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First report of the ambrosia beetle *Xylosandrus compactus* and associated fungi in the Mediterranean maquis in Italy, and new host–pest associations

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In September 2016, a survey conducted in the Circeo National Park revealed an outbreak and serious damage caused by the black twig borer (*Xylosandrus compactus*) and its associated fungi in the Mediterranean maquis. Among the affected hosts, *Quercus ilex*, *Viburnum tinus*, *Ruscus aculeatus*, *Pistacia lentiscus*, *Laurus nobilis* and *Ceratonia siliqua*, showed flagging and wilting of branches and, in younger individuals, the death of the whole plant occurred. In total, 18 different fungal taxa were found associated with the insect. These included *Ambrosiella xylebori*, *Geosmithia pallida*, *Fusarium* spp., *Epicoccum nigrum* and *Bionectria* sp. This is the first report in Europe of *X. compactus* and associated ambrosia fungi in a natural environment.

Introduction

In 2011, *Xylosandrus compactus* was first recorded in Europe (Garonna *et al.*, 2012) in urban parks of the Campania region of Italy. Later the species was found in Tuscany, Liguria and Sicily, and in South-east France mainly on *Quercus ilex*, *Laurus nobilis* and *Ceratonia siliqua*.

This paper reports on the situation in summer 2016, when a serious decline and wilting of Mediterranean maquis was recorded on the Circeo Promontory in the Circeo National Park, Central Italy, in the Latium Region (Fig. 1). The affected area extends for more than 13 hectares. A large number of evergreen maquis species, including *Q. ilex*, *Viburnum tinus*, *Ruscus aculeatus*, *Pistacia lentiscus*, *L. nobilis* and *C. siliqua*, showed flagging and wilting of branches up to 2–3 cm in diameter, or, in younger individuals, death of the whole plant.

Present study: description and results

In September 2016, a survey was conducted in the 'Quarto freddo' of the Circeo Promontory (41°14′50″ N 13°02′35 E) to evaluate symptoms and damage on different hosts and to collect samples from affected individuals. Small holes (0.8–1 mm in diameter) were observed on the majority of samples associated with external symptoms of wilting. Once the samples were processed in the laboratory, tunnels were found in association with holes, with the presence of larvae, pupae and adult beetles. Large longitudinal necroses were always associated with the tunnels and extending upward and downward along the branches. Thirty adult beetles were collected from tunnels found in branches of *Q. ilex*, *V. tinus*, *R. aculeatus*, *L. nobilis*, *P. lentiscus* and *C. siliqua*. According to morphological characteristics, the species was determined as the black twig borer, *X. compactus* Eichhoff (Coleoptera: Curculionidae, Scolytinae) (Fig. 2).

The species is widely distributed in Africa, Asia and South America and has been introduced in the Pacific Islands, New Zealand, Hawaii and the South-Eastern United States (Rabaglia *et al.*, 2006). However, *X. compactus* is believed to be native to East Asia (Wood, 1982; Chong *et al.*, 2009). The black twig borer is a primary phytophagous pest of over 200 hosts causing extremely serious infestations in domestic and natural environments. Females attack healthy twigs, boring into the living tissues of trees and shrubs (Ngoan *et al.*, 1976). Adult females are shiny black, with a body length of 1.6–1.8 mm. Males, which are incapable of flying, are reddish black and smaller than females, measuring 0.9–1.3 mm in length (Hara & Beardsley, 1979). *Xylosandrus compactus* is an arrhenotokus species in which males are born from unfertilized eggs and females from fertilized ones. After mating, which primarily occurs between siblings just after adult emergence, the male remains in the gallery while the female leaves the tunnel through the entry hole and colonizes branches of new hosts, boring an entry hole and a subsequent brood gallery (Hara & Beardsley, 1979; Greco & Wright, 2015). Two larval instars per generation were observed in laboratory rearing (Ngoan *et al.*, 1976),

Fig. 1 Damage caused by *Xylosandrus compactus* and associated fungi on Mediterranean maquis plant species in the Circeo Promontory (August 2016).

