INTRODUCTION

Ambrosia beetles (Coleoptera: Curculionidae) represent about 3,400 species within the subfamily Scolytinae and 1,400 species within the Platypodinae mostly living in the tropics (Hulcr et al., 2015). They bore into the xylem and feed on their symbiotic fungi, which they transport in specialized mycetangia (Vega & Biedermann, 2020). Mutualism between beetles and ambrosia fungi has independently
evolved several times (Johnson et al., 2018; Vanderpool et al., 2018), and ambrosia beetles represent a key research system for studies on symbioses (Hulcr et al., 2020). Ambrosia beetles, especially in the tribe Xyleborini, are highly successful as invasive species in new habitats (Gomez et al., 2018; Hulcr & Stelinski, 2017; Rassati et al., 2016). Their broad host range, cryptic habitat in woody tissue, haplodiploid reproduction, and sib-mating, along with massive international transport of wood products and woody dunnage have favored dispersal among continents (Gohli et al., 2016; Lantschner et al., 2020; Meurisse et al., 2019). More than 50 species are established outside their native range (Lantschner et al., 2020), including serious pests of trees growing in natural and managed habitats (Hughes et al., 2017; Ranger et al., 2016; Umeda et al., 2016).

Most ambrosia beetles colonize dying or recently dead hosts (Hulcr & Stelinski, 2017), but some species preferentially attack living but stressed trees (Hulcr et al., 2017; Ranger et al., 2015; Wang et al., 2021). To discern among stressed and healthy trees most ambrosia beetles exploit olfactory cues, in particular ethanol (Oliver & Mannion, 2001; Ranger et al., 2010, 2012, 2015, 2021; Reding et al., 2011; Werle et al., 2019), which is induced and emitted by trees stressed by abiotic (Kelsey et al., 2014; La Spina et al., 2013; Ranger et al., 2013, 2019) or biotic (Kelsey et al., 2013; McPherson et al., 2008) factors. Ethanol within host tissues can also influence ambrosia beetle colonization. Apparently-healthy trees baited with ethanol are attacked but not colonized by Xylosandrus germanus (Blandford) or other ambrosia beetles, while X. germanus foundresses establish fungal gardens and offspring within stems of trees irrigated with dilute ethanol solutions (Ranger et al., 2018). Ethanol incorporated into agar based media also promotes the growth of certain ambrosia beetle nutritional fungal symbionts and inhibits the growth of antagonistic fungi (Lehenberger et al., 2021; Ranger et al., 2018).

Ambrosia beetle host selection and colonization are also affected by the amount of ethanol associated with stressed trees (Kelsey & Joseph, 1997, 1999; Klimetzek et al., 1986; Ranger et al., 2011). Kelsey et al. (2013) documented four times more ambrosia beetle attacks above ethanol-infused sapwood tissue than in the opposite side of the same log. Xylosandrus germanus and Xyleborinus saxesenii were differentially attracted to bolts soaked in varying concentrations of ethanol; the number of entry holes decreased with increasing ethanol concentration for X. germanus and increased for X. saxesenii (Rassati et al., 2020). Only X. germanus established successful galleries, and the number of emerged X. germanus adults increased and then decreased with increasing ethanol concentrations (Rassati et al., 2020), following the pattern observed for the growth of its fungal symbiont (Ranger et al., 2018). These results suggest that different ambrosia beetle species are more attracted by a certain ethanol concentration over others, which might correspond to the optimal concentration at which their colonization is maximized.

Although most ambrosia beetles attack a broad range of species (Hulcr et al., 2007), certain tree species may be preferentially attacked over others (Egonyu et al., 2017; Mayfield & Hanula, 2012; Rassati et al., 2016; Reding & Ranger, 2020). For example, X. germanus preferentially attacks bolts of chestnut (Castanea sativa Mill.) (Rassati et al., 2016) or red maple (Acer rubrum L.) (Reding & Ranger, 2020) over a number of other broadleaved species. Xylosandrus crassiusculus entrance holes were more than five times higher on California bay laurel (Umbellularia californica (Hook. & Arn.) Nutt) than on camphor tree (Cinnamomum camphora (L.) J. Presl), and only a few holes or none at all were found on seven other species (Mayfield et al., 2013). Different tree species emit volatiles that might enhance or inhibit attraction of ambrosia beetles (Burbano et al., 2012; Martini et al., 2015; Owens et al., 2017; Pham et al., 2020; VanDerLaan & Ginzel, 2013; Yang et al., 2018). Moreover, Castrillo et al. (2012) demonstrated that X. germanus produces more progeny on sawdust-based artificial diet made with European buckthorn (Rhamnus cathartica L.) over American beech (Fagus americana L.), black walnut (Juglans nigra L.), and red oak (Quercus rubra L.). Menocal et al. (2018) showed better performance of Xyleborus bipinnatus Eichhoff on medium made from avocado (Persea americana Mill.) than medium made from silkbay (Persea humilis Nash.). Differential success of ambrosia beetle colonization on different tree species may reflect success of their fungal symbionts.

