Research Article

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Lethal and sublethal effects of synthetic and bioinsecticides toward the invasive ambrosia beetle *Xylosandrus compactus*

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Abstract

BACKGROUND: Exotic ambrosia beetles are emerging widespread pests of several wild and managed trees and shrubs. *Xylosandrus compactus* (Eichhoff) is one of the most invasive species causing damage to a broad range of host plants. Little information is available on its control, including the impact of insecticides. Bioassays were conducted to evaluate the potential of four bioinsecticides and seven synthetic insecticides in controlling *X. compactus*. Beetle mortality and sublethal effects on tunneling, cultivation of the mutualist fungus and reproduction were assessed.

RESULTS: Concentration–mortality curves were determined for all tested insecticides. Lambda-cyhalothrin was the most toxic insecticide, showing the lowest estimated 90% and 50% lethal concentrations (LC_{90} and LC_{50}), followed by deltamethrin and thiamethoxam. Acetamiprid caused the highest levels of mortality and brood size reduction under extended laboratory conditions. Moreover, acetamiprid, thiamethoxam and lambda-cyhalothrin caused the greatest mortality and, together with deltamethrin, strongly affected progeny occurrence inside infested galleries and beetle brood size. Among the bioinsecticides, pyrethrins significantly affected beetle survival under laboratory conditions, but not brood size in extended laboratory bioassays. Some of the tested insecticides had significant lethal and sublethal effects only when beetles were exposed to fresher residues, highlighting differences in toxicity persistence.

CONCLUSION: This study provides first baseline toxicity data for synthetic insecticides and bioinsecticides with different modes of action and origin toward *X. compactus*, and the first evidence that several insecticides can cause multiple sublethal effects on this pest. These findings can help in building suitable integrated pest management packages against this pest.

Supporting information may be found in the online version of this article.

Keywords: biopesticides; integrated pest management; invasive species; neonicotinoids; pyrethroids; Xyleborini

1 INTRODUCTION

Several species of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) are rapidly spreading across the globe owing to increasing global trade in wood products and climate change.[1–4] Indeed, reports of the invasion of non-indigenous ambrosia beetles in new areas are constantly growing, with more than 50 species belonging to the tribe Xyleborini already established outside their native range.[5,6] Among them, different species from the genus *Xylosandrus* are emerging pests that are able to cause serious damage to several trees and shrubs growing in forests, nurseries, orchards, and urban areas.[7,8] In particular, *Xylosandrus compactus* (Eichhoff), *X. crassiusculus* (Motschulsky), and *X. germanus* (Blandford) are the main widespread invasive species of this genus.[8–11]

Xylosandrus compactus, commonly known as the black twig borer, is native to Southeast Asia and is currently widely distributed in Africa, Asia, southeastern USA, South America and several European countries.[12] Contrary to most beetles of this group, this pest is able to attack both stressed and apparently healthy plants of 220 species belonging to 62 different families, including trees, shrubs and agricultural crops.[8,13,14] *Xylosandrus compactus* females preferentially colonize twigs and small branches where they bore galleries and cultivate species-specific ambrosia fungi that represent the only food source for their progeny.[15,16]

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Dispersing beetle females are consistently associated with *Ambrosiella xylebori* Brader ex Arx (Microascales: Ceratocystidaceae), the beetle primary mycetangial mutualist.[17–20] However, several other microorganisms have been found in association with *X. compactus*, such as members of the *Fusarium solani* complex, *Acremonium* sp. and different other fungi including plant pathogens, commensals or even antagonists.[21–23]

The beetle exhibits a cryptic life cycle because different biological stages develop, by feeding on mutualistic fungi, protected inside the plant xylem. Similar to other ambrosia beetles belonging to the tribe Xyleborini, only beetle females are able to fly, find susceptible hosts and cause damage to plant tissues.[13,24,25] Males are flightless and rarely leave infested maternal galleries where mating among siblings occurs.[13,16] The cryptic nature of the beetle life cycle has a crucial role in the development of effective management strategies, which need to be primarily targeted at dispersing females before they successfully make tunnels in host plant xylem.[8] However, little has been documented about effective control strategies against *X. compactus* and no integrated pest management packages have been developed to date.

Various opportunistic predators and parasitoids are reported to feed on different beetle biological stages under field or laboratory conditions. However, none of the tested species was reported to effectively suppress pest populations under field conditions.[26–31] On the other hand, mycoparasitic fungi and antagonistic bacteria, such as *Trichoderma* spp. (Ascomycota: Hypocreales) and *Bacillus* spp., have been reported to affect *X. compactus* brood production.[32] The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) and *Aspergillus flavus* Link (Eurotiales: Trichocomaceae) have also been reported to affect *X. compactus* survival.[33,34]

Chemical control of *Xylosandrus* species is mainly based on preventive insecticide application to susceptible host trees, as reported mainly for *X. crassiusculus* and *X. germanus*.[7,8] However, there is a general lack of information about the potential chemical control of *X. compactus*. As reviewed by Gugliuzzo et al.[8] the little evidence currently available in the literature is based on the use of chlorpyrifos (currently banned in many countries) on flowering dogwood in Florida, causing more than 80% of *X. compactus* mortality,[35] and the use of abamectin by injection into the trunk of carob trees, which was not completely effective against the beetle in Spain.[36] Moreover, potential sublethal effects of insecticides toward this group of insects are worthy of investigation. Indeed, sublethal effects are a key component of insecticide toxicology being major population dynamic stressors in the exposed organisms, including insect target pests.[37,38]

In this context, this study aims to provide baseline toxicity data for insecticides with different modes of action (MoA) and origin toward *X. compactus*. Specifically, we assessed the potential of different bioinsecticides and synthetic insecticides to control the invasive ambrosia beetle in laboratory and extended laboratory bioassays.

2 MATERIALS AND METHODS

2.1 Beetle laboratory rearing

Overwintered beetle females were used to start the *X. compactus* laboratory colony. For this, carob branches exhibiting symptoms of beetle infestation were collected in Ragusa (36°53′20″N, 14°33′19″E), Italy, during winter 2018 and placed inside plastic boxes, brought to the laboratory, and maintained at 25 ± 2°C and 60% ± 10% relative humidity (RH). In the following weeks, newly emerging *X. compactus* females were collected and observed under a stereomicroscope for morphological identification.[39] Collected beetle females were then transferred on sterile filter paper to Petri dishes and kept for 3 days. Meanwhile, to accelerate the loss of potential microbial contaminants, beetles were subjected to three cycles of 12 h on moist filter paper followed by 12 h of dry filter paper.[40]

Two *X. compactus* laboratory colonies were developed on two different susceptible host plants, namely carob (*Ceratonia siliqua* L.) and laurel (*Laurus nobilis* L.), used in the laboratory and extended laboratory bioassays, respectively. Concerning laboratory bioassays, beetles were reared by using carob healthy twigs (cultivar Latinissima) collected from wild unmanaged trees (Catania, Italy). Selected twigs (diameter of 5–7 mm) were cut into 15-cm sections and their ends were sealed with Parafilm® strips to minimize drying. The obtained twig sections were then soaked in an aqueous solution of 10% ethanol for 2 h to increase their attraction to beetle females.[32] Soon after the immersion, twig sections were dried for 30 min under a laminar flow hood before moving to individual sterile glass tubes (25 × 250 mm culture tubes). For the extended laboratory bioassays, beetles were reared using the methodology described for laboratory bioassays but with laurel as the host plant, using healthy stems (diameter of 7–14 mm) collected at the campus of the University of Catania (Catania, Italy). Beetle females were surface-sterilized using 70% ethanol and deionized water to further reduce the occurrence of microbial contaminants on the body surface,[40] and then released inside glass tubes.

