 31 badanych winnic, w ośmiu nie stwierdzono występowania *P. ficus*, 16 winnic było opanowanych w stopniu słabym do umiarkowanego, a pozostałe winnice zostały silnie opanowane przez szkodnika. Z tego względu gatunek ten jest zaliczany do głównych szkodników winnic i powinien być obiektem zrównoważonego zwalczania w celu zapobiegania poważnym stratom w uprawach winorośli. Wyniki prowadzonych badań wykazały również, występowanie 6 gatunków mrówki w tunezyjskich winnicach wina deserowego, opanowanych przez wełnowce. Należały do nich następujące gatunki: *Tetramorium semilaeve* (André), *Pllagiolepis schmittzii* (Forel), *Tapinoma nigerrimum* (Nylander), *Crematogaster schmidti* (Mayr), *Pheidole pallidula* (Nylander) oraz *Pheihole teneriffana* (Forel). Spośród wymienionych gatunków cztery ostatnie uznano jako owady pełniące rolę opiekuńczą w stosunku do wełnowców na plantacja winorośli. Gatunek *T. nigerrinum* był najsilniej związany a tarcznikami należącymi do Pseudococcidae. Praca zawiera nowe doniesienia dotyczące terenów objętych uprawą winorośli w Tunezji, które stanowią przyczynek do ulepszenia programów Integrowanej Ochrony w winnicach.