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# RESULTS OF FOREIGN EXPLORATION FOR NATURAL ENEMIES OF *PLANOCOCCUS FICUS (HOM.: PSEUDOCOCCIDAE)*, A NEW INVASIVE MEALYBUG IN CALIFORNIA VINEYARDS

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**SUMMARY:**
Mealybugs are pests and virus vectors of grapevines in many regions of the world. Newly introduced into California the vine mealybug, *Planococcus ficus* (Homoptera; Pseudococcidae) damages vines, but could also become a major threat to figs, apples, citrus and tropical crops. Classical biological control is one way to control the pest. Foreign exploration for natural enemies of the *P. ficus* complex is underway in the Mediterranean basin where it is thought to originate. The efficacy and behaviour of potential natural enemies will be evaluated for future release into California. In addition, collections of *P. ficus* will be genetically characterized and its centre of origin determined. This article presents the objectives and first results of this programme.

Key-words: invasive species, biological control, grapevine, natural enemies, genetics

**RESUME:**
**RÉSULTATS DES PROSPECTIONS DES ENNEMIS NATURELS DE *PLANOCOCCUS FICUS (HOM.: PSEUDOCOCCIDAE)*, UNE NOUVELLE ESPÈCE ENVAHISSANTE DANS LE VIGNOBLE CALIFORNIEN**

Les cochenilles farineuses sont des ravageurs et des vecteurs sur la vigne dans de nombreuses regions du monde. Nouvellement introduite en Californie, *Planococcus ficus* (Homoptera; Pseudococcidae) pose de sérieux problèmes dans les vignobles, mais pourrait également devenir une menace sur les arbres fruitiers, tels que figuiers, pommiers, agrumes et cultures tropicales. La lutte biologique classique est une des methodes de contrôle de cette peste agricole. Des missions de prospection sont actuellement menées pour la recherche d'auxiliaries naturels de *P. ficus* dans le bassin méditerranéen, ou elle semble être originaire. L'efficacité et le comportement des auxiliaires potentiels sera evalué pour envisager des lâchers futurs en Californie. De plus, des échantillons de *P. ficus* vont etre caractérisés génétiquement afin d'une part d'etudier la diversité des populations rencontrées, et d'autre part de préciser l'aire d'origine precise de l'insecte. Cet article présente les objectifs et les premiers résultats de ce programme.

Mots clés: espèce envahissante, cochenilles farineuses, lutte biologique, vigne, auxiliaires, génétique

## INTRODUCTION

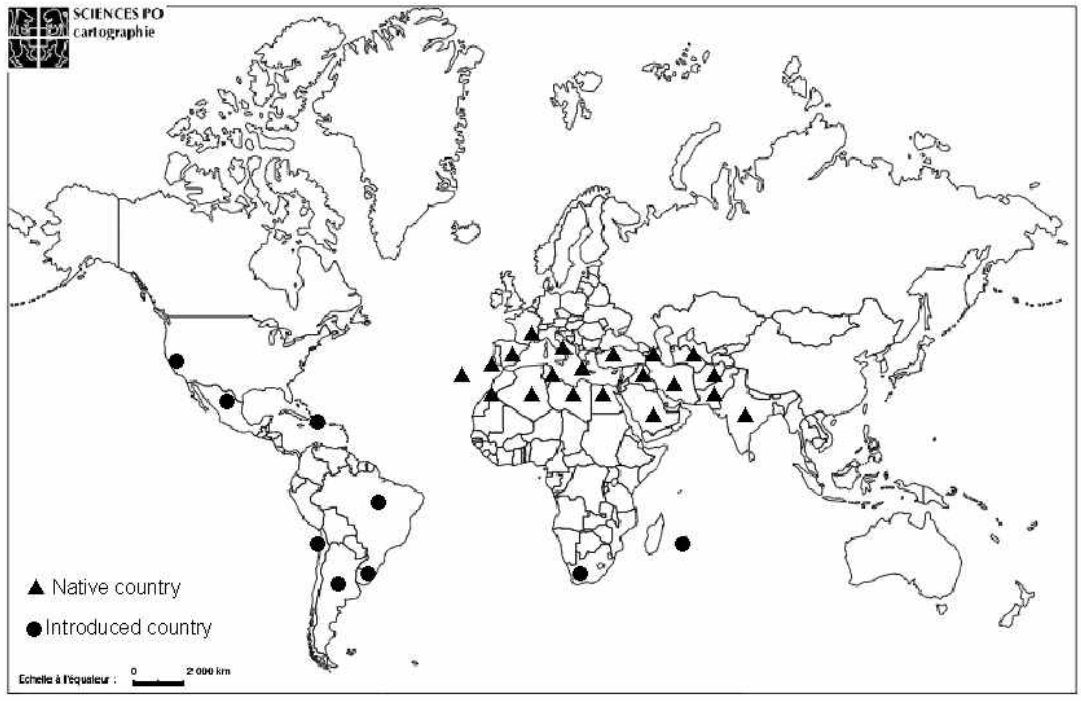
Scale insects are considered as the first pests in agriculture worldwide (Sforza, 2000), and may cause serious damage to vineyards under warm climate areas due to their polyvoltinism. In California, several mealybug species (Homoptera: Pseudococcidae), e.g. the long-tailed mealybug (*Pseudococcus longispinus*), and obscure mealybug (*P. affinis*) have long been present in vineyards, but they are usually minor pests not requiring chemical control (Geiger et al., 2001). The recent introduction in California of *Planococcus ficus* (Bentley, 1998), also called the Vine Mealybug (VMB), has drastically modified the strategy of controlling mealybugs in California agriculture. This is a pest of grapevines in many parts of the world, including its introduced range, but also in its native Eurasian range (Duso, 1989; Sforza, 2000).