Between five and eight *X. compactus* females were released in each rearing tube. After beetle release, the tubes were closed with cellulose acetate plugs, placed in darkness at 25 ± 2°C and 60% ± 10% RH and moistened when necessary. Coetaneous newly emerging adults of the progeny, occurring 5 to 6 weeks after the foundresses release inside tubes, were collected, managed as indicated above and used for bioassays.

2.2 Tested insecticides

The commercial bioinsecticides and synthetic insecticides, differing by MoA and origin, that were tested in both bioassays are shown in Table 1. Because the use of insecticides for the control of *X. compactus* in newly invaded European countries is not yet authorized, we tested various commercial synthetic insecticides and bioinsecticides commonly used in Italy and many other countries around the world as tools to control (in nursery and/or field conditions) Scolytinae or similar target pests.[41–43]

2.3 Laboratory bioassays

For each tested insecticide, seven increasing concentrations were tested, according to preliminary observations aimed at identifying the minimum concentration causing a mortality level not significantly different from the untreated control (distilled water only) and including highest label rates.[44] Bioassays were conducted using carob twig sections (diameter of 5–7 mm and 15 cm long) appositely sealed at the ends with Parafilm®, immersed for 2 h in 10% ethanol and left to dry for 30 min. These twig sections were then immersed and swirled into the different insecticide solutions or water (control) for 10 s before drying for 30 min. Each treated twig section was then transferred into a sterile glass tube (25 × 250 mm culture tubes). *Xylosandrus compactus* females from the laboratory rearing, and managed as indicated above, were released inside glass tubes. Specifically, five beetle females were released for each replicate (tube). There were 8 replicates (tubes) and 40 beetles for each tested concentration and

TABLE 1. Overview of synthetic and bioinsecticides tested against *Xylosandrus compactus* in laboratory and extended laboratory bioassays

Active ingredient (a.i)	Trade name	Concentration a. i. 100 g^{-1}	Label rate	Chemical family	Insecticide Resistance Action Committee mode of action classification
Bioinsecticides					
Azadirachtin	Neemazal®-T/S (Biogard)	1 g	0.030 mg L^{-1}	Botanical	Compounds of unknown or uncertain mode of action
Beauveria bassiana ATCC 74040	Naturalis® (Biogard)	0.0185 g	0.0006 mg L^{-1}	*B. bassiana* strains	Fungal agents of unknown or uncertain mode of action
Pyrethrins	Biopiren plus® (Biogard)	2 g	0.040 mg L^{-1}	Pyrethrins	Sodium channel modulators
Spinosad	Laser™ (Corteva)	44.2 g	0.332 mg L^{-1}	Spinosyns	Nicotinic acetylcholine receptor allosteric modulators – Site I
Synthetic insecticides					
Abamectin	Cal-ex® 1.9 EW (Cheminova)	1.89 g	0.019 mg L^{-1}	Avermectins	Glutamate-gated chloride channel allosteric modulators
Acetamiprid	Epik® SL (Sipcam)	4.67 g	0.140 mg L^{-1}	Neonicotinoids	Nicotinic acetylcholine receptor competitive modulators
Chlorantraniliprole	Coragen® (Cheminova)	18.4 g	0.037 mg L^{-1}	Diamides	Ryanodine receptor modulators
Deltamethrin	Decis® Evo (Bayer)	2.42 g	0.024 mg L^{-1}	Pyrethroids	Sodium channel modulators
Lambda-cyhalothrin	Karate Zeon® 1.5 (Syngenta)	1.47 g	0.022 mg L^{-1}	Pyrethroids	Sodium channel modulators
Sulfoxaflor	Closer™ (Corteva)	11.9 g	0.048 mg L^{-1}	Sulfoximines	Nicotinic acetylcholine receptor competitive modulators
Thiamethoxam	Actara® 25 WG (Syngenta)	25 g	0.100 mg L^{-1}	Neonicotinoids	Nicotinic acetylcholine receptor competitive modulators

untreated control. Tubes were closed using wet cellulose acetate plugs, stored in darkness and maintained at 25 ± 2°C and 60% ± 10% RH for 14 days.

Insecticide lethal effects (cumulative mortality) were evaluated 24 h, 48 h and 14 days after adult female release. Beetle adults were considered dead when they did not react after being touched with a paintbrush. Moreover, evidence of tunneling (gallery establishment), mutualist growth, and progeny occurrence were recorded to detect any potential sublethal effects of the tested substances. For this, 14 days after beetle release inside tubes, twig sections were carefully dissected and examined under a stereomicroscope. Brood size (the number of eggs and larvae) was recorded for each beetle female that survived the treatment and was able to establish a maternal gallery and cultivate the fungal mutualist.

2.4 Extended laboratory bioassays

The same insecticides tested in the laboratory were used in extended laboratory bioassays by spraying laurel plants with the highest label rates (Table 1).[45] In particular, potted laurel plants (pot diameter = 21 cm) were: (i) grouped in randomized blocks, (ii) physiologically stressed by flooding, and (iii) sprayed until runoff with the different insecticides or water only (control). Untreated laurel plants were approximately 3 years old and 1.5–1.8 m tall. Flood stress was imposed starting from 4 days before treatment using a pot-in-pot system and was maintained throughout the duration of the bioassays to simulate stress conditions and make trees susceptible to beetle attack.[46,47] For this purpose, empty 24 cm (diameter) pots were first lined with plastic bags. Potted laurel plants were then placed in the plastic lined plot and irrigated until there was standing water around the base of the tree. Each block (12 in total), including 15 potted plants, was sprayed with insecticides solutions from a distance of 0.5 m using a 2 L hand sprayer (Dea 2000 Volpi®, Italy) until runoff and left to dry.

