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THAUMATOTIBIA LEUCOTRETA (MEYRICK) (LEPIDOPTERA TORTRICIDAE) INTERCEPTED FOR THE FIRST TIME IN ITALY

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Mazza G., Strangi A., Marianelli L., Del Nista D., Roversi P.F. - *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera Tortricidae) intercepted for the first time in Italy.

In June 2014, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera Tortricidae) was found during the inspection of the Italian Phytosanitary Service of Tuscany Region in the port of Leghorn, in a container from South Africa containing Navel oranges. The degree of damage is high, reaching the 2% of the harvested fruits. This is the first interception of this polyphagous pest for Italy.

KEY WORDS: False Codling Moth, *Citrus sinensis*, phytosanitary citrus pests, interception, Italy.

Globally, *Citrus* fruit is the highest value fruit crop in international trade and the biggest component of South African exported subtropical fruit, as reviewed in MAGWAZA (2013). Worldwide, South Africa is the third largest exporter of fresh *Citrus* fruit after Spain and Turkey (AFRICA, 2012). Unfortunately, this fruit is easily prone to develop various types of diseases and is attacked by several pests decreasing postharvest fruit market value, since external appearance is the primary specification used to evaluate quality of fresh *Citrus* fruit (ALQUEZAR *et al.*, 2010). South Africa hosts a wider range of insect pests on *Citrus* than elsewhere in the world, largely due to climatic conditions (URQUHART, 1999); one of the major pest of economic concern in southern Africa is the polyphagous false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera Tortricidae). This lepidopteran pest infests mainly *Citrus* in this region, but is recorded on approximately 24 cultivated and 50 wild plant species (VENETTE *et al.*, 2003; KIRKMAN *et al.*, 2007).

This quarantine pest of international concern is a multivoltine species, native to the Ethiopian zoogeographic province and present in much of Sub-Saharan Africa (VENETTE *et al.*, 2003). Damage is mainly caused by larvae feeding inside the fruits at any stage, nuts, maize ears or cotton bolls. Feeding damage can also lead to the development of secondary infections mediated by fungi and bacteria. The insect can be found on its host crops throughout the year in tropical and sub-tropical climates (without diapause), with overlapping generations. Larvae feed inside the fruits and usually then fall to the soil surface to pupate in a cocoon of silk and soil fragments (GILLIGAN *et al.*, 2011).

In June 2014, at the port of Leghorn (43°34'N; 10°18'E) 1.600 cartons (about 24.000 kilos) of Navel oranges (*Citrus sinensis*), coming from South Africa, were stopped by the Phytosanitary Service of Tuscany Region because of the damages presented (Fig. I, 1). The trip from South Africa lasted about 20 days at a temperature of 4-5°C. No reliable information is available concerning the treatments carried out on these fruits in the place of origin.

The Port of Leghorn is one of the major points of entry into Italy for commodities coming from non-EU countries, subject to the monitoring of plant health (TOSCANA, 2014). In 2013, the Phytosanitary inspection activities carried out in the Port of Leghorn authorized the entry into the European Union of about 92.000 tons of fresh fruit which corresponds to an estimated economic value of 70 million euros, of which 42 million is *Citrus*, 27 million is Pomaceae and 1 million stone fruit and other fruits. Altogether, the amount of fresh fruit imported from South Africa, Argentina and Chile is about 90% of the total. Specifically *Citrus* fruits, which are imported during the period from May to October, come mainly from South Africa (60%) and Argentina (25%) (TOSCANA, 2014).

During the inspection 20 cartons randomly chosen containing 65 oranges each (for a total of 1.300 orange fruits) were singly examined. The 2% of fruits presented the yellowish-brown rind around the oviposition hole and one larva of Lepidoptera Tortricidae, in each orange, that fed just below the fruit surface (Fig. I, 2). It is noteworthy that 2% is also the current fruit loss level in South Africa due to this pest (EPPO, 2013).