### DISTRIBUTION AND ORIGIN

*Planococcus ficus* is a serious exotic pest in California that was first found in the Coachella Valley in 1994 in table grape vineyards (Bentley, 1998). Four years later, significant infestations of VMB were discovered in Kern County. Until recently, infestations of VMB were relatively localized. By 2002, it had emerged as a serious pest in many parts of the state including Santa Barbara, Napa and Sonoma Counties.

VMB has been considered an economic pest of grape since the late twentieth century and is found throughout much of the Mediterranean region. It is primarily located in Italy, Spain, France, Israel and Egypt (Figure 1). It is also present and considered as an introduced species in several countries of South America, and South Africa.

Figure 1: World distribution of *Planococcus ficus* (map built up from literature survey)
*Carte de distribution mondiale de* Planococcus ficus *(carte construite à partir des données de la littérature).*



## BIOLOGY

Only male VMB have wings and can fly; the females are wingless, and remain on their host plant. The VMB has 5 to 7 generations per year, enabling populations to grow very rapidly. Each female can deposit up to 700 eggs (average is approximately 300) in an ovisac. VMB produces a tremendous amount of honeydew, and waxy residue resembling melted candle wax. It is considered a polyphagous insect, occurring on a wide variety of plant families in its native range, including Vitaceae (*Vitis vinifera*), Moraceae (*Ficus carica*), Salicaceae (*Salix* sp.), Rosaceae (*Malus domestica*), and Punicaceae (*Punica granatum*) (Ben Dov, 1994). Unlike other mealybugs, all life stages (larger life stage is 2,5 to 3 mm in length) of the VMB can be present year-round on its host plant. There is no overwintering stage. On vine, during winter months, eggs, crawlers, nymphs, and adults are found under bark, within developing buds, and on roots. By late spring and summer, mealybugs can be found on all parts of vine, including leaves and clusters. In addition, morphologically, VMB is closely related to the citrus mealybug, *Planococcus citri*, and in many cases both species were misidentified. Like for other scale insects, ants are tending VMBs to obtain honeydew on which they feed, then the ants may protect the VMB from predators and parasites.

## DAMAGE AND RISKS

Although it has only been found on grapes so far in California, alternate hosts are reported. It can feed on subtropical (grapes, figs, apples, and citrus) and tropical crops (dates, bananas, avocados, and mangos) as well as a number of common weeds, such as malva, burclover, black nightshade, sowthistle, and lambsquarter. However, major damage occurs on vine. Mealybugs are phloem feeders and are capable of feeding on many different parts of the vine: trunk, canes, leaves, grape clusters and sometimes the roots. They negatively impact crop quality and yield by contaminating clusters with egg sacs, larvae, adults, and honeydew. Often, the honeydew leads to mold growth, defoliation and fouled fruit. The VMB is particularly troublesome because it excretes significantly more honeydew than other mealybug species. This leads to large amounts of mold on clusters and leaves; thus, making it exceedingly difficult to clean the fruit at harvest. Even a small infestation of VMB can quickly result in large economic losses if left unchecked. In California, new outbreaks were discovered in young vineyards that were less than 3 years old.

In addition, mealybugs, including VMB, vector viral diseases of grapevines in different viticultural regions of the world (Rosciglione & Gugerli 1989, Engelbrecht & Kasdorf 1990; Cabaleiro & Segura, 1997; Sforza et al. 2003).