The stems of treated laurel plants were exposed in the laboratory to dispersing coetaneous beetle females to estimate toxicity persistence over the time, at three time intervals after treatment (T0, day of treatment; DAT3, 3 days after treatment; DAT7, 7 days after treatment). For each of the three exposure times, five plants from each treatment were transferred to the laboratory and the main stem was cut into two different sections (diameter of 14–18 mm and 15 cm long). Stem sections were then sealed at the ends with Parafilm® and transferred singly to sterile glass tubes (25 × 250 mm culture tube) before beetle release. There were ten replicates (stem sections) for each tested insecticide (plus an untreated control) for each of the three tested time intervals. Five beetle females, appositely managed as indicated above, were released for each replicate. Tubes were closed with cellulose acetate plugs soon after beetle release, stored in darkness and maintained at 25 ± 2°C, 60% ± 10% RH for 14 days.

Lethal and sublethal effects caused by the tested insecticides on *X. compactus*, at different exposure intervals (residual effect), were evaluated using the same methodology as the laboratory bioassays (Section 2.3). Beetle cumulative mortality was evaluated at 24 h, 48 h and 14 days after beetle release inside tubes, whereas evidence of tunneling (gallery establishment), mutualist growth, progeny occurrence, and brood size (number of progeny produced by beetle females) were recorded 14 days after beetle release by carefully dissecting the infested stem sections.

2.5 Data analysis

The normality and homogeneity of variance of the dependent variables were checked through Kolmogorov–Smirnov and

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Shapiro–Wilk tests and the data set was log-transformed whenever needed. The mean percentages of: (i) beetles producing galleries (evidence of tunneling), (ii) galleries with mutualist growth, and (iii) beetles producing progeny were calculated. The brood size (mean number of progeny produced by foundresses) was also calculated. For extended laboratory bioassays, the brood size was calculated considering only those beetle females surviving insecticide treatment and untreated control groups. Thiamethoxam was excluded from the data analysis (concerning brood size) at T0 and DAT3 because no progeny occurred for this treatment (100% beetle mortality) at these two exposure times.

A Probit analysis was performed to estimate the 50% and 90% lethal concentrations (LC_{50} and LC_{90}) of the different insecticides after 48 h of exposure in the laboratory. Values were considered significantly different whether their 95% fiducial limits did not overlap.[48] Considering the latent period of infection needed by entomopathogenic fungi to develop in the host, lethal concentrations (after 48 h of exposure) of the tested *B. bassiana* were not calculated. For each treatment, beetle mortality was corrected using corresponding control mortalities by means of Abbott's formula if required. Because the data did not fulfil the assumptions for analysis of variance (ANOVA), for lethal and sublethal effect assessment in laboratory bioassays, the non-parametric Kruskal–Wallis test followed by Dunn's post hoc test ($p < 0.05$) were carried out for multiple mean comparisons among concentrations of the same insecticide. One-way ANOVA followed by Tukey's HSD post hoc tests ($p < 0.05$) were carried out with the data on lethal and sublethal effects in extended laboratory bioassays. Statistical analyses were carried out using IBM® SPSS® Statistics for Macintosh, Version 23.0.0.0 (IBM Corp., Armonk, NYUSA).

3 RESULTS

3.1 Laboratory bioassays

3.1.1 *Acute toxicity of insecticides in the laboratory*

The Probit models were fitted to estimate the lethal concentrations for each tested insecticide. No significant difference was found between observed and expected values (Table 2). Among the tested bioinsecticides, pyrethrins and spinosad caused the highest cumulative mortality levels, reaching 100% beetle mortality when tested at the two highest concentrations (Figure S1 and Table S1). By contrast, the *B. bassiana* treatment significantly affected beetle survival only after 14 days of exposure, when tested at the three highest concentrations (Figure S1 and Table S1). Azadirachtin slightly affected *X. compactus* survival, reaching a maximum of 55% beetle mortality when tested at the highest concentration (Figure S1 and Table S1). Azadirachtin also presented low toxicity to the beetle, as highlighted by the high LC_{90} values estimated for this compound (Table 2). Lowest estimated LC_{90} and LC_{50} values were obtained with pyrethrins, being the most toxic among the tested bioinsecticides (Table 2).

Acetamiprid, deltamethrin, lambda-cyhalothrin and thiamethoxam caused the highest cumulative mortality levels among tested synthetic insecticides (Figure S2 and Table S1), reaching 100% *X. compactus* mortality (after 48 h of exposure) and presenting the lowest estimated LC_{90} values (Table 2). Chlorantraniliprole caused an evident increase in beetle mortality only after 14 days of exposure at the highest tested concentrations (Figure S2 and Table S1). However, estimated LC_{90} values for this active ingredient showed relatively low toxicity against the invasive pest

TABLE 2. Baseline toxicity of synthetic and bioinsecticides tested against *Xylosandrus compactus* colonizing previously treated carob (*Ceratonia siliqua* L.) twig sections

Insecticide	*n*	Slope ± SE	χ^2 (df)	*p*-value	Lethal concentration (a.i. mg L^{-1})	95% Confidence limits (a.i. mg L^{-1})	LC/FR
Abamectin	280	0.356 ± 0.339	44.113 (54)	0.829	LC_{50} = 0.323	0.105–6.142	17.090
					LC_{90} = 18.938	1.770–13 326.462	1002.011
Acetamiprid	280	3.180 ± 0.325	25.690 (54)	1.000	LC_{50} = 0.038	0.031–0.047	0.271
					LC_{90} = 0.143	0.109–0.206	1.021
Azadirachtin	280	0.029 ± 0.322	35.489 (54)	0.976	LC_{50} = 0.909	0.228–65.690	30.300
					LC_{90} = 66.141	3.963–586 984.948	2204.700
Chlorantraniliprole	280	0.738 ± 0.279	27.744 (54)	0.999	LC_{50} = 0.163	0.090–0.497	4.837
					LC_{90} = 3.802	0.993–60.212	112.283
Deltamethrin	280	4.510 ± 0.444	24.122 (54)	1.000	LC_{50} = 0.008	0.007–0.010	0.372
					LC_{90} = 0.033	0.025–0.047	1.405
Lambda-cyhalothrin	280	8.623 ± 1.166	18.543 (54)	1.000	LC_{50} = 0.002	0.002–0.003	0.136
					LC_{90} = 0.006	0.005–0.008	0.272
Pyrethrins	280	4.256 ± 0.423	23.300 (54)	1.000	LC_{50} = 0.012	0.010–0.014	0.300
					LC_{90} = 0.045	0.034–0.065	1.125
Spinosad	280	1.184 ± 0.155	29.019 (54)	0.998	LC_{50} = 0.145	0.110–0.191	0.437
					LC_{90} = 1.172	0.754–2.251	3.535
Sulfoxaflor	280	0.432 ± 0.269	42.274 (54)	0.876	LC_{50} = 0.330	0.159–1.476	6.996
					LC_{90} = 8.844	1.829–292.156	187.353
Thiamethoxam	280	4.382 ± 0.518	14.759 (54)	1.000	LC_{50} = 0.011	0.009–0.014	0.110
					LC_{90} = 0.042	0.032–0.062	0.420

Note: Probit analysis was performed to estimate the 50% and 90% lethal concentrations (LC_{50} and LC_{90}) of the different insecticides after 48 h of exposure in the laboratory.
Abbreviations: χ^2, chi-square testing goodness of fit of concentration–mortality response; df, degrees of freedom; LC/FR, ratio between lethal concentration and field rate reported in the formulation label against the target pest; *n*, number of total replicates; SE, standard error.

