RESEARCH ARTICLE

Seasonal changes in population structure of the ambrosia beetle *Xylosandrus compactus* and its associated fungi in a southern Mediterranean environment

Antonio Gugliuzzo, Giulio Criscione, Antonio Biondi, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia*

Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy

* giovanna.tropeagarzia@unic.it

Abstract

Exotic ambrosia beetles are increasing in Europe due to global trade and global warming. Among these xylomycetophagous insects, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae) is a serious threat for several Mediterranean host plants. Carob trees growing in Sicily (Italy) have been extensively attacked by beetles leading to rapid tree decline. Although *X. compactus* has been found in Europe for several years, most aspects of its ecology are still unknown. We thus studied the population structure and dynamics of *X. compactus*, together with its twig size preference during a sampling of infested carob trees in southeast Sicily. In addition, fungi associated with insects or galleries were isolated and characterized. The results showed that, in this newly-colonized environment and host plant, adult *X. compactus* overwinters inside twigs and starts to fly and reproduce in mid spring, completing five generations before overwintering in late fall. The mean diameter of carob twigs infested by the beetle varied significantly over the seasons, with the insect tending to infest larger twigs as season progresses. The mean number of adults/gallery was 19.21, ranging from 6 to 28. The minimum temperature significantly affected the overwintering adult mortality.

*Ambrosiella xylebori* and *Fusarium solani* were the main symbionts associated with the pest in this study. *Acremonium* sp. was instead recorded for the first time in Europe inside *X. compactus* galleries. Several other fungi species were also found for the first time in association with *X. compactus*. Our findings provide useful insights into the sustainable management of this noxious pest.

Introduction

The number of exotic insect pests established in Europe is constantly increasing, mainly due to global trade and global warming [1–7]. These organisms often cause economic damage as well as an increase on pesticide applications with the consequent side effects toward non-target organisms, the environment and human health [8–12]. Among insect pests which have
invaded the Mediterranean Basin over the last decades, alien wood-boring beetles are of primary importance due to the high diversity of host plants and the suitable climate [13–17]. The mild winters followed by dry and warm summers of Mediterranean regions, favour the spread and establishment of alien Scolytinae [18], including several xylomycetophagous ambrosia beetle species belonging to the genus Xylosandrus [19]. In particular, four of these species are native to Asia and are now widespread in Italy. Xylosandrus morigerus (Blandford) is known as a plant nursery pest, while X. crassiusculus (Motschulsky) and X. germanus (Blandford) are considered pests of various cultivated and wild host species [20–23].

Since its first report in 2012 [24, 25], Xylosandrus compactus (Eichhoff), also known as the black twig borer, has been reported as an emerging pest for several plants of the Mediterranean maquis [26], as well as for various trees and ornamental shrubs that are widespread in southern Europe [19, 27, 28]. Among these, Laurus nobilis L. [25] and carob tree (Ceratonia siliqua L.) have been reported as preferred host plants [29]. Unusual heavy pest infestations on large branches and trunks, associated with serious decline and wilting, have been recently observed on carob trees in Sicily (Southern Italy) [29–31]. Carob is a thermophilous arboreal species characteristic of the olea-lentisc and carob groups (belonging to the Oleo sylvestris-Ceratonion siliquae alliance) [32] and it is a widespread and long-living tree in Mediterranean woodland vegetation [33, 34]. This tree species, largely diffused in dry areas of Sicily, provides to farmers several products and by-products including the flour extracted from the seeds (Locust Bean Gum, LBG) used in food industry as thickening agent (E410) [35–37].

Xylosandrus compactus, as well as the other ambrosia beetles, develop by feeding exclusively on fungi cultivated by females inside galleries [38–40]. In insect-fungus mutualisms, symbiotic fungi can degrade the defensive substances of a plant and/or directly produce antagonistic compounds against other microorganisms that may co-occur in the galleries [41]. Among the symbiotic fungi reported in association with X. compactus, three species seem to be the most recurrent, i.e., Ambrosiella xylebori Brader ex Arx, A. macrospora (Francke-Grosm.) L.R. Batra and Fusarium solani (Mart.) Snyd. & Hans [26, 42–45]. On the other hand, several other fungal species, including Geosmithia pallida, Epicoccum nigrum and Bionectria sp., have been found in association with the black twig borer on Mediterranean maquis plants [26]. However, the symbiotic community composition may be spatio-temporal dependent. Bateman et al. [46] isolated A. xylebori almost exclusively from the mycetangium (a fungus spore-carrying organ), and Fusarium spp. mainly from the body surface, clearly demonstrating that the different fungi are spatially segregated on the insect’s body. Skelton et al. [47] demonstrated that closely related symbionts are interchangeable by offering alternative fungal symbionts from different Ambrosiella clade in experimental galleries inoculated with X. compactus. However, Li et al. [48] showed that aposymbiotic specimens, deprived of Ambrosiella, in any case develop empty mycetangia.

Although X. compactus has been in Europe for several years, causing much damage to wild and cultivated plants, its population structure over the seasons in the newly-invaded areas has not yet been investigated. Similarly, there is little information on the fungal communities of X. compactus in such areas. The annual population trend of the beetle was thus monitored, and the fungi associated with the insect or occurring inside the infested galleries were identified and characterized.