In the present study, we examined the effect of ethanol concentration and host tree species on host selection and colonization of ambrosia beetles. We hypothesized that different host tree species and different ethanol concentrations within the host tissues would influence interspecific differences in the attraction and colonization of ambrosia beetles. These results are discussed in relation to a niche partitioning mechanism whereby host tree and ethanol concentration might assist in reducing interspecific competition for resources of ambrosia beetle species with overlapping flight activity period.

2 | MATERIALS AND METHODS

2.1 | Study site, bolt preparation and experimental scheme

The study was conducted in a broadleaf forest (45° 17’ 14’’N; 11° 41’ 9’’E) located on the northern slope of Monte Fasolo (260 m a.s.l.) in the Euganean Hills area, Veneto region, north-eastern Italy (Figure S1). The forest is dominated by O. carpinifolia and Quercus pubescens Willd., with lesser amounts of C. sativa, Fraxinus ornus L., and Robinia pseudoacacia L., and was selected because it hosts a diversity of both native and exotic ambrosia beetle species. The climate is characterized by an annual mean temperature of approximately 14°C and precipitation of approximately 800 mm (Table S1).

In mid-May 2020, 96 bolts (diam. 6.0 ± 1.3 cm, length 30 ± 2.3 cm) were cut from branches of eight tree species (12 bolts per species), that is Acer platanoides L., C. sativa, Carpinus betulus L., Corylus avellana L., F. ornus, O. carpinifolia, Quercus robur L., and Picea abies (L.) H. Karst. (Figure S1 and Figure S2a). All are widespread in temperate forests of Europe and are hosts for many ambrosia beetles. For each selected tree species, bolts were obtained from three different standing trees (four bolts per tree). For
each bolt, a 10-cm deep hole was then made on one end using a 1.5-cm-diameter drill (hole vol. = 17.3 cm³). Six bolts of each species were randomly selected to receive a 5% (v:v) aqueous ethanol solution and six to receive 90% aqueous ethanol solution. The 5% concentration was selected based on previous host selection and colonization studies on X. saxesenii and Xylosandrus spp. (Ranger et al., 2018; Rassati et al., 2020), and the 90% concentration was selected because it is highly attractive to many ambrosia beetle species (Klingeman et al., 2017). The aqueous ethanol solution was poured into the drilled hole, which was then plugged with a silicon cap (Figure S1). Bolts were brought to the field on 22 May. Using plastic cable ties attached to an eye bolt screwed into the wood near the silicon cap (Figure S2a), bolts were hung approximately 2 m from the ground, a height at which ambrosia beetles are abundant (Marchioro et al., 2020; Miller et al., 2020). Bolts of the eight different tree species were hung 5 m apart in randomized complete blocks, with alternating blocks for each ethanol concentration 40 m apart (Figure S1). We did not test the two ethanol concentrations within the same block because we wanted to avoid potential repellent effects of the highest ethanol concentration. Bolts were checked every week until 10 July when bolts were brought back to the lab. At each check, the ethanol solutions were replenished, and entry holes bored during the previous week were circled with a permanent marker using a different colour for each week (Figure S2b).

2.2 | Analysis of ambrosia beetle attacks and colonization

In the laboratory adult females blocking entry holes (Biedermann & Taborsky, 2011; Nuotclà et al., 2019) were carefully excised avoiding damage to the entry hole and then were identified to species. Width of adult beetles was measured with a stage micrometer under a stereomicroscope. Pins of a thickness comparable to the width of beetles of each species were used to measure entry hole diameter. This methodology allowed us to reliably assign entry holes to X. saxesenii and X. crassiusculus. The other two colonizing species, A. dispar and the auger beetle, Scobicia chevrieri (Villa & Villa), have the same width and entry hole diameter. For the latter species, we analysed only galleries in which we found and identified an adult beetle. At the end of September, bolts were peeled using an electric planer (Figure S2c,d) and galleries were classified as either developed or superficial (Figure S3), indicating successful or unsuccessful attack, respectively.