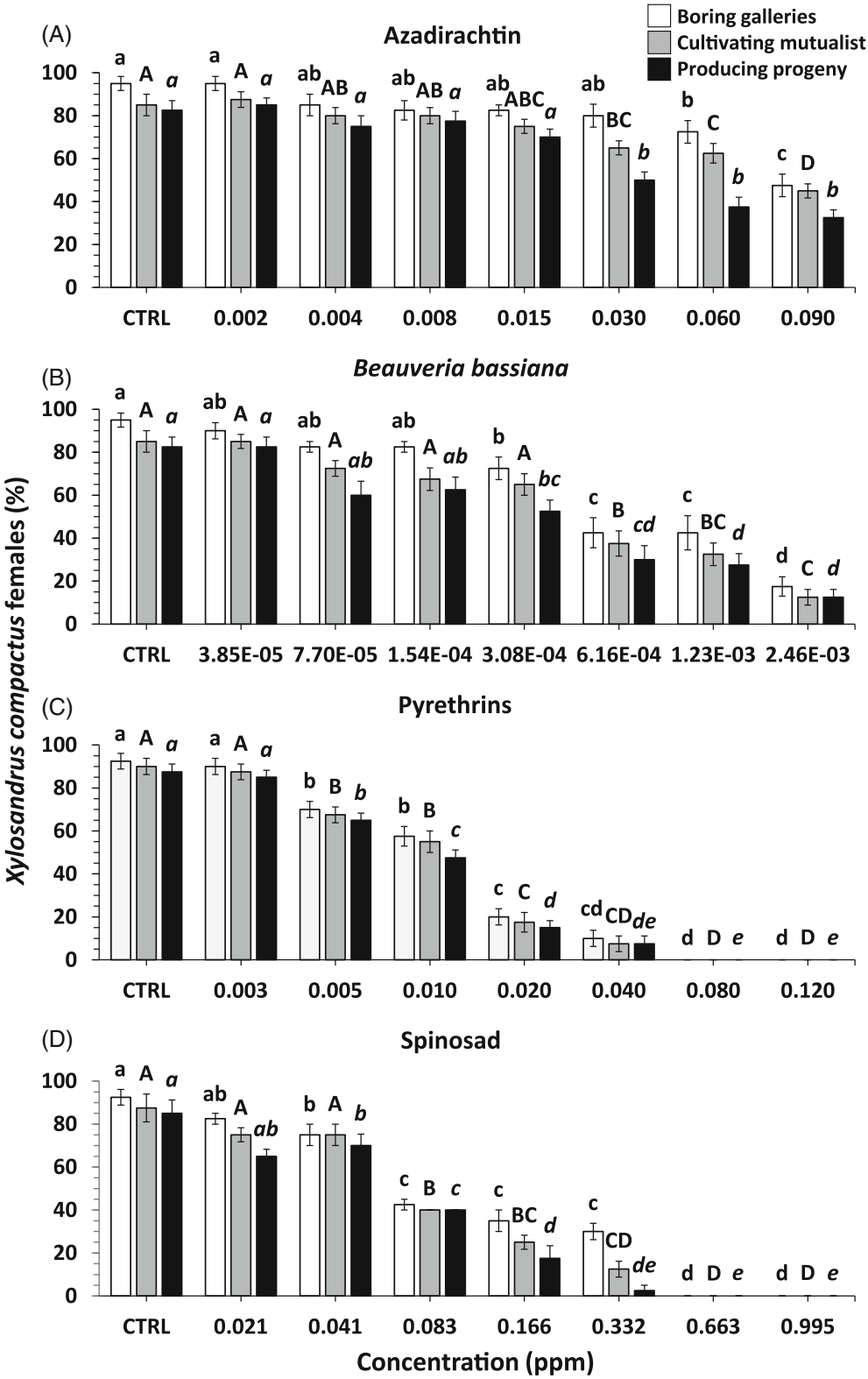

FIGURE 1. Impact of different concentrations of four bioinsecticides on *Xylosandrus compactus* gallery establishment (percentage of beetles boring galleries), mutualist growth and progeny occurrence (percentage of beetles cultivating mutualist and producing progeny inside infested galleries) after 14 days of exposure to treated carob twig sections. Within the data for each studied trait, means (±SE) with different letters are significantly different according to Kruskal–Wallis *H* test followed by Dunn's post hoc test for multiple comparisons at $p \leq 0.05$.

(Table 2). Abamectin and sulfoxaflor were least toxic against the beetle, showing the highest estimated LC_{90} and LC_{50} values (Table 2) and the lowest cumulative mortality levels (Figure S2). Lambda-cyhalothrin was instead the most toxic insecticide showing the lowest estimated LC_{90} and LC_{50} values, followed by deltamethrin and thiamethoxam (Table 2).

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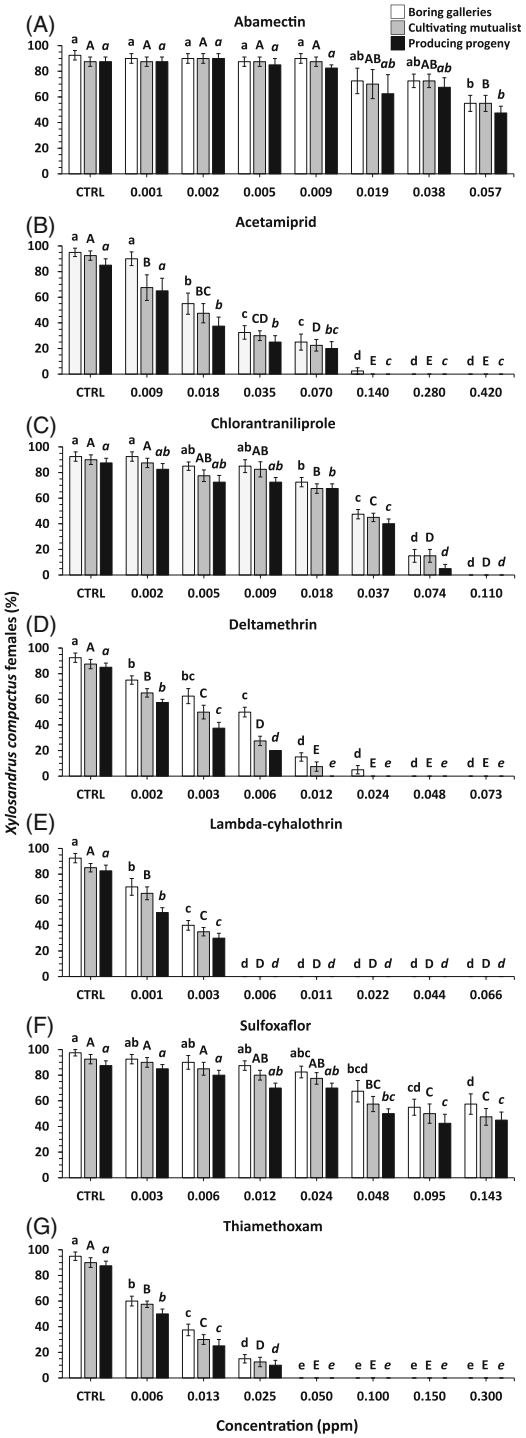