Materials and methods

Beetle samplings and dissection of the samples

The study was carried out by sampling unmanaged carob trees (var. Latinissima) from the beginning of November 2017 to the end of December 2018, in the town of Scicli (18m a.s.l.,
located in south east Sicily (Italy). This sampling site was characterized by an area of about 10 ha of a semi-urban environment next to a natural landscape, typical of the Mediterranean environment, where many natural carob trees grow spontaneously without any anthropic intervention. In this location, the *X. compactus* first appeared in summer 2017 [29]. Climatic data were provided by the Sicilian Agrometeorological Service (SIAS). The data (minimum, maximum and daily average temperatures) were obtained from the nearest climatic station located 5 km from the sampling site (51 m a.s.l., 36°45'41.3"N, 14°40'50.5"E).

A yearly population trend of *X. compactus* on carob trees was estimated by sampling and dissecting twigs (20 twigs/biweekly) that showed infestation signs, such as the presence of an ambrosia beetle entrance hole, wilting, defoliation and wood necrosis near the entrance holes [22, 29]. Samplings were carried out every 15 days in order to increase the likelihood of sampling all the different biological stages of each pest generation, given this beetle completes its life cycle in about one month [38]. For each sampling date, four twigs/tree (approximately 1.5–2 m above the ground) were sampled from all the cardinal points of five randomly chosen trees located at a distance of about 50 m from each other. Each sampling included ten small twigs (diameter \( \leq 7 \) mm; length \( \leq 50 \) cm), usually infested by one or few females, and ten larger twigs (diameter \( \geq 8 \) mm; length \( > 50 \) cm), usually attacked by multiple females [22, 30, 38]. Twig diameter was measured before the samplings using a Vernier caliper.

**Fungal isolation and identification**

Fungi that were inhabiting the beetle’s external body surface or growing in the infested twig galleries of each sampled twig were isolated. Sections of symptomatic twig tissues were excised from the lesions surrounding the beetle gallery and disinfected with 1.5% sodium hypochlorite solution, rinsed in sterile water, and placed on potato dextrose agar (3.9% PDA, Oxoid). To prevent bacteria growth, medium plates were amended with 100 mg/liter of streptomycin sulphate (Sigma-Aldrich). Samples were incubated at 25 \( \pm \) 1°C, or until the fungal growth was evident. Fungi inside the galleries were also isolated by scraping off a portion of the fungal biomass with a sterile wood-stick and transferring it onto PDA. For isolation of the beetle fungal communities, 2–3 not disinfected beetles per sample were plated on PDA. Per each sampling date, from two to six fungal isolates were obtained from twigs or insects.

The fungi colonies were transferred onto new plates to obtain pure cultures. Single conidium or hyphal tip culture fungi were then established on PDA for all fungal colonies. After being stored in an incubator in the dark at 25 °C for up to two weeks, morphotypes were assigned based on macromorphology (i.e., color, size comparison/growth rate, texture). These cultures were separated into possible ambrosia and other fungi by examining the colony characteristics [44, 46]. Representative isolates of mean species were also identified with molecular analysis. All isolates were stored at -20 °C in 20% (v/v) glycerol at the Department of Agriculture, Food and Environment of the University of Catania, Italy.
Molecular identification of the fungal isolates was performed by sequencing internal transcribed spacer regions of the rDNA and 5.8S region (ITS). Genomic DNA was extracted from 35 isolates using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI, USA). The ITS of the nuclear ribosomal RNA operon was amplified with primers ITS5 and ITS4 for all isolates and species [50].

The PCR products were sequenced in both directions by Macrogen Inc. (South Korea). The DNA sequences generated were analysed, and consensus sequences were computed with Mega 7 [51]. BLAST searches were used to compare the obtained sequences with other sequences in the NCBI database.

Data analyses

The raw insect data obtained were preliminarily examined in order to detect the first two dates in a row with adults only infesting the sampled twigs, i.e., the beginning of the insect reproductive diapause. Mid-December 2017 (see the Results section) was thus set as the starting sampling date for analysing the yearly structure of the X. compactus population. Data were thus divided into the following four seasons: from Dec 15 2017 to March 14 2018 (winter), from March 15 2018 to Jun 14 2018 (spring), from Jun 15 2018 to Sep 14 2018 (summer) and from Sep 15 2018 to Dec 14 2018 (autumn).

Raw datasets were then tested for normality and homogeneity of variance using Kolmogorov-Smirnov D test and Cochran’s test, respectively, and no data transformation was needed. Data were analysed by factorial ANOVA (at a level of significance of $p \leq 0.05$), using season as the independent factor. The dependent variables were the specimen numbers belonging to each biological stage (egg, larval, pupal and adult) in the whole sample, the mean diameter of infested twigs, and the number of adults/gallery. A Bonferroni post-hoc test was conducted in order to compare the mean diameter of infested twigs over the different seasons. The temperature dependent mortality of overwintering adults was described by a linear regression model, studying the proportion of dead adults (number of dead adults/number of sampled adults) per sampling date as a function of the minimum temperature trend of the two weeks before the sampling. The trend was estimated by calculating, per sampling date, the mean minimum daily temperature of the fourteen days preceding each sampling. Statistical analyses were carried out using SPSS 22.0 software (IBM Corp., Armonk, NY, USA).