while three instars per generation were recorded in the field in Hawaii (Hara, 1977; Greco & Wright, 2015). Ngoan *et al.* (1976) report that it is difficult to distinguish the generations because they overlap from April to September, with all life stages usually occurring together in the same galleries. Damage is related to both wood boring and the introduction of ambrosia fungi, which are of crucial importance for larval development (Hara & Beardsley, 1979). These ambrosia fungi, which are often pathogenic to the host trees, are frequently associated with the insect (Bosso *et al.*, 2012). In order to determine the fungi associated with the black twig borer, adults were transferred alive onto potato dextrose agar + streptomycin (PDAst) medium in 9-mm Petri dishes and allowed to move freely; developing fungal colonies were transferred individually onto PDA plates and maintained at room temperature. In addition, isolation was attempted from *L. nobilis*, *C. siliqua*, *P. lentiscus*, *Q. ilex* and *V. tinus*. Small fragments of tissue were collected in aseptic conditions from tunnel walls and from the margins of the necrosis, and directly plated on PDAst medium. DNA was extracted from pure fungal culture grown in potato dextrose broth (PDB) and the internal transcribed spacer region amplified according to White *et al.* (1990). PCR products were sequenced in both directions (Macrogen, KR). The sequence results were compared with other DNA sequences by using BLAST against the NCBI nucleotide database. In total, 18 different fungal taxa were found associated with the insect and/or tunnels and necrosis. Among the most frequent taxa found, the following species were identified: *Ambrosiella xylebori* Brader ex

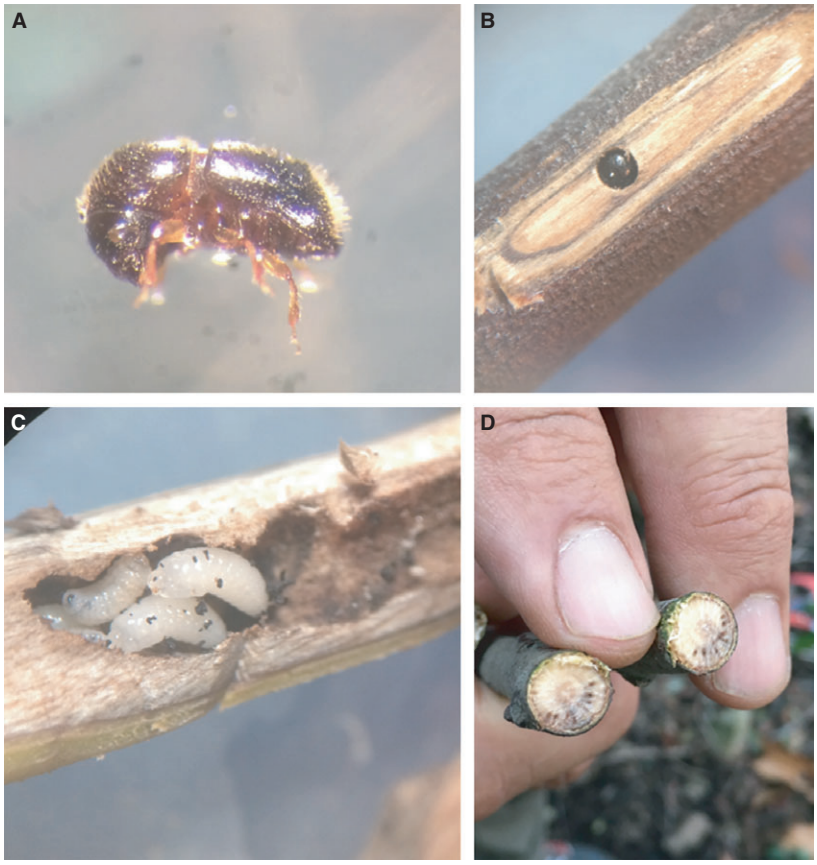

Fig. 2 (A) Adult female of *Xylosandrus compactus*. (B) Entrance hole of adult female. (C) Larvae of *X. compactus* in a tunnel. (D) Wood discoloration associated with tunnels.