FIGURE 2. Impact of different concentrations of seven synthetic insecticides on *Xylosandrus compactus* gallery establishment (percentage of beetles boring galleries), mutualist growth and progeny occurrence (percentage of beetles cultivating mutualist and producing progeny inside infested galleries) after 14 days of exposure to treated carob twig sections. Within the data for each studied trait, means (±SE) with different letters are significantly different according to Kruskal–Wallis *H* test followed by Dunn's post hoc test for multiple comparisons at $p \leq 0.05$.

3.1.2 *Sublethal effects of insecticides in the laboratory*

Gallery establishment, mutualist growth and progeny occurrence were significantly affected by the tested concentration of all

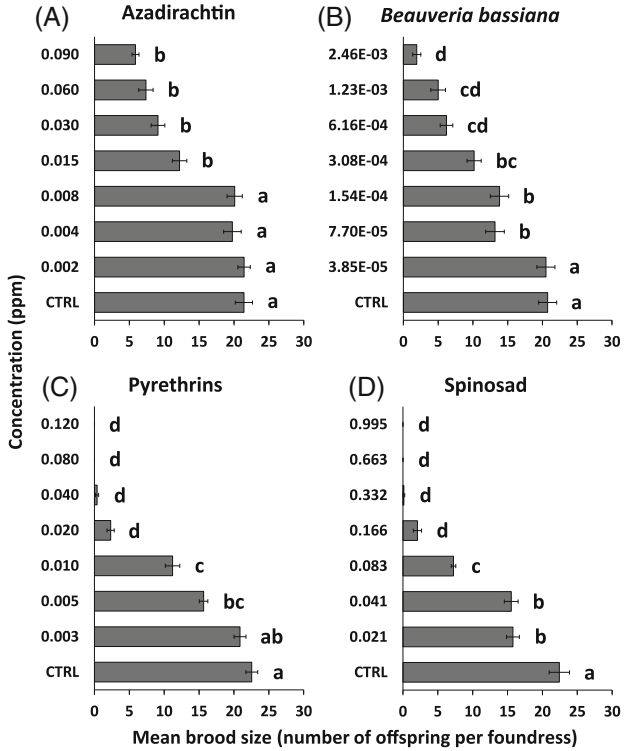

FIGURE 3. Impact of different concentrations of four bioinsecticides on *Xylosandrus compactus* brood size (mean number of progeny produced by beetle females) after 14 days of exposure to treated carob twig sections. Means (±SE) with different letters are significantly different according to Kruskal–Wallis *H* test followed by Dunn's post hoc test for multiple comparisons at $p \leq 0.05$.

bioinsecticides (Figure 1) and synthetic insecticides (Figure 2). In particular, the percentage of *X. compactus* females boring galleries significantly decreased to 0% for pyrethrins (df = 7,56; $H = 58.19$; $p < 0.001$) and spinosad (df = 7,56; $H = 58.88$; $p < 0.001$) treatments at the two highest concentrations (Figure 1C,D). Similar results were found for the percentage of beetle females cultivating the mutualist and producing progeny for both bioinsecticides. These latter parameters were also significantly affected by the *B. bassiana* treatment, with 12.5% of beetle females cultivating the mutualist (df = 7,56; $H = 49.57$; $p < 0.001$) and producing progeny (df = 7,56; $H = 47.44$; $p < 0.001$) when exposed to the highest tested concentration.

Among synthetic insecticides, lambda-cyhalothrin and thiamethoxam caused a drastic reduction in the percentage of beetle females cultivating mutualist (df = 7,56; $H = 62.20$; $p < 0.001$ and df = 7,56; $H = 59.29$; $p < 0.001$, respectively) and producing progeny (df = 7,56; $H = 62.21$; $p < 0.001$ and df = 7,56; $H = 56.61$; $p < 0.001$, respectively) also when tested at low concentrations (Figure 2E,G). Moreover, no mutualist growth and progeny production were found for *X. compactus* females exposed to high concentrations of acetamiprid and deltamethrin (Figure 2B,D).

Mean brood size was significantly affected by the tested concentration of all bioinsecticides (Figure 3) and synthetic insecticides (Figure 4). In particular, pyrethrins (df = 7,56; $H = 59.15$; $p < 0.001$) and spinosad (df = 7,56; $H = 59.99$; $p < 0.001$) caused the greatest reduction in brood size among the tested bioinsecticides (Figure 3). Concerning synthetic insecticides, the greatest impact in terms of *X. compactus* brood size reduction (Figure 4)