## FACTORS OF SPREAD

It is important to note that this pest is easily transported from infested to non-infested areas via contaminated farm equipment, worker clothing, birds and wind. Several reasons are advanced for its relative spreading success:

1. VMB reproduces at a higher rate than other species, enabling small numbers of mealybugs to reach damaging levels in one season. This greatly increases the population size, and it leads to overlapping generations.
2. VMB excretes much more honeydew than other species. The stickiness of all the plant parts also facilitates spread of VMB from vineyard to vineyard on equipment and worker clothes,
3. VMB can feed on all parts of the vine throughout the year. By hiding under bark or on the roots, VMB is better protected from most foliar insecticides, from high summer temperatures, and from parasitoids and other natural enemies.
4. VMB is not native to California, so it has fewer natural enemies than other indigenous mealybugs
5. VMB has a wide host range. It can feed on subtropical and tropical crops as well as a number of common weeds, making control difficult throughout the season.

### BIOLOGICAL CONTROL

The vine mealybug is closely related to the citrus mealybug, and shares a number of parasitoid species that are present in California. Additionally, foreign exploration for new parasitoids added to the parasitoid complex. One of these is *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) which is the most common parasitoid in the VMB native range. It was introduced in the 1940s against citrus mealybug. The adult female is about 2 mm in length and golden-brown with long antennae that are black at the base and then white to the ends. The winged male is smaller (1 mm), dark-colored with hairy antennae. In field trials, this parasitoid attacked 70-95% of the exposed vine mealybugs in August and September samples in the San Joaquin Valley of California. Several others wasps were collected in Eurasia and middle East: *Leptomastidea abnormis, Leptomastix flavus, L. dactylopii, and Coccideoxenoides peregrinus* (Anonymous, 2003). Of these, *L. abnormis* was realeased in Fresno county in 2001, but revealed to be less effective than *A. pseudococci* with only 6% parasitism. Field studies and evaluation are still ongoing projects, particularly at UC California and CDFA (California Department of Food and Agriculture)

### OBJECTIVES OF THE PRESENT STUDY

This study firstly aims to collect additional natural enemies specific to VMB for future evaluation at EBCL and releases in California against local invasive populations of VMB, paying a particular attention to climate matching between Eurasian countries and California; Secondly to pinpoint the center of origin of VMB by genetic characterization and phylogenetic studies in its native range. Any ecological data will be collected for a better knowledge of VMB biology.

It is proposed that the first year will be devoted to foreign exploration and identification of the VMB populations and their natural enemies found; followed by evaluation of natural enemies in the following years and shipments of selected biocontrol organisms to North American cooperators.

## MATERIAL AND METHODS

### COLLECTING PERMITS

For its status of pest of grapevine, an “Official Letter of authorisation” under the “Directive 95/44/CE” was requested to the service of Ministery of Phytosanitary Cooperation, France, to enable collecting, rearing, and studying *P. ficus* for scientific purpose. The present study is then covered by a specific declaration listed as 05LR035. Scientific contacts were obtained in each country surveyed, and collecting permits obtained where necessary.

### AREA SURVEYED

Based on geographical and climatic similarities with California, 7 countries were investigated in 2005 (Table 1).

Table 1: Area surveyed in 2005 with collection dates, and host plants selected
*Zone de prospection en 2005 avec les périodes et les plantes-hôtes correspondantes*

| Country | Region | Main Cities | Period | Host plants |
|---|---|---|---|---|
| Morocco | Anti Atlas, West coast & South Rif | Ouarzazate Rabat, Meknès, Fès | Mid May | *Ficus carica, Vitis vinifera* |
| Spain | Catalogna Valenciana Andalucia | Barcelona Alicante, Cazorla Huelva | Early June | *Ficus carica* |
| Portugal | Algarve, Alentejo | Faro, Odemira, Evora | Early June | *Ficus carica, Vitis vinifera* |
| Italy | Apuglia, Lazio | Bari, Rome | Mid June | *Ficus carica* |
| Turkey | Western zone, central Anatolia | Istanbul, Bursa, Denizli, Isparta, Safranbolu | End of June | *Ficus carica, Vitis vinifera* |
| France | Languedoc-Roussillon | Languedoc, Ardèche, Charentes, Dordogne | July-August | *Ficus carica, Vitis vinifera* |
| Greece | Crete, Peloponesis | Heraklion, Korinthos | June-August | *Vitis vinifera* |

#### HOST PLANTS AND COLLECTING TECHNIQUES

Two host plants were specifically surveyed, e.g. fig tree (*F. carica*) and grapevines (*V. vinifera*). In the field, plants were selected randomly, and those presenting ant colonies were analyzed in depth. Ants were collected together with mealybugs and will be part of a further study. Abandonned vineyards and organic vineyards were preferred. Depending on the latitude and the period of the year, different parts of the plants were surveyed; trunk, bark crevaces, stems, and leaves were investigated in detail. When momies are found, they are placed indivually in vials until emergence of adults.