Results

Yearly beetle population structure

During the first three sampling dates, there was a decreasing trend in the proportion of immature beetles, i.e., 37, 14.3 and 0% of the sampled individuals were juveniles, in mid-November, late-November and early December 2017, respectively (Fig 1). In line with this, on Dec 15 2017, the sampled beetles were all adults, and therefore this was considered as the starting date for the yearly analyses. The population trend of X. compactus was affected by the season, as shown in Fig 1. The specimen percentage of the four developmental stages varied significantly over the seasons (eggs: $F_{3,198} = 3.446, p = 0.018$; larvae: $F_{3,198} = 10.405, p < 0.001$; pupae: $F_{3,198} = 7.991, p < 0.001$; adults: $F_{3,198} = 3.094, p = 0.028$).

During the winter (from mid December 2017 to early April 2018), only adults were found inside the galleries. Beetle eggs occurred from April to November 2018 with five major peaks: in early May (41.8%), mid-June (20.5%), late July (25.4%), early September (23.3%) and early October 2018 (21.1%). Larvae were sampled from late April to late November with four major peaks in mid-May (33.9%), late June (39.2%), early August (57%) and late September (33.8%), and a minor one in late October 2018 (21.2%). The amount of pupae, recovered from late May
to late November, followed the same trend as the larvae (Fig 1). Adults were always found infesting the sampled twigs, however their numbers were constant during the winter but variable from spring to autumn, with major peaks in early June (58.2%), late July (56.5%), and early September 2018 (47.7%).

The mean diameter of infested carob twigs was 7.14 mm (±0.34), but varied significantly according to the season ($F_{3,198} = 17.061; p < 0.001$) (Fig 2). Results of the post-hoc Bonferroni test showed that the mean diameter of infested twigs during the summer was significantly

---

Fig 1. Annual trend of the *Xylosandrus compactus* population structure on carob trees in Sicily (Southern Italy) from November 2017 to December 2018. Mean percentage of eggs, larvae, pupae and adults of *X. compactus* found inside the sampled carob twigs. During winter only inactive (overwintering) adults were found. The dashed line represents the biweekly mean temperature trend. Seasons: W = winter, Sp = spring, Su = summer, A = autumn.

https://doi.org/10.1371/journal.pone.0239011.g001

Fig 2. Mean (±SE) diameter of carob twigs infested by *Xylosandrus compactus* from November 2017 to December 2018 in Sicily (Southern Italy). Seasons: W = winter, Sp = spring, Su = summer, A = autumn.

https://doi.org/10.1371/journal.pone.0239011.g002
different from the other seasons (summer vs spring, summer vs autumn, and summer vs winter: \( p < 0.001 \)). However, no significant difference was found between the mean infested diameter during spring, autumn and winter (spring vs autumn: \( p = 0.984 \); spring vs winter and autumn vs winter: \( p = 1.000 \)). Specifically, during the winter, it ranged from 7.17 ± 0.87 mm in January to 10.86 ± 1.47 mm in March. While, during the summer, the mean diameter of infested twigs ranged from 4.50 ± 0.22 mm in June to 5.50 ± 0.34 mm in August. This value also decreased during the spring and was higher during the winter (Fig 2).

There was no significant difference between the number of adults/gallery among the seasons (\( F_{3,136} = 0.137; p = 0.938 \)). The mean value of adults/gallery during the monitoring period was 19.21 ± 0.40 (mean ± SE), ranging from 18.91 ± 0.80 in winter to 19.58 ± 0.70 in autumn. Specifically, the minimum and maximum number of adults of *X. compactus* found inside a single gallery was 6 and 28, respectively.

The highest percentage of *X. compactus* dead adults, inside the infested twigs, was recorded from December to March (Fig 3), when the minimum temperatures (weekly mean) were consistently lower than 10 °C. The mortality peak (39.82% dead adults) occurred in the second half of January after several weeks with minimum daily temperatures lower than 5 °C. Results of the linear regression model (Fig 4) of the proportion of dead adults as a function of the minimum temperature trend showed that the temperature affected significantly the mortality of overwintering adults (\( R^2 = 0.72; F_{1,13} = 33.434; p < 0.001 \)).

**Fungal isolation, identification and characterization**

A total of 54 fungal isolates belonging to different genus and species were obtained from galleries and beetles, and were identified using morphological and/or molecular analyses. In total, eight different species were associated with the beetle and/or galleries. Based on culture morphology, most isolates (13) were identified as *A. xylebori* and produced aleurioconidiophore
with single aleurioconidium, in agreement with the description of this species by Brader (1964) and von Arx & Hennebert (1965) [52, 53].