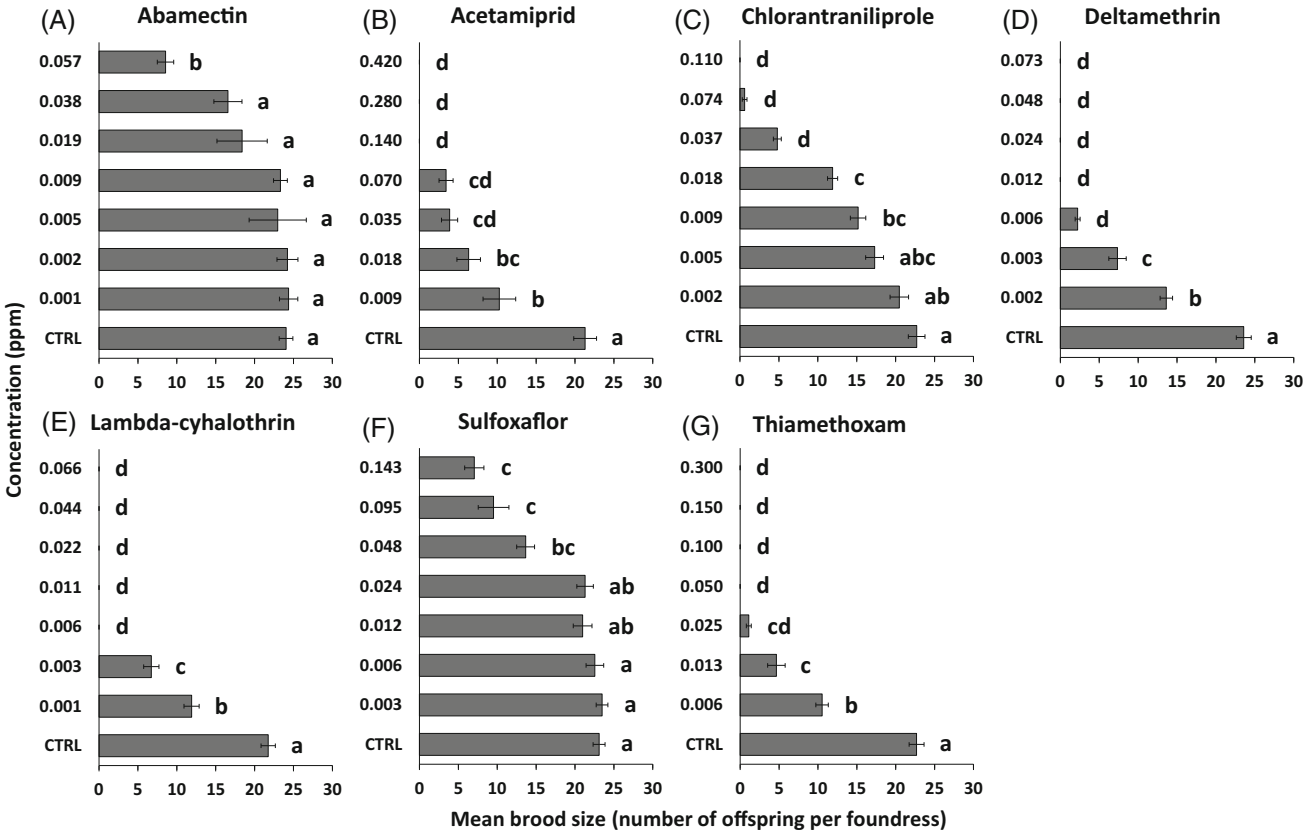

FIGURE 4. Impact of different concentrations of seven synthetic insecticides on *Xylosandrus compactus* brood size (mean number of progeny produced by beetle females) after 14 days of exposure to treated carob twig sections. Means (±SE) with different letters are significantly different according to Kruskal–Wallis *H* test followed by Dunn's post hoc test for multiple comparisons at $p \leq 0.05$.

was obtained with acetamiprid (df = 7,56; $H = 52.01$; $p < 0.001$), deltamethrin (df = 7,56; $H = 62.09$; $p < 0.001$), lambda-cyhalothrin (df = 7,56; $H = 62.10$; $p < 0.001$) and thiamethoxam (df = 7,56; $H = 57.15$; $p < 0.001$).

3.2 Extended laboratory bioassays

3.2.1 Lethal effects of insecticide label rates in extended laboratory conditions

Among the tested insecticides, only acetamiprid, lambda-cyhalothrin and thiamethoxam significantly affected the survival of *X. compactus* females after 24 h of exposure ($F_{11,108} = 21.78$; $p < 0.001$), concerning beetles exposed to laurel stems on the day of treatment (T0). No significant difference was found between the control and the other tested synthetic and bioinsecticides (Figure S3). In particular, a mortality rate of 6.00% ± 3.06% was recorded for the untreated control, whereas acetamiprid caused the highest average mortality (70.00% ± 5.37%). Increasing mortality rates were found after 48 h ($F_{11,108} = 42.16$; $p < 0.001$) and 14 days ($F_{11,108} = 30.11$; $p < 0.001$) of exposure, with the highest cumulative mortality caused by pyrethrins (14 days: 50.00% ± 5.37%) among the bioinsecticides, and by acetamiprid (48 h: 94.00% ± 3.06%; 14 days: 98.00% ± 2.00%), lambda-cyhalothrin (48 h: 58.00% ± 4.67%; 14 days: 80.00% ± 6.67%) and thiamethoxam (48 h: 80.00% ± 5.16%; 14 days: 100%) among the synthetic insecticides (Figure S3 and Figure 5).

A similar trend was observed for beetles exposed to laurel stems 3 days after treatment (DAT3), with the survival of *X. compactus* females exposed to chemical residues significantly affected by the treatment after 24 h ($F_{11,108} = 18.17$; $p < 0.001$), 48 h ($F_{11,108} = 45.97$; $p < 0.001$) and 14 days ($F_{11,108} = 42.34$; $p < 0.001$) of exposure. Highest cumulative mortality was found with pyrethrins (14 days: 54.00% ± 7.33%) among the bioinsecticides, and acetamiprid (48 h: 90.00% ± 4.47%; 14 days: 96.00% ± 2.67%), lambda-cyhalothrin (48 h: 46.00% ± 5.21%; 14 days: 84.00% ± 4.99%) and thiamethoxam (48 h: 86.00% ± 4.27%; 14 days: 100%) among the synthetic insecticides (Figure S3 and Figure 5).

When laurel stems were exposed to *X. compactus* females 7 days after treatment (DAT7), only acetamiprid and thiamethoxam residues significantly affected their survival (24 h: $F_{11,108} = 9.07$; $p < 0.001$; 48 h: $F_{11,108} = 20.64$; $p < 0.001$; 14 days: $F_{11,108} = 16.36$; $p < 0.001$). In particular, acetamiprid caused the highest average mortality both after 24 h (40.00% ± 5.16%), 48 h (74.00% ± 6.00%) and 14 days (86.00% ± 6.00%) of exposure (Figure S3 and Figure 5).