#### REARING OF MEALYBUGS

The aim of rearing *P. ficus* is to establish healthy colonies for testing and evaluating parasitoids collected from the field. Mealybug colonies are maintained on potato sprouts in small jars (Samways & Mapp, 1983; Sforza et al, 2003). One colony of a French population of *P. ficus* is maintained in a climatic chamber (25°C, RH 80%, L:D 16:8), and a colony from Morocco is maintained in quarantine (23°C, HR 90%, L:D 16:8). Three potatoes are deposited in a 30X30x10cm plexigas box; each potato is maintained horizontal using 3 toothpicks to prevent contact with the bottom of box; long, flat and cultivated in organic conditions potatoes are preferred. Crawlers, ovisacs and females are deposited on the top of each potato. Weekly observations are necessary for checking if mealybugs did not fall down the potatoes, and for replacing potatoes where necessary.

#### IDENTIFICATIONS AND STORAGE CONDITIONS

In each collection, 10 young females are hand picked from host plants and stored in 70% alcohol vials for identification. Parasitoids and ants are stored in the same conditions. In each site, 10 additional mealybugs are collected in 95% alcohol for genetic analysis.

## PRELIMINARY RESULTS

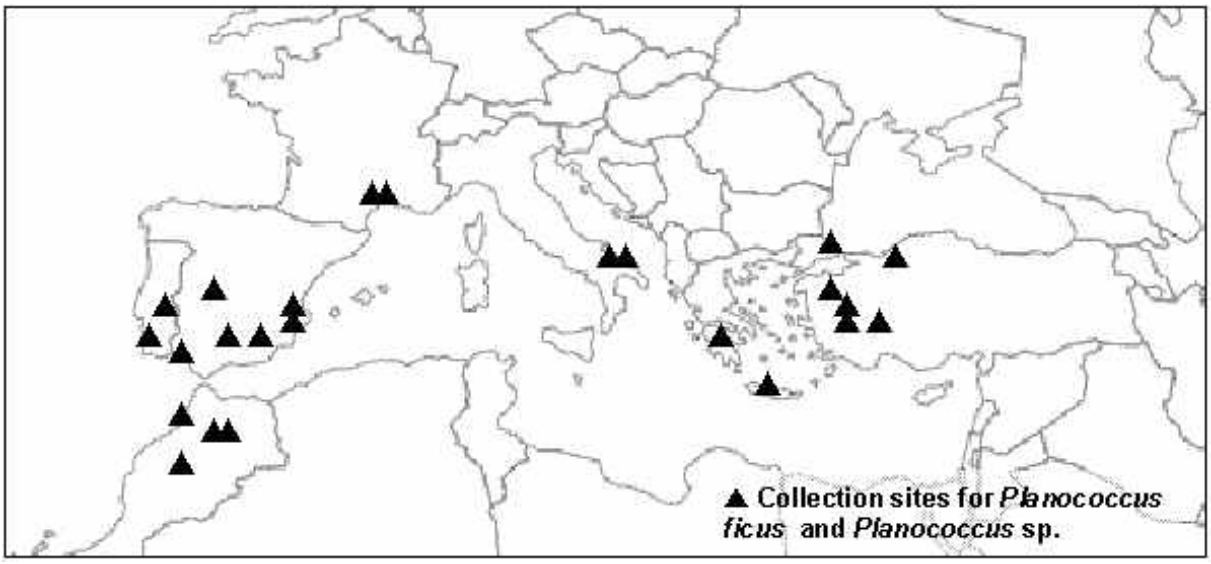
#### IDENTIFICATIONS AND DISTRIBUTION

At the time of writing, all insects (VMB, parasitoids, and predators) collected from 7 countries in 2005 are not yet identified, but those from Morocco and France are *P. ficus*, whether they were collected from *F. carica* or *V. vinifera* (Figure 2). On fig trees, VMB was found either in cultivated landscapes, or on isolated trees in wild areas along roads, or even in the centre of a big city (Istanbul, Turkey). Additional scale insects were observed during this study, e.g.

*Parthenolecanium corni* (Coccidae) and *Targionia vitis* (Diaspididae) on vines (Morocco), and high infestation of *Pseudococcus longispinus* (Pseudococcidae) on ornemantal Ficus sp. (Rabat, Morocco), *Saissetia* sp. (Coccidae) on *Ficus carica* (Morocco), and one Diaspididae (possibly *Hemiberlesia* sp.) on *F. carica* (Morocco).

