IDENTIFICATION OF TWO ENTOMOPATHOGENIC FUNGI NATURALLY INFECTING *PTEROCHLOROIDES PERSICAE* (CHOLODKOVSKY 1899) (HEMIPTERA, APHIDIDAE) IN PEACH ORCHARDS IN TUNISIA

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ABSTRACT

Symptoms of mycosis of *Pterochloroides persicae* (Cholodkovsky 1899) (Hemiptera, Aphididae) in a peach and almond orchard were recorded in April 2011. The causal agent was morphologically identified. Polymerase chain reaction (PCR) of the internal transcribed spacer region (ITS1 and ITS4) was used for molecular identification and verification of the morphological determination. Both methods gave consistent results and we recorded for the first time the natural occurrence of two fungal species belonging to the order Hypocreales (Phylum Asocomycota); *Beauveria bassiana* Viull (Hypocreales, Cordycipitacae) and *Metacordyceps liangshanensis* (Hypocreales, Clavicipitaceae).

Keywords: *Beauveria bassiana*, *Metacordyceps liangshanensis, Pterochloroides persicae*, Tunisia

Introduction

The giant brown peach aphid *Pterochloroides periscae* (Cholodkovsky 1899) (Hemiptera, Aphididae) is a worldwide pest of peach, almond, plum, apricot, apple and citrus (Stoetzel and Miller 1998; Ateyyat and Abu-Darwish 2009; Mdellel et al. 2011). It occurs in Europe, Asia, North America and some Mediterranean countries (Kairo and Poswal 1995; Blackman and Eastop 2000). In Tunisia, it was first recorded in 1987 at Sfax (Southern of Tunisia) on almond trees (El-Trigui and El-Shérif 1989) and is considered to be a serious pest of stone fruit trees, especially peach, almond, plum and apricot (Ben Halima-Kamel and Ben Hamouda 2004, 2005; Mdellel et al. 2011). This aphid causes severe damage to its host plants at all growth stages and often kills trees (Kairo and Poswal 1995). In order to control this pest, many of its natural enemies have been identified and used as biological control agents, of which *Pauesia antennata* Mujerji (specific parasitoid of *P. persicae*) and *Coccinella algerica* Kovar are considered to be the most important (Rakshani et al. 2005, Mdellel et al. 2012). Similarly, other natural's enemies can be so used, such as the entomopathogenic fungi. Indeed, fungal diseases of insects are common and widespread and many of them are considered to be important factor regulating pest insect populations (Carruthers and Soper 1987).

Entomopathogenic fungi are recorded as important causes of aphid mortality all over the world (Latge and Papierok 1988). Currently, at least 90 genera and more than 700 species of entomopathogenic fungi infecting invertebrates have been identified, most of which are associated with insects, but only 100 of these have been or are currently being developed for insect control (Wraight et al. 2001). Intensive studies have increased our understanding of the distribution and epidemiology of fungi in insect populations and their use as biological control agents of pests of agricultural crops (Pell et al. 2001). In fact, in temperate regions, Hyphomycetes are important fungal pathogens of aphids (Barta and Cagan 2006). Thus sampling of host individuals can reveal information about the prevalence of fungal species as pathogens in natural host population. The use of entomopathogenic fungi in biological control is a new field in Tunisia. The objective of the present study is to identify isolates of entomopathogenic fungi from *P. persicae* cadavers naturally infected with fungi.

Materials and Methods

Sampling

This investigation of mycoses in *P. persicae* populations was done in three regions of Tunisia, in the north: at Ariana (SidiThabet: 36°54′31″ N, 10°02′33″ E) an arid area and at two regions on the coast of Tunisia: the first at the High Agronomic Institute at Chott Mariem (35°52′31″ N, 10°34′16″ E) and second at Jammel (35°38′24″ N, 10°45′36″ E). The last two regions have an arid climate. Aphid cadavers showing symptoms of infection with fungi (Figs. 1 and 2) were placed in ventilated plastic boxes and carried to the laboratory.

Fungal Identification

Fungal cultures were obtained from aphid cadavers by isolating them on potato dextrose agar (PDA) af-

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Fig.1 *Pterochloroides persicae* population on peach showing symptoms of infection with entomopathogenic fungi.