3.2.2 Sublethal effects of insecticide label rates in extended laboratory conditions

Treatment significantly affected the percentage of beetle females boring galleries (T0: $F_{11,108} = 28.52$; $p < 0.001$; DAT3: $F_{11,108} = 38.45$; $p < 0.001$; DAT7: $F_{11,108} = 16.36$; $p < 0.001$), cultivating the mutualist (T0: $F_{11,108} = 24.25$; $p < 0.001$; DAT3: $F_{11,108} = 32.70$; $p < 0.001$; DAT7: $F_{11,108} = 10.91$; $p < 0.001$) and producing progeny (T0: $F_{11,108} = 22.00$; $p < 0.001$; DAT3:

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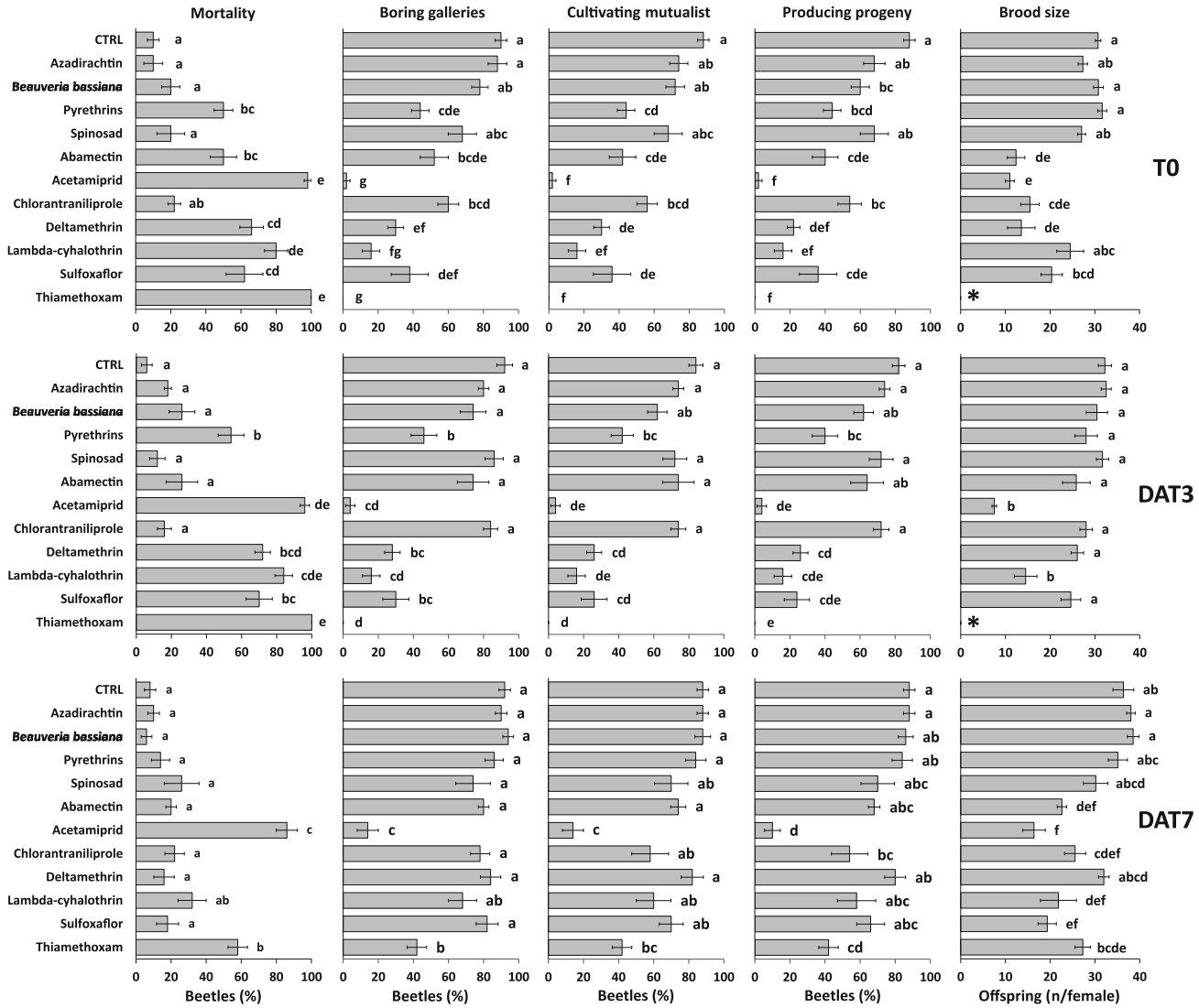

FIGURE 5. Impact of different synthetic and bioinsecticides on *Xylosandrus compactus* survival, gallery establishment (percentage of beetles boring galleries), mutualist growth and progeny occurrence (percentages of beetles cultivating mutualist and producing progeny inside infested galleries), and brood size (mean number of progeny produced by surviving beetle females only) after 14 days of exposure to treated laurel stems. Beetle exposure to treated laurel stems was conducted at three time intervals after the treatment (T0, day of treatment; DAT3, 3 days after treatment; DAT7, 7 days after treatment). Means (±SE) with different letters are significantly different according to Tukey's HSD post hoc test at $p \leq 0.05$. * Thiamethoxam was excluded from the data analysis (concerning brood size) at T0 and DAT3 because there was no progeny for this treatment (100% beetle mortality) at these two exposure time intervals.

$F_{11,108} = 29.29$; $p < 0.001$; DAT7: $F_{11,108} = 11.55$; $p < 0.001$). In particular, acetamiprid and thiamethoxam showed the greatest impact against beetle females by strongly affecting gallery establishment, mutualist growth and progeny production at all the tested time intervals (Figure 5).

The tested insecticides differently affected the brood size produced by *X. compactus* foundresses that survived to treatment at all tested time intervals (T0: $F_{10,82} = 18.53$; $p < 0.001$; DAT3: $F_{10,79} = 9.80$; $p < 0.001$; DAT7: $F_{11,102} = 11.34$; $p < 0.001$). No difference was found between the mean progeny of the untreated control and tested bioinsecticides at T0, DAT3 and DAT7 (Figure 5). On the contrary, among tested synthetic insecticides, acetamiprid caused the greatest brood size reduction, namely 64.21%, 76.73% and 54.95% less than the control at T0, DAT3 and DAT7, respectively.

4 DISCUSSION

Development of effective management strategies targeting invasive ambrosia beetles is greatly hampered by the cryptic nature of these fungus-farming insects, which are able to complete their life cycle protected within galleries excavated in wood. As a consequence, their control mainly relies on preventing gallery establishment by dispersing females.[8] For this reason, the chemical control of invasive *Xylosandrus* ambrosia beetles, *X. crassiusculus* and *X. germanus*, is conducted by preventive insecticide application to host trees.[7] However, very little has been documented on the potential efficacy of insecticides against other *Xylosandrus* species.[8]

The synthetic insecticides and bioinsecticides tested in the current study showed variable efficacy in protecting twigs and stems of susceptible plants from attack by the ambrosia beetle

X. compactus. In particular, the lowest infestation numbers among all treatments were obtained by testing pyrethroids (deltamethrin and lambda-cyhalothrin) and neonicotinoids (acetamiprid and thiamethoxam). To the best of our knowledge, this is the first evidence of the effectiveness of these substances against *X. compactus*. Pyrethroids such as bifenthrin and permethrin are recommended in ornamental tree nurseries for managing ambrosia beetles.[49] However, despite these two pyrethroids are being considered among the most effective insecticides against *X. crassiusculus* and *X. germanus*, results were often inconsistent.[46,50–53] Attacks of *X. germanus* were also significantly affected by lambda-cyhalothrin in a single apple tree trial, but its efficacy in other locations and environmental conditions remains to be properly assessed.[54] Moreover, Ranger et al.[55] observed variable efficacy in preventing attacks by ambrosia beetles, including *Xylosandrus* spp., by testing deltamethrin-treated nets covering the main stem of flood-stressed *Cercis canadensis* L. trees.