The following species were identified from the other isolates: *F. solani* (9 isolates), *Xenoacremonium recifei* (10), *Clonostachys rosea* (12), *Acremonium* sp. (1), *Cytospora* sp. (4), *Aureobasidium pullulans* (3), and *Penicillium* sp. (2). To confirm the taxonomic identification, BLAST searches were performed for representative isolates of each species. ITS sequences of isolates CR 32 and CR 18 showed 99.6% and 100% homology with the holotype strain of *A. xylebori* (CBS 110.61) and *F. solani* (FKKM2), respectively. Similarly, the ITS sequences of CR 31 and CR 58 showed 99.2% and 100% identity with *X. recifei* (CBS 137.35) and *C. rosea* (CBS 149.52), respectively.

*Fusarium* and *Ambrosiella* species were the most prevalent isolates from galleries, i.e., 25.71% and 37.14%, respectively. On the other hand, *X. recifei* and *C. rosea* were isolated from both galleries and beetles. Occasionally, *Acremonium* sp., *Cytospora* sp. and *Penicillium* sp. were obtained from galleries, while *A. pullulans* were obtained from beetles.

**Discussion**

This study provides the first data on the population structure and dynamics of *X. compactus* in Europe, and specifically on carob trees growing in Sicily (Southern Italy). The results of the annual beetle population trend show that, in the monitored environment, *X. compactus* overwinters inside twigs as adult and brood production begins in spring, after female emergence. Different generations were thus identified thanks to the study of the proportion of biological stages over the seasons (Fig 1). Egg peaks occurred in early May, mid-June, late July, early September and early October. These findings strongly suggest that the pest completed five
generations, being active and reproducing from April and starting to overwinter in late autumn. The same number of peaks was found for the larval stage, and the time interval between adult peaks (about 6 weeks) was sufficient for X. compactus to complete its biological cycle, to find a new host plant/twig and establish a new gallery. In agreement with our findings, Ngoan et al. (1976) and Hara (1977), found that the X. compactus life cycle, from egg to adult, lasted 28.5 days [38, 54]. In addition, the female progeny left parental galleries from 7 to 9 days after pupal ecdisys, starting to lay eggs from 4 to 14 days after initial boring in a new twig [39].

The results of this study support the data obtained by sampling flying X. compactus adults using ethanol-baited traps in an earlier study by Gugliuzzo et al. (2019) [29]. These authors reported that the first flight of the year occurred in April, with temperatures consistently higher than 20 °C, and that the flight activity stopped in autumn, when the daily mean temperature decreased rapidly. In addition, the presence of only adults inside the twigs during the cold season confirms that the pest overwintered as adults, in line with a study that investigated the unusual behaviour of beetles on carob branches and trunks [30]. Sheltered and inactive groups of X. compactus adults were also found by Pecancho et al. (2012) after a survey conducted on infested laurel plants in north western Italy [27]. Similarly, in Florida X. compactus were found to overwinter as adults inside successfully-infested twigs of flowering dogwood [38]. On the other hand, in Uganda the pest infesting robust a coffee was continuously active and all the biological stages occurred throughout the year [55].

Several studies report that this species prefers to attack small twigs and lateral small branches with a diameter <7 mm [22, 38, 56]. Our results showed that the mean infested diameter of carob twigs varied significantly over the seasons, reaching the minimum and maximum values during the summer (<4 mm) and the winter (>10 mm), respectively. In addition, during the cold season, the percentage of dead adults correlated negatively with the minimum temperature trend. This suggests that thicker twigs may represent a good shelter for X. compactus in order to survive the coldest periods. However, specific investigations are needed to verify these hypotheses. The beetle’s movement to larger twigs before overwintering indicates that pest control should be carried out by pruning and disposing the pruned material. Likewise, the preference shown by the beetle for small twigs during the summer suggests these twigs should be monitored and should be the focus of any possible control strategies.

In our study, the number of adults found inside carob galleries with only X. compactus adults ranged from 6 to 28 individuals per gallery. Considering galleries infested by both adults and young instars, other authors have reported that the number of specimens/gallery ranged from 3 to 36 on L. nobilis [24], 1 to 40 on Cornus florida L. [38] and 1 to 41 on Coffea canephora P. [22]. Similar data were also found by Gugliuzzo et al. (2019) on infested large branches and trunks of carob [29], where the mean number of adults/gallery was 19.98.

We found that the symbiotic fungus A. xylebori was the main ambrosia species associated with the black twig borer and isolated from galleries, as reported by Vannini et al. (2017) in Italy [26]. This species has been isolated from several host species in association with X. compactus worldwide [46, 57–60] and it has also been described in association with other beetle species, including congeneric taxa [61] and other taxa that are more distantly related [62]. Von Arx & Hennebert (1965) designated a type for the genus and species based on Brader’s isolate (CBS 110.61) [53]. In addition, as reported by Mayers et al. [60], Brader [52] and von Arx & Hennebert [53] described two types of aleurioconidiophores produced by the Ambrosiella species: one with disarticulating monilioid conidiophore cells, breaking off with attached aleuroconidia, and a second, straight, hyphoid aleurioconidiophore with a single, attached aleuroconidium. Ambrosiella xylebori has been described with the second conidiophore type.