Since no adults were captured and the taxonomic identification of larvae is particularly problematic, we combined morphological with molecular analyses (TIMM *et al.*, 2008). From morphological point of view we followed the description and the distribution of the setae illustrated in KOMAI (1999). For the molecular analyses three samples (labelled Sample 1, 2 and 5) were collected and stored in 95% ethanol. Subsequently, in the laboratory of CRA-ABP, larvae were put in vials containing plastic beads, frozen in liquid nitrogen and homogenized (Precellys 24, Precellys). DNA was extracted using ZR Tissue & Insect DNA MicroPrep kit (ZymoResearch) according to manufacturer protocol. Amplification of standard barcode fragment was obtained according with protocol published by FOLMER *et*

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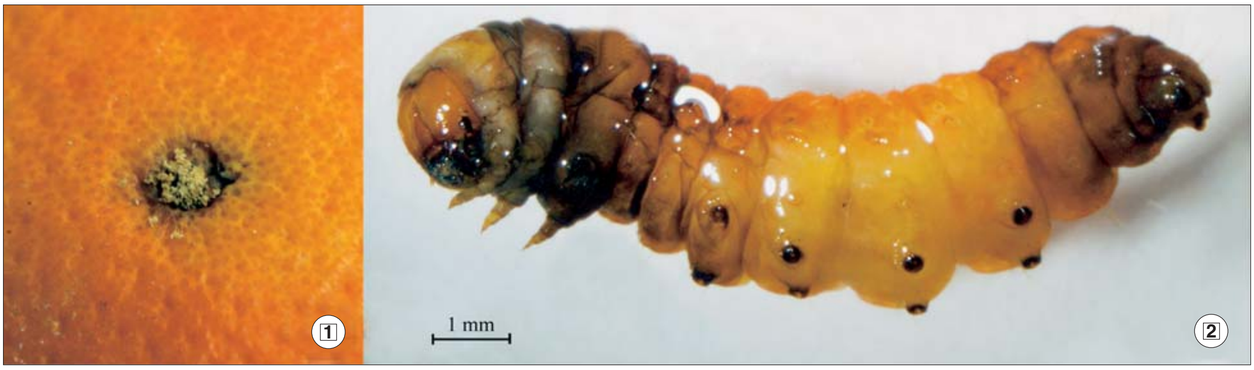

Fig. I – 1) External damage; 2) Larva of *Thaumatotibia leucotreta.*

al. (1994). Amplicons were sequenced at the Centro di Servizi per le Biotecnologie di Interesse Agrario Chimico ed Industriale (CIBIACI), Università degli Studi di Firenze, Italy.

Sequences were manipulated with BioEdit and aligned using ClustalOmega. A DNA fragment 386 bp long located at 5' end of Cytochrome Oxidase 1 (CO1) gene was used for the subsequent analyses. Maximum Likelihood (ML), Neighbour-Joining (NJ), Minimum Evolution (ME), Maximum Parsimony (MP) and UPGMA trees were inferred using MEGA 6.0 (TAMURA *et al.*, 2013) performing 500 bootstrap replicates. Tree based on bayesian Inference (BI) was constructed using BEAST v. 1.8.0 (DRUMMOND *et al.*, 2007), analyses were run for 10 million generations, sampling every 1000 generations (first million discarded as burn-in). Four independent Markov chain Monte Carlo (MCMC) analyses were performed starting from a randomly chosen tree. Maximum clade credibility tree was summarized.

In both the analyses, all the samples were identified as belonging to *Thaumatotibia leucotreta* (Fig. II).