Some neonicotinoids, including thiamethoxam, were found to be not as effective in preventing attacks of *Xylosandrus* ambrosia beetles in some other studies.[43,50] By contrast, our results show that both acetamiprid and thiamethoxam were highly toxic against *X. compactus*, also when tested at low concentrations. Estimated lethal concentrations of these substances had lower or similar values compared to the field rate (FR; mg L^{-1}) (label dose), and the LC_{90}/FR ratio ranged from 0.42 for thiamethoxam to 1.02 for acetamiprid (Table 2). A combination of thiamethoxam and lambda-cyhalothrin was found as useful to suppress Scolytinae species in areas infested by the ambrosia beetle *X. glabratus* Eichhoff.[41] Similar results were obtained by Carrillo et al.[42] but the persistence of the tested formulation (thiamethoxam + lambda-cyhalothrin) under field conditions was significantly lower than other treatments consisting of pyrethroids only. High-level insecticidal activity was also recently found by testing newly synthesized neonicotinoids against another ambrosia beetle, namely *X. affinis* Eichhoff, highlighting the need to develop new analogs showing high toxicity against this ecological group of insects.[56]

Overall, in the current study, the most toxic insecticide against the beetle was the pyrethroid lambda-cyhalothrin (LC_{90} = 0.006 mg L^{-1}, LC_{90}/FR ratio = 0.27). Similar estimated LC_{90} values were found between pyrethrins (LC_{90} = 0.045 mg L^{-1}, LC_{90}/FR ratio = 1.13) and deltamethrin (LC_{90} = 0.033 mg L^{-1}, LC_{90}/FR ratio = 1.41). However, the tested neonicotinoids (acetamiprid and thiamethoxam) showed higher residual contact toxicity among all treatments when tested in extended laboratory trials (Figure S3 and Figure 5), indicating longer residual efficacy by these substances against *X. compactus*. These results corroborate those by Doerr et al.[57] who found acetamiprid and thiamethoxam to be among the most effective insecticides in affecting the survival of the bark beetle *Scolytus rugulosus* (Müller, 1818) during residual toxicity bioassays 21 days after treatment.

The rapid decrease in residual efficacy obtained in this study when testing pyrethrins and the pyrethroids deltamethrin and lambda-cyhalothrin (Figure S3) suggests the need for further studies to determine the frequency of protective sprays required to ensure effective control of this pest. Moreover, the potential toxicity of other pyrethroids, already evaluated in several US states against different ambrosia beetle species, should be tested against *X. compactus* in the newly invaded areas. For example, a reduction in *X. germanus* attacks up to 31 days post-application was observed in permethrin residual efficacy trials to protect ethanol-injected *Magnolia* trees.[51] Moreover, Brown et al.[53] found permethrin residues to be optimal in preventing *X. crassiusculus* attacks on ethanol-filled *Liriodendron tulipifera* L. bolts up to 17 days post-application. Otherwise, bifenthrin efficacy lasted only about 10 days, suggesting the need of more frequent applications.[51]

Despite our promising results concerning the efficacy of synthetic insecticides and bioinsecticides against *X. compactus* under laboratory and extended laboratory conditions, their efficacy remains to be properly assessed under nursery and field conditions. In this context, several aspects such as formulation type, extreme climatic conditions, application mode and frequency, among others should be considered as potential factors affecting the efficacy of different insecticides.[53,58] More importantly, nursery and field applications should be strictly timed with the beetle seasonal flight activity, which differs according to local climatic conditions.[25] Consequently, monitoring beetle populations by means of ethanol-baited traps or ethanol-soaked bolts represents a crucial step for the success of chemical control of this species.[52,59–62]

Although there is potential need to use insecticides against harmful ambrosia beetles in specific pest management context, especially when aimed at protecting high-value trees under stress conditions, their use could negatively affect non-target organisms, such as predators, parasitoids and pollinators, even at sublethal concentrations.[37,63–66] For this reason, the prevention of host tree stress should be considered as a fundamental to each management strategy targeting *Xylosandrus* species. Moreover, the use of eco-friendly biological control strategies, for example biopesticides, should be preferred to the use of broad-spectrum synthetic insecticides. In this context, we found pyrethrins as the most toxic bioinsecticide among those tested, but providing partial protection against the pest when tested in extended laboratory trials. The *B. bassiana* strain (ATCC 74040) we tested caused significant *X. compactus* mortality (52.5% and 77.5% after 14 days of exposure at the two highest tested concentrations) and reduced brood size only when tested under laboratory conditions; no efficacy was found under extended laboratory bioassays. This could be due to environmental factors affecting this fungus under field conditions (RH, ultraviolet light, temperature, host plant),[67] as well as to the selectivity of the tested strain for different target insects. Direct spray applications to *X. germanus* foundresses with the same *B. bassiana* strain significantly affected the beetle survival (60% or higher mortality) and brood production under laboratory conditions.[68] This entomopathogenic fungus was also highly virulent against *X. crassiusculus*, causing 50% mortality when tested at less than half of the dosage required for *X. germanus*.[69]

Our results show also how tested insecticides can cause sublethal effects on *X. compactus* individuals both when tested at low concentrations in laboratory bioassays and under extended laboratory conditions (using label rates). For example, acetamiprid not only caused the highest levels of mortality in extended laboratory bioassays, but also drastically reduced the number of offspring produced by beetle females that survived to the treatment (Figure 5), representing one of the most promising insecticides among those tested in this study for the chemical control of *X. compactus*. The tested bioinsecticides largely differed in their sublethal effects on the beetle. In particular, despite azadirachtin and *B. bassiana* showing slight negative effects on *X. compactus* survival, both bioinsecticides caused a significant reduction in offspring production by beetle females when exposed to high active

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ingredient concentrations in laboratory bioassays. By contrast, pyrethrins and spinosad, proved to cause acute mortality at the highest tested concentrations, negatively affected tunneling and mutualist cultivation by beetle females, as well as progeny production. These observations are consistent with already revealed properties of tested botanical insecticides showing repellent effects and thus probably reducing the beetle acceptance for treated stems.[70,71]

In conclusion, this study is the first providing data on the baseline toxicity and various sublethal effects caused by insecticides with different MoA and origin toward *X. compactus*. The obtained results will be validated through field studies with the aim to develop new sustainable pest management strategies targeting invasive ambrosia beetles.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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