Similarly, A. xylebori isolates in our collection produced single aleuroconidia from simple aleuroconidiophores, which likely do not disarticulate, and ITS sequences showed a high
homology with the holotype strain (CBS 110.61). Although the role of these fungi still needs to be determined, some knowledge is already available in literature. Ambrosiella xylebori is a primary symbiotic fungus which is typically transported in the mycetangium of X. compactus and supports insect growth in the host tree. All known Ambrosiella spp. produce a fruity aroma [63], and these chemical volatiles may play a key role in attracting ambrosia beetles within the galleries [64].

In addition to the primary symbiont, some often non-mycangial fungi, are also associated with ambrosia beetles [46]. In this study, we isolated a Fusarium species from infested galleries, here identified as F. solani. Fusarium solani is a name given to a complex "Fusarium solani Species Complex" (FSSC) of over 45 morphologically cryptic species [65] and a recent study showed that FSSC is actually another genus of the Nectriaceae family named Neocosmospora [66]. Members of this genus have been reported in association with X. compactus and other ambrosia beetles, and they are often reported as pathogenic to the host tree [22, 38, 44, 67], and to other woody crops (i.e., avocado) in Sicily [68]. A recent study also demonstrated that X. compactus females are attracted to several bioactive volatile compounds released by F. solani [67]. Wood tunneling by X. compactus females interrupts the transmission of water and nutrients within the plant, leading to wilting of the infested plant part within weeks [22, 38]. In addition, secondary pathogens, fungal symbionts, and a plant response likely contribute to the dieback [69].

The other fungi isolated from the sampled galleries were Acremonium sp., already reported by Bateman et al. [46], and Penicillium sp., reported in association with X. germanus [19]. The latter appears to be passively introduced during gallery excavation [70]. By contrast, to the best of our knowledge, this study reports for the first time the association of Cytospora sp., A. pullulans, X. recifei and C. rosea with this ambrosia beetle. Cytospora species are canker and dieback pathogens of woody hosts [71, 72], whereas A. pullulans, X. recifei and C. rosea have not been reported as pathogens, and their role still needs to be determined and merits further study. As reported by Hofstetter et al. (2006), some fungi consistently carried by bark beetles have been found to be commensal or even antagonistic [73]. In addition, the low isolation frequency of some isolates suggests either contamination or incidental association related to abiotic factors, which may influence the presence of fungi associated with X. compactus and the occurrence during the isolation process [42, 43, 59]. Considering that the fungi identification of this study is based on ITS sequences, a companion investigation is currently ongoing with the aim of sequencing additional loci of the fungal isolates associated with X. compactus. Such further study will focus on molecular characterization and multi-locus phylogeny allowing a definitive fungi identification at the species level.

Taken as a whole, our findings highlight that in this newly colonized environment the pest is able to complete five generations per year. Moreover, our results allowed to intercept the key steps of the population phenology and the spring first flight of the beetle. Thus, these data provide useful knowledge for ameliorating the monitoring and sustainable control of X. compactus, namely, winter pruning and removal of infested twigs coupled with summer monitoring of younger twigs may represent an effective and environmentally-friendly strategy for X. compactus control on carob trees.

This study was carried out in one of the very first areas (south east Sicily, Southern Italy) among those recently invaded by X. compactus in Europe, and on the main host plant severely affected by this pest in the described area, i.e. carob tree. However, considering the high invasive potential and wide host range of X. compactus, further investigations on its population dynamics and symbiotic associations in other environments and host plants would provide more definitive insights into the invasion process, multitrophic interactions and real potential damage of X. compactus in the Mediterranean Basin.
Supporting information

S1 Table. Raw datasets presented without restriction.
(XLSX)

Acknowledgments

This work was supported by the Regione Siciliana, Assessorato Regionale dell’Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea, Dipartimento Regionale dell’Agricoltura, Servizio 4 Servizio Fitosanitario Regionale e Lotta alla Contraffazione and by the University of Catania through the research agreements “Difesa fitosanitaria nei confronti dello scolittide del Carrubo Xylosandrus compactus (Eichhoff)” (5A725192026) and the project “Emergent Pests and Pathogens and Relative Sustainable Strategies”, respectively. Antonio Gugliuzzo received a PhD grant (PhD course in Agricultural Food and Environmental Science) from the University of Catania. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceptualization: Antonio Gugliuzzo, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia.

Data curation: Antonio Gugliuzzo, Giulio Criscione, Antonio Biondi, Dalia Aiello, Alessandro Vitale.

Formal analysis: Antonio Gugliuzzo, Antonio Biondi, Giovanna Tropea Garzia.

Funding acquisition: Antonio Biondi, Alessandro Vitale, Giovanna Tropea Garzia.

Investigation: Antonio Gugliuzzo, Giulio Criscione, Dalia Aiello, Alessandro Vitale, Giovanna Tropea Garzia.

Methodology: Antonio Gugliuzzo, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia.

Project administration: Giancarlo Polizzi, Giovanna Tropea Garzia.

Resources: Giancarlo Polizzi, Giovanna Tropea Garzia.

Supervision: Giancarlo Polizzi, Giovanna Tropea Garzia.

Validation: Antonio Gugliuzzo, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia.

Visualization: Antonio Gugliuzzo, Antonio Biondi, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia.