This insect was recently inserted in EPPO A2 List,

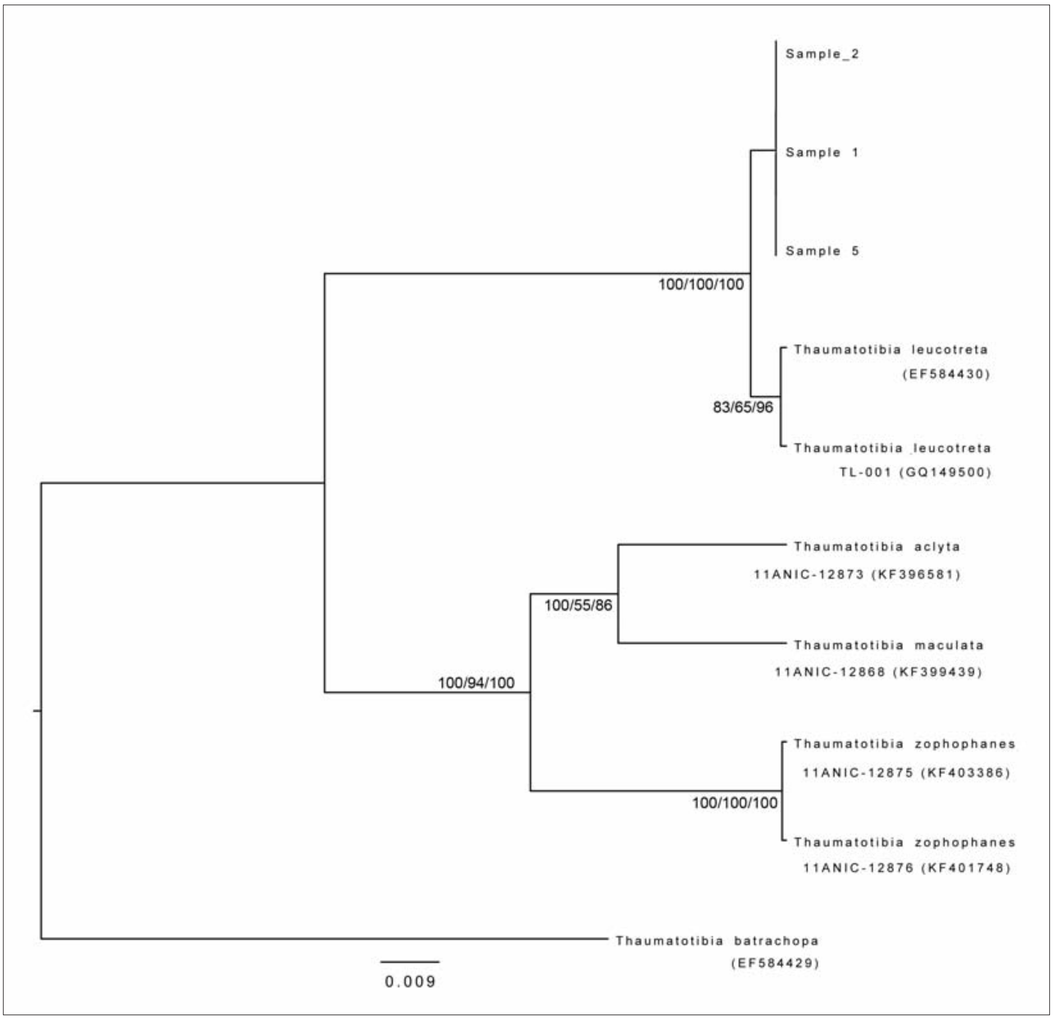

Fig. II – Consensus phylogram from the Bayesian analysis of CO1 386 bp fragment (-lnL = -892.2). Support values are shown for MP, ML and BI respectively.

because it was locally present in Israel where it was found in 1984 on macadamia nuts (EPPO, 2013). In 2009, this pest was detected in the Netherlands on glasshouse of *Capsicum chinense*, and subsequently eradicated. Finally, the insect has been occasionally intercepted by several lepidopterists in some Northern European countries (Denmark, Finland, Sweden and the UK) (EPPO, 2013). This is the first detection of this pest for Italy and the second for the Southern Europe after the several interceptions in Spain (EPPO, 2013).

In spite of DAIBER (1978) suggests that this pest may not perform well in Mediterranean climates, other authors reported that this pest may be able to establish in areas where the average annual low temperature is >-10°C (PPQ, 1993). Moreover since this pest has a broad host range and suitable host plants are both common and abundant, a relatively high probability of pest establishment exists if the pest were introduced into a suitable situation. The port of Leghorn is the first point of entry and do not necessarily represent the intended final destination of infested material.

It is noteworthy that *Citrus* is mainly imported in the period June-October and that the frequency of imports coincides with the presence of the pest in the area of origin and in this period is more likely to meet suitable conditions and to find available hosts for this pest in the EPPO Region (EPPO, 2013).

The pest, as in our case, has been regularly detected as larvae (at all development stages) in imported consignments. This demonstrates that survival is possible during current transport conditions. In fact, consignments are usually transported by ship, mostly with fruit pulp temperature in the range of +4 to +10°C (Transport Information Service http://www.tis-gdv.de/tis_e/ware/inhaltx.htm#6) and despite the low temperature in the container, only temperature close to 0°C for prolonging time are able to kill larvae and thus explain the interception of live ones (HOFMEYR *et al.*, 1998).

Since this species is commonly intercepted at ports of entry in North America (GILLIGAN *et al.*, 2011) and regularly detected between 2004 and 2010 on imported consignments in Spain and the Netherlands (EPPO, 2013), in addition to the high number of live larvae intercepted, vigilance and early detection methods are critical to minimize the probability of this species introduction and establishment in Southern Europe.

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