Figure 2 : Collection sites for *Planococcus ficus* and *Planococcus* sp. in the Mediteranean basin from May to August 2005

*Collections de* Planococcus ficus *et* Planococcus *sp. Dans le bassin Méditeranéen de mai à août 2005*




**BIOLOGICAL DATA**

Two host plants were found for VMB: *Ficus carica*, and *Vitis vinifera* in Morocco (cv. Sugar one), in Spain (cv. Italia, Aledo), and in southern Portugal (cvs. Cardinal, Red Globe, and Doña Maria), At sea level, in May in Morocco, June in Portugal, Spain, Italy and Turkey, VMB was still under the bark of host-plants. The highest elevation for VMB was found at 730m (near Fès, Morocco) on fig trees. At each collection date, egg laying already started with sometimes newly emerged crawlers starting spreading out of the trunk crevaces. In a single case, one VMB female was found egg laying on a leaf (Alicante, Spain), but it is most likely an accidental behaviour. First observations of larvae on green parts were made in mid July in Crete, then females on grape berries in late July (Pers. comm N. Roditakis). In Southern France, VMB was found in departments of Herault and Gard, but not in Ardèche so far. Surveys made in Northwestern areas (Charentes, Dordogne) on fig trees did not reveal presence of VMB. Usually, when a fig tree is infested, colonies are always abundant (egg laying in mid August).

**NATURAL ENEMIES**

From May to July, just a few parasitoid wasps were found from VMB momies: 1 Anagyrus sp (to be identified) was collected from central Morocco, near Marrakech in a cultivated vineyards infested by VMB, and individuals of *Anagyrus* (most likely *A. pseudococci*) (1 male and 1 female) were collected from Crete in the same conditions in July. Parasitism is considered to be over 60% in vineyards of Crete (Pers. comm N. Roditakis). Hyperparasitoids are also collected from momies.

In vineyards, in Morocco (*V. vinifera* cv. Sugar one) and southern Portugal (cvs. Red Globe, and Dona Maria), larvae of the mealybug ladybird (possibly *Cryptolaemus montrouzieri)* were observed in clusters of VMB. In addition, egg predators were observed in all countries surveyed; pink larvae of 2mm-long of cecidomyid midge were found feeding actively in VMB ovisacs. First adults emerged on mid-July in Crete (Pers. comm N. Roditakis). Similar predators were found in Southern France on fig trees.

**ASSOCIATION WITH ANTS**

Like for VMB identifications, we do not have species names of each ant species collected in the 7 countries surveyed. Except for 1 tree in southern Italy (Apuglia region), all fig trees bearing mealybugs were associated with ants. Generally, ants are very aggressive, and spread high quantity of formic acid for protecting mealybugs from the invador/predator (the entomologist in this case!). In some cases, mealybugs were not found although ants were, as they are tending aphids, psyllids, and flatids. Generally, presence of ants is a very good indicator for presence of mealybugs and may save time for field survey.

## CONCLUSION AND DISCUSSION

These preliminary data over a 3-month survey in 7 countries show the natural distribution of VMB from the western to the eastern Mediteranean basin. Two main plants, vine and fig tree, were found on which VMB is common and aboundant. We can strongly hypothesize that the total, if not all mealybugs, collected from fig trees are VMB and most likely the ones from vines are VMB too, as it is commonly believed that 80% of *Planococcus* from vines are *P. ficus*. There is a high uncertainty with identifications of *Planococcus* spp., as some papers stated working with *P. citri* that revealed to be *P. ficus* afterwards; that aims to carefully consider the taxonomical aspect for this project.

For the genetic purpose, additional countries need to be investigated but in regard of the geopolitical constraints for western Europeans to travel in the Middle East and western Asia, collaborations with local scientists are welcome. In addition, building collaboration is crucial for field work as vineyards are commonly restricted areas in which surveys and collecting are not authorized unless being introduced by a local scientist or technician from universities and plant protection. That is why collecting VMB from fig trees makes things less complicated than from vines, as trees are easy and free to access in fallow lands and roadsides.

In a few months, we were able to collect several parasitoids and predators, that need to be firstly identified and then evaluated in field and controlled conditions. For that purpose, the setting of a rearing at EBCL, inside and outside our quarantine, is a useful tool for host specificty testing.

The next step will be to survey diversity of natural enemies and prepare a list of potential new parasitoids specific to VMB collecting from locations climatically matching with California.

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