Writing – original draft: Antonio Gugliuzzo, Alessandro Vitale.

Writing – review & editing: Antonio Gugliuzzo, Antonio Biondi, Dalia Aiello, Alessandro Vitale, Giancarlo Polizzi, Giovanna Tropea Garzia.

References

1. Roques A, Rabitsch W, Rasplus JY, Lopez-Vaamonde C, Nentwig W, Kenis M. Alien terrestrial invertebrates of Europe. In: Handbook of alien species in Europe. Springer, Dordrecht; 2009. pp. 63–79.
2. Poland TM, Rassati D. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J Pest Sci. 2019; 92(1): 37–49.
3. Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM et al. No saturation in the accumulation of alien species worldwide. Nat Commun. 2017; 8(1): 1–9.

4. Biondi A, Guedes RNC, Wan FH, Desneux N. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, Tuta absoluta: past, present, and future. Annu Rev Entomol. 2018; 63: 239–258.

5. Větek G, Károlyi B, Mézáros A, Horváth D, Korányi D. The invasive brown marmorated stink bug (Halyomorpha halys) is now widespread in Hungary. Entomol Gen. 2018; 38(1): 3–14.

6. Bras A, Avtzis DN, Kenis M, Li H, Větek G, Bernard A, et al. A complex invasion story underlies the fast spread of the invasive box tree moth (Cydalima perspectalis) across Europe. J Pest Sci. 2019; 92: 1187–1202.

7. Lesieur V, Lombaert E, Guillemaud T, Courtial B, Strong W, Roques A, et al. The rapid spread of Leptoglossus occidentalis in Europe: A bridgehead invasion. J Pest Sci. 2019; 92: 1329–1342.

8. Guedes RNC, Roditakis E, Campos MR, Haddi K, Bielza P, Siqueira HAA, et al. Insecticide resistance in the tomato pinworm Tuta absoluta: patterns, spread, mechanisms, management and outlook. J Pest Sci. 2019; 92: 557–565.

9. Rodriguez-Flores MS, Seijo-Rodriguez A, Escuredo O, del Carmen Seijo-Coello M. Spreading of Vespula velutina in northwestern Spain: influence of elevation and meteorological factors and effect of bait trapping on target and non-target living organisms. J Pest Sci. 2019; 92(2): 239–258.

10. Desneux N, Decourtye A, Delpuech JM. The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol. 2007; 52: 189–200.

11. Philipp G, Neveen A, Marwa A, Basel AYA. Occurrence of pesticide residues in fruits and vegetables for the Eastern Mediterranean Region and potential impact on public health. Food Control. 2020; 107457.

12. Marini L, Haack RA, Rabaglia RJ, Toffolo EP, Battisti A, Faccoli M. Exploring associations between international trade and environmental factors with establishment patterns of exotic Scolytinae. Biol Invasions. 2011; 13(10): 2275–2288.

13. Rassati D, Lieutier F, Faccoli M. Alien wood-boring beetles in Mediterranean regions. In: Insects and diseases of Mediterranean forest systems. Springer, Cham; 2016. pp. 293–327.

14. Meurisse N, Rassati D, Hurley BP, Brockerhoff EG, Haack RA. Common pathways by which non-native forest insects move internationally and domestically. J Pest Sci. 2019; 92(1): 13–27.

15. Javal M, Roques A, Haran J, Hérad F, Keena M, Roux G. Complex invasion history of the Asian longhorned beetle: fifteen years after first detection in Europe. J Pest Sci. 2019; 92(1): 173–187.

16. Contarini M, Vannini A, Giarruzzo F, Faccoli M, Morales-Rodriguez C, Rossini L, et al. First record of Xylosandrus germanus (Blandford) (Coleoptera: Curculionidae: Scolytinae) in the Mediterranean scrubland in Southern Italy, and its co-presence with the co-generic species X. compactus (Eichhoff) and X. crassiusculus (Motschulsky). EPPO Bulletin. 2020. In press.

17. Francardi V, Penncchio F, Santini L, Rumine P, Paoli A, Navarra A, et al. First report of Xylosandrus compactus on Laurus nobilis in Tuscany. Giornate Fitopatologiche 2012, Milano Marittima (RA), 13–16 marzo 2012: 443–446.
26. Vannini A, Contarini M, Faccoli M, Valle MD, Rodriguez CM, Mazzetto T, et al. First report of the ambrosia beetle *Xylosandrus compactus* and associated fungi in the Mediterranean maquis in Italy, and new host–pest associations. EPPO Bulletin. 2017; 47(1): 100–103.

27. Pennacchio F, Santini L, Francardi V. Biocological notes on *Xylosandrus compactus* (Eichhoff) (Coleoptera Curculionidae: Scolytinae), a species recently recorded into Italy. Redia. 2012; 95: 67–77.

28. Francardi V, Noal A, Francescato S, Pinto R, Bruni A, Loffredi L, et al. Coexistence of *Xylosandrus crassiusculus* (Motschulsky) and *X. compactus* (Eichhoff) (Coleoptera Curculionidae Scolytinae) in the National Park of Circeo (Lazio, Italy). Redia. 2017; 100: 149–155.