Arx & Hennebert (from *L. nobilis*, *C. siliqua* and *P. lentiscus*) considered the main ambrosia species associated with black twig borer (Bateman *et al.*, 2016); *Geosmithia pallida* (G. Sm.) M. Kolarík, Kubátová & Paoutová (from *Q. ilex*, *P. lentiscus* and *V. tinus*), an emerging pathogen of live oak in the United States (Lynch *et al.*, 2014) showing affinities with a wide range of wood-boring insects, such as bark beetles and sawyer beetles (Kolařík *et al.*, 2004); *Fusarium* spp. (from *Q. ilex* and *L. nobilis*), already reported in association with *X. compactus* and in general with ambrosia beetles (Greco & Wright, 2015); *Epicoccum nigrum* (from *Q. ilex*) reported as associated with *Xylosandrus crassiusculus* and *Xyleborus affinis* (Kostovcik *et al.*, 2015); and *Bionectria* sp. (from *L. nobilis*).

Discussion

As described above, *X. compactus* has previously been recorded in Italy and South-East France mainly on *Q. ilex*, *L. nobilis* and *C. siliqua.* However, this is the first record for the Latium region and the first record in Europe reporting an outbreak and serious damage caused by the black twig borer and its associated fungi in the Mediterranean maquis. This is also the first record of *X. compactus* on *R. aculeatus* and *P. lentiscus* and the first record of the association of *X. compactus* with the fungi *G. pallida* and *E. nigrum*. Although the role of these associated fungi has still to be determined, some of them, such as *Fusarium* spp., *E. nigrum* and *G. pallida*, are plant pathogens. The presence in the natural environment of the black twig borer and its associated fungi on a wide range of hosts is an extremely alarming signal and should be considered as a real threat to natural ecosystems in Europe. For these reasons, the authors strongly recommend the inclusion of *X. compactus* in the EPPO Alert List.

Premier signalement en Italie, dans le maquis méditerranéen, du scolyte *Xylosandrus compactus* et des champignons associés; nouvelles associations hôte-ravageur

En septembre 2016 une enquête menée par le Parc National de Circeo a permis d'identifier dans le maquis méditerranéen un foyer ainsi que des dégâts importants causés par scolyte noir des rameaux (*Xylosandrus compactus*) et ses champignons associés. Parmi les espèces hôtes touchées, *Quercus ilex*, *Viburnum tinus*, *Ruscus aculeatus*, *Pistacia lentiscus*, *Laurus nobilis* et *Ceratonia siliqua* présentaient des symptômes de décoloration et de flétrissement des branches, ainsi que, chez les jeunes individus, la mort de la totalité de la plante. Au total, 18 taxons différents de champignons ont été trouvés associés à cet insecte. Cela comprend *Ambrosiella xylebori*; *Geosmithia pallida*; *Fusarium* spp.; *Epicoccum nigrum*; et *Bionectria* sp. Cela constitue le premier signalement de *Xylosandrus compactus* et de ses champignons associés, en Europe, dans un environnement naturel.

Первое сообщение о короеде *Xylosandrus compactus* и связанных с ним грибах в средиземноморском маквисе в Италии, а также о новых сочетаниях хозяин-вредитель

В сентябре 2016 г. обследование, проводившееся в средиземноморском маквисе, в Национальном парке Цирцео, выявило вспышку и серьезный ущерб, нанесенный черным точильщиком побегов (*Xylosandrus compactus*) и связанными с ним грибами. Среди затронутых растений-хозяев *Quercus ilex*, *Viburnum tinus*, *Ruscus aculeatus*, *Pistacia lentiscus*, *Laurus nobilis* и *Ceratonia siliqua* показали увядание ветвей, а у более молодых особей имело место отмирание всего растения. В общей сложности 18 различных грибных таксонов были сочтены связанными с этим насекомым. Они включали *Ambrosiella xylebori*, *Geosmithia pallida*, *Fusarium* spp., *Epicoccum nigrum* и *Bionectria* spp. Это сообщение представляет собой первую регистрацию *Xylosandrus compactus* и связанных с ним грибов в естественной среде в Европе.

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