2.3 | Statistical analysis

Generalized linear mixed-effects models (GLMMs) were used to analyse flight and attack activity for each species. The number of entry holes of a given species bored during each week (Poisson distribution) was the dependent variable, the week number since the starting day (and both quadratic and cubic terms) was the independent variable. The models were fitted using the ‘glmer’ function in the package ‘lme4’ (Bates et al., 2017) implemented in R (R Core Team, 2019). Overdispersion and residual distribution were checked via the ‘DHARMa’ package (Hartig, 2017). To determine the influence of tree species and ethanol concentration on host selection and colonization we used linear mixed-effects models (LMMs). For host selection, the number of entry holes of a given species visible on the bolt surface was used as dependent variable whereas tree species (categorical variable), ethanol concentration (categorical variable) and their interaction were independent variables. The interaction term was included in the model for X. saxesenii and X. crassiusculus but not for A. dispar and S. chevrieri due to the lack of data points for certain treatments. For colonization, the percentage of developed galleries of a given species per bolt was the dependent variable whereas tree species and ethanol concentration were independent variables. The effect of the interaction between the latter two variables was not tested due to the lack of data points for certain treatments. When necessary, the number of entry holes or the percentage of developed galleries was either log- or square root-transformed to satisfy the assumption of normality. The latter models were fitted using the ‘lmer’ function from the ‘lme4’ package (Bates et al., 2017) implemented in R (R Core Team, 2019). All post hoc pairwise comparisons among means were performed using the ‘glht’ function from the ‘multcomp’ package (Hothorn et al., 2016) with Tukey correction. In all three analyses, block was a random variable. Statistical significance was always set at the 0.05 level.

3 | RESULTS

Bolts were attacked by two native ambrosia beetles (A. dispar and X. saxesenii) and one exotic ambrosia beetle (X. crassiusculus), and the auger beetle, S. chevrieri.

The majority of 706 entry holes had a diameter corresponding either to X. crassiusculus (329) or X. saxesenii (110). We were able to assign 96 of the remaining 267 holes to either S. chevieri (57 holes) or A. dispar (39 holes). Xylosandrus crassiusculus, X. saxesenii, A. dispar and S. chevieri attacked 68%, 44%, 25% and 26% of the bolts, respectively.

Only 108 of 706 entry holes resulted in developed galleries, 94 for X. crassiusculus, seven for X. saxesenii, six for S. chevieri, and one for A. dispar. Xylosandrus crassiusculus also had the highest success rate (28.5%), followed by S. chevieri (10.5%), X. saxesenii (6.3%) and A. dispar (2.5%).

The temporal analysis of entry holes showed a significant non-linear trend for A. dispar (Figure 1a), X. crassiusculus (Figure 1c), and S. chevieri (Figure 1d) (Table S2), with a peak in mid-June for all three species. A significant non-linear trend was found also for X. saxesenii (Figure 1b, Table S2), but the peak was less evident and was followed by a slight further increase at the end of the sampling period. For X. crassiusculus, the percentage of developed galleries per bolt was higher in June (33.4%) than in July (7.4%).
3.1 | Effect of ethanol concentration and host tree species on host selection

Host tree species and ethanol concentration significantly affected the number of *A. dispar* entry holes (LMMs, $F = 2.33, p = 0.03$ and $F = 21.62, p < 0.001$, respectively), with significantly more entry holes on *O. carpinifolia* than on *P. abies*, and no difference between *O. carpinifolia* and the other species (Figure 2a). In addition, the number of entry holes was significantly higher in bolts filled with 90% ethanol than with 5% ethanol (Figure 2b).

Host tree species and ethanol concentration also significantly affected the number of *X. crassiusculus* entry holes (LMMs, $F = 2.38, p = 0.02$ and $F = 12.85, p < 0.01$, respectively), with no interaction between host tree species and ethanol concentration. The number of entry holes was significantly higher on *Q. robur* than on *P. abies*, whereas no difference was observed between *Q. robur* and the other species (Figure 2e). In addition, the number of entry holes was significantly higher in bolts filled with 5% ethanol than with 90% ethanol (Figure 2f).

The number of *X. saxesenii* entry holes was affected by host tree species (LMM, $F = 11.23, p < 0.001$, Figure 2c), but not ethanol concentration (LMM, $F = 0.020, p = 0.88$, Figure 2d) or the interaction between ethanol concentration and host tree species. The number of entry holes was significantly higher on *Q. robur* than on all the other species except *F. ornus* (Figure 2c).

Finally, the auger beetle *S. chevrieri* was affected by both host tree species (LMM, $F = 5.32, p < 0.001$, Figure 2g) and ethanol concentration (LMM, $F = 49.09, p < 0.001$, Figure 2h). The number of entry holes was significantly higher on *A. platanoides* than on *C. betulus* and *F. ornus* (Figure 2g), as well as on bolts filled with 90% ethanol than with 5% ethanol (Figure 2h).