29. Gugliuzza A, Criscione G, Tropea Garzia G. Unusual behavior of *Xylosandrus compactus* (Coleoptera: Scolytinae) on Carob trees in a Mediterranean environment. Insects. 2019; 10(3), 82.

30. Gugliuzza A, Criscione G, Siscaro G, Russo A, Tropea Garzia G. First data on the flight activity and distribution of the ambrosia beetle *Xylosandrus compactus* (Eichhoff) on carob trees in Sicily. EPPO Bulletin. 2019; 49(2): 340–351.

31. Gugliuzza A, Mazzeo G, Mansour R, Tropea Garzia G. Carob pests in the Mediterranean region: biodiversity, natural enemies and management options. Phytoparasitica. 2019; 47(5): 605–628.

32. Baumel A, Mirleau P, Viruel J, Bou Dagher Kharrat M, La Malfa S, Ouahmane L, et al. Assessment of plant species diversity associated with the carob tree (*Ceratonia siliqua*, Fabaceae) at the Mediterranean scale. Plant Ecol Evol. 2018; 151(2): 185–193.

33. Di Guardo M, Scollo F, Ninot A, Rovira M, Hermoso JF, Distefano G, et al. Genetic structure analysis and selection of a core collection for carob tree germplasm conservation and management. Tree Genetics & Genomes. 2019; 15(3): 41.

34. Viruel J, Le Galliot N, Pironon S, Feliner GN, Suc JP, Lakhal-Mirleau F, et al. A strong east-west Mediterranean divergence supports a new phylogeographic history of the carob tree (*Ceratonia siliqua*, Leguminosae) and multiple domestications from native populations. J Biogeogr. 2020; 47(2): 460–471.

35. Savarino G, Barbagallo RN. Carob processing in Sicily: technological aspects and products. Industrie Alimentari. 2009; 48(496): 36–45.

36. La Malfa S, Avola C, Brugaletta M, La Rosa G, Muratore G. Morphological and technological characterization of different carob cultivars in Sicily. Acta Hortic. 2012; 940: 207–212.

37. Ferrauto G., Pavone P. Palynological, physico-chemical and organoleptic characteristics of carob tree (*Ceratonia siliqua* L.) honey from Sicily. Int J Food Sci Technol. 2013; 48(8): 1596–1602.

38. Ngoan ND, Wilkinson RC, Short DE, Moses CS, Mangold JR. Biology of an introduced ambrosia beetle, *Ambrosiella xylebori* (Coleoptera: Curculionidae: Scolytinae) on Carob trees in a Mediterranean environment. EPPO Bulletin. 2017; 47(1): 100–103.

39. Bhat SS, Sreedharan K. Association of *Xylosandrus crassiusculus* (Eichhoff), a pest of robusta coffee. Journal of Coffee Research. 1988; 18(1): 54–57.

40. Hara AH, Beardsley JW. The biology of the black twig borer, *Xylosandrus compactus* (Eichhoff), in Hawaii. Proc Hawaii Entomol Soc. 1979; 18: 55–70.

41. Biedermann PH, Klepzig KD, Taborsky M. Fungus cultivation by ambrosia beetles: behavior and laboratory breeding success in three xyleborine species. Environ Entomol. 2009; 38(4): 1096–1105.

42. Biedermann PH, Vega FE. Ecology and Evolution of Insect-Fungus Mutualisms. Annu Rev Entomol. 2020; 65:431–455.

43. Muthappa BN, Venkatasubbaiah P. Association of *Ambrosiella macrospora* with *Xylosandrus compactus*, the shot-hole borer of robusta coffee in India. Journal of Coffee Research. 1981; 11(2): 54.

44. Bhat SS, Sreedharan K. Association of *Ambrosiella xylebori* Brader, with the shot-hole borer *Xylosandrus compactus* Eichhoff, a pest of robusta coffee. Journal of Coffee Research. 1988; 18(1): 54–57.

45. Bosso L, Senatore M, Varlese R, Ruocco M, Garonna AP, Bonanomi G, et al. Severe outbreak of *Fusarium solani* on Quercus ilex vectored by *Xylosandrus compactus*. J Plant Pathol. 2012; 94(4) (suppl.): S4.99.

46. Mayers CG, Harrington TC, Masuya H, Jordal BH, McNew DL, Shih HH, et al. Patterns of coevolution between ambrosia beetle mycangia and the Ceratocystidaeae, with five new fungal genera and seven new species. Persoonia. 2020; 44: 41–66.

47. Bateman C, Šigut M, Skelton J, Smith KE, Hulcr J. Fungal associates of the *Xylosandrus compactus* (Coleoptera: Curculionidae, Scolytinae) are spatially segregated on the insect body. Environ Entomol. 2016; 45(4): 883–890.

48. Skelton J, Johnson AJ, Jusino MA, Bateman CC, Li Y, Hulcr J. A selective fungal transport organ (mycangium) maintains coarse phylogenetic congruence between fungus-farming ambrosia beetles and their symbionts. Proc R Soc B. 2019; 286(1894): 20182127.