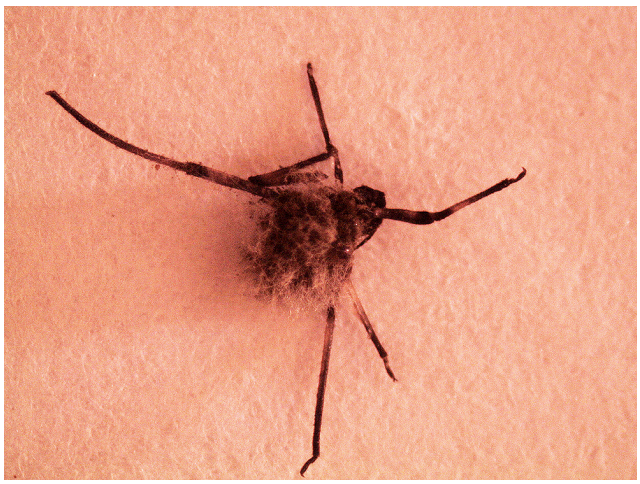

Fig. 2 *Pterochloroides persicae* cadaver showing symptoms of having been killed by a fungal infection.

ter surface-sterilization following the procedures used by Goettel et al. (2000). Microscopic descriptions were made using cultures on PDA and MA (Malt agar). Mycelia were mounted in lacto phenol cotton blue (0.01%) and observed under phase contrast using a Leitz DMRB optical microscope (40×/0.65 PH2 and 100×/1.25 OIL PH3). Fungal preparations were photographed using a Coolpix 5000 5.0 MP digital camera. The fungi were identified (based on morphological characteristics) under a stereoscopic microscope (Nikon SMZ 800 with light source Intralux 4000-1) with the aid of the taxonomic keys of Lawrence (1997), Humber (1997) and Barnett and Hunter (2006).

Genomic DNA extraction from mycelia was done using the method described by Reader and Broda (1985). DNA was amplified by PCR using the complementary primers ITS1F and ITS4 and a MyCycler TM thermocycler System (Bio-Rad, Madrid, Spain) according to the manufacturer's instructions (Promega, Madison, WI, USA). The sequences were compared with those available in the GenBank database using BLAST search analysis (sequences were initially aligned using DNASTAR Inc. (Madison, WI, USA).

Results and Discussion

Two entomopathogenic fungi naturally infecting *P. persicae* were identified: *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales, Cordycipitaceae) and *Metacordyceps liangshanensis* (Ascomycota: Hypocreales, Clavicipitaceae). These two species are reported for the first time infecting *P. persicae*. Nevertheless, Tsinovskii and Egina (1972), cited by Cross and Poswal (1996), report *Entomophthora thaxteriana* Petch infecting *P. persicae*, which is now named *Conidiobolis obscuris*, and is the cause of substantial mortality in several species of aphids. In addition, in Israel, Ben Zev (1988) report another species of entomopathogen attacking this aphid called: *Taxterosporium tubinatum* Kenneth.

Several studies have demonstrated that *B. bassiana* infects hundreds of species of hosts belonging to many insect orders (Nicolai et al. 2006). Gurulingappa et al. (2011) demonstrate the efficiency of this fungus against *Aphis gossypii* Glover. These authors report that it reduces the longevity and fecundity of aphids, and is thus potentially an excellent biological control agent. Similarly, Sevim et al. (2012) note that much effort has been put into research on the development of *B. bassiana* as a biological control agent (for inundative and inoculative biological control) for use in agriculture and forestry in temperate regions. In South Korea, Kim et al. (2013) have demonstrated also that spraying green peach aphids with a filtrate of cultures of *B. bassiana* results in a high mortality (78%) of the aphid three days after the treatment. Akmal et al. (2013) tested the efficiency of *B. bassiana* against several species of aphids (*Schizaphis graminum* (Rondani 1852), *Rhopalosiphum padi* (L., 1758), *Brevicoryne brassicae* (L., 1758) and *Lipaphis erysimi* (Kaltenbach 1843) in the field and showed it can be used as a biocontrol agent for managing these aphids. *M. liangshanensis*, Kepler et al. (2013) infects species of Lepidoptera in USA. Currently, the relative virulence of infections by *B. bassiana* and *M. liangshanensis* in *P. persicae* is unknown, but future pathogenicity assays will clarify whether they are both highly virulent biological control agents of *P. persicae*.

In conclusion, the present study provides the first report of *B. bassiana* and *M. liangshanensis* infecting *P. persicae* populations infesting peach and almond trees in Tunisia, but further research is needed on the relative efficiency of these two entomopathogenic fungi against *P. persicae* and their potential as biological control agents.

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