3.2 | Effect of ethanol concentration and host tree species on colonization

Only *X. crassiusculus* constructed a sufficiently large number of developed galleries to justify analysing the effect of host tree species and ethanol concentration on colonization. The percentage of developed galleries per bolt was affected by host tree species (LMM, $F = 3.41, p < 0.01$, Figure 3a), being significantly higher on *O. carpinifolia* than on *A. platanoides*, *C. ovellana*, *C. betulus*, *F. ornus* and *P. abies* but similar to *C. sativa* and *Q. robur* (Figure 3a). The percentage of developed galleries was significantly higher in bolts filled with 5% ethanol than with 90% ethanol (LMM, $F = 11.92, p < 0.01$; Figure 3b).

4 | DISCUSSION

In addition to supporting the importance of ethanol in host selection and colonization by ambrosia beetles (Lehenberger et al., 2021; Oliver & Mannion, 2001; Ranger et al., 2018, 2021; Reding et al., 2011) our
results demonstrate that these processes are also affected by ethanol concentration and host species, with major differences among ambrosia beetle species. Certain species seek high or low ethanol concentration emitted by their hosts, whereas others are less selective. Moreover, some species have more prominent host preference than others. Finally, for *X. crassiusculus* we show that tree species can affect colonization.

We found that bolts infused with two different concentrations of ethanol (5% and 90%) differentially affected host selection of the three ambrosia beetles and the auger beetle that were active under field conditions over the time span of this study. In particular, *A. dispar* and *S. chevrieri* preferentially attacked bolts infused with ethanol at the highest concentration (90%). *X. crassiusculus* preferentially attacked bolts with the lowest ethanol concentration (5%), whereas *X. saxesenii* was indifferent to ethanol concentration. All three species are known to be attracted to ethanol-emitting trees (Ranger et al., 2016, 2021; Reed et al., 2015; Tanasković et al., 2016), but our results suggest that the beetles use ethanol concentration as an indicator of host tree susceptibility, and possibly as a means to reduce interspecific competition.

The preference of *A. dispar* and *S. chevrieri* for bolts infused with 90% ethanol can be related to their habit of colonizing dying or severely stressed trees. This pattern is in line with previous studies showing that both species are strongly attracted to ethanol, with attraction increasing with ethanol concentration (Byers et al., 2020; Klimetzek et al., 1986; Schroeder & Lindelöw, 1989). In contrast, the preference of *X. crassiusculus* for bolts infused with 5% ethanol suggests a preference for trees in the early stages of physiological stress. This differential host preference is in agreement with the higher ability of *Ambrosiella hartigii* Batra, the fungal symbiont of *A. dispar*, to tolerate ethanol in host tissue than *Ambrosiella roeperi* T.C. Harr. & McNew, the fungal symbiont of *X. crassiusculus* (Lehenberger et al., 2021; Ranger et al., 2018). This scenario would explain why exotic *X. crassiusculus* has become a major pest of stressed living trees in ornamental hardwood nurseries in the USA (Ranger et al., 2016, 2021) and would also explain why *A. dispar* and *X. crassiusculus* do not simultaneously attack the same trees or logs despite their overlapping flight activity (Frank &
Our study provides support for the potential role of ethanol concentration and host tree species in determining the ecological niche
of ambrosia beetle species. We only tested two extremes of ethanol concentration (5% versus 90%) and on only three ambrosia beetle species, and we did not measure the amount of ethanol actually present in or emitted by host tissues. Testing more ethanol concentrations on a larger number of ambrosia beetle species would allow further unravelling of the role of ethanol in defining species-specific preferences in terms of ethanol concentration in wood tissues might be more prone to exchange fungal symbionts, which could lead to novel beetle-fungus associations with unpredictable impact (Carrillo et al., 2014; Rassati et al., 2019; Wingfield et al., 2016). In addition, identifying the most attractive ethanol concentration for different ambrosia beetle species could increase the efficacy of monitoring and surveillance programs (Addesso et al., 2019; Reding et al., 2011), as well as open new opportunities for push and pull tactics and mass-trapping (Ranger et al., 2021). Given that bark thickness and structure were recently shown to affect hole boring of Euwallacea kuroshio Gomez & Hulcr and Platypus quercivorus (Murayama), respectively (Boland & Woodward, 2021; Kuma et al., 2021), future studies should also investigate the effect of bark and wood properties on host selection and colonization of other ambrosia beetle species.

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CONFLICT OF INTEREST
Declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
DR, CMR, and GC conceived research and wrote the manuscript. DR and GC conducted the experiments and statistical analyses. MF contributed material. DR and MF secured funding. All authors read, contributed to, and approved the manuscript.

DATA AVAILABILITY STATEMENT
Raw data are accessible at the following link: http://researchdata.cab.unipd.it/id/eprint/428. Rassati, 2021

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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