49. Li Y, Ruan YY, Stanley EL, Skelton J, Hulcr J. Plasticity of mycangia in *Xylosandrus ambrosia* beetles. Insect Sci. 2019; 26(4): 732–742.
50. White TJ, Bruns T, Lee SJ, Johnson JH, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990; 18(1): 315–322.

51. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016; 33(7): 1870–1874.

52. Brader L. Etude de la relation entre le scolyte des rameaux du cafeir, Xyleborus compactus Eichh. (X. morstatti/Hag.), et sa plante-hôte. Mededelingen van de Landbouwhogeschool Wageningen, Nederland. 1964; 64: 1–109.

53. Von Arx JA, Hennebert GL. Deux champignons ambrosia. Mycopathol Mycol Appl. 1965; 25: 309–315.

54. Hara AH. Biology and rearing of the black twig borer, Xylosandrus compactus (Eichhoff) in Hawaii. M. Sc. Thesis, University of Hawaii, Honolulu. 1977.

55. Egonyu JP, Ahumuzza G, Ogari I. Population dynamics of Xylosandrus compactus (Coleoptera: Curculionidae: Scolytinae) on Coffea canephora in the Lake Victoria Crescent agroecological zone of Uganda. Afr Zool. 2016; 51(3): 121–126.

56. Chong JH, Reid L, Williamson M. Distribution, host plants, and damage of the black twig borer, Xylosandrus compactus (Eichhoff), in South Carolina. J Agric Urban Entomol. 2008; 26(4): 199–209.

57. Alamouti SM, Tsui CKM, Breuil C. Multigene phylogeny of filamentous ambrosia fungi associated with ambrosia and bark beetles. Mycol Res. 2009; 113: 822–835.

58. Hayato M. Note on the dieback of Cornusflorida caused by Xylosandrus compactus. Bull Forestry Forest Prod Res Inst. 2007; 6: 59–63.

59. Kuo HC. Plant-fungus-beetle interactions: Case studies in Hawaiian endemic Xyleborus species and the black twig borer. Ph.D. dissertation, University of Hawaii at Manoa, Honolulu. 2010.

60. Mayers CG, McNew DL, Harrington TC, Roep RA, Fraedrich SW, Biedermann PH, et al. Three genera in the Ceratocystidiaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. Fungal Biol. 2015; 119(11): 1075–1092.

61. Gebhardt H, Weiss M, Oberwinkler F. Dryadomyces semassae: A nutritional fungus associated with ambrosia beetles of the genus Amsa (Coleoptera: Curculionidae, Scolytinae). Mycol Res. 2005; 109: 687–696.

62. Batra LR. Ambrosia fungi e a taxonomic revision, and nutritional studies of some species. Mycologia. 1967; 59: 976–1017.

63. Harrington TC. The genus Ceratocystis. Where does the oak wilt fungus fit? 2009. In: Billings RF, Appel DN (eds), Proceedings of the 2nd National Oak Wilt Symposium Texas Forest Service Publication 166, Austin, Texas, pp. 21–35.

64. Hulcr J, Mann R, Stelinski L. The scent of a partner: ambrosia beetles are attracted to volatiles from their fungal symbionts. J Chem Ecol. 2011; 37: 1374–1377.

65. O’Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME, et al. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the Fusarium solani species complex. J Clin Microbiol. 2008; 46(8): 2477–2487.

66. Lombard L, Van der Merwe NA, Groenewald JZ, Crous PW. Generic concepts in Nectriaceae. Stud Mycol. 2015; 80: 189–245.

67. Egonyu JP, Torto B. Responses of the ambrosia beetle Xylosandrus compactus (Coleoptera: Curculionidae: Scolytinae) to volatile constituents of its symbiotic fungus Fusarium solani (Hypocreales: Nectriaceae). Arthropod Plant Interact. 2018; 12: 9–20.

68. Guarnaccia V, Sandoval-Denis M, Aiello D, Polizzi G, Crous PW. Neocosmospora perseae sp. nov., causing trunk cankers on avocado in Italy. Fungal Syst Evol. 2018; 1(1): 131–140.

69. Paine TD, Raffa KF, Harrington TC. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu Rev Entomol. 1997; 42: 179–206.

70. Ranger CM, Biedermann PH, Phuntumart V, Beligala GU, Ghosh S, Palmquist DE, et al. Symbiont selection via alcohol benefits fungus farming by ambrosia beetles. Proc Natl Acad Sci. 2018; 115(17): 4447–4452.

71. Lawrence DP, Holland LA, Nouri MT, Travadon R, Abramsian A, Michailides T, et al. Molecular phylogeny of Cytospora species associated with canker diseases of fruit and nut crops in California, with the descriptions of ten new species and one new combination. IMA Fungus. 2018: 9: 333–370.

72. Aiello D, Polizzi G, Gusella G, Fiorenza A, Guarnaccia V. Characterization of Eutypa lata and Cytospora pistaciae causing dieback and canker of pistachio in Italy. Phytopathol Mediterr. 2019; 58(3): 699–706.

73. Hofstetter RW, Cronin JT, Klepzig KD, Moser JC, Ayres MP. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia. 2006; 147: 679–691.