

Title

Response of the oak ambrosia beetle *Platypus quercivorus* (Coleoptera: Platypodinae) to volatiles from fresh and dried leaves

Authors

Duy Long Pham¹, Yasuto Ito², and Michimasa Yamasaki^{3*}

¹ Department of Forestry, Vietnam Ministry of Agriculture and Rural Development (ORCID: 0000-0002-0810-474X)

² Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, Shisou-shi, Hyogo, Japan (ORCID: 0000-0002-4443-0454)

³ Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Kyoto-shi, Kyoto, Japan (ORCID: 0000-0002-0724-9234)

*Corresponding author

Michimasa Yamasaki

E-mail: riseiyam@gmail.com

ORCID: 0000-0002-0724-9234

Acknowledgments

We thank Dr. N. Mori and Mr. Y. Hirozane for their technical assistance in the GC-MS operation. Our thanks were extended to all members of the Laboratory of Forest Biology for their support during the study. This study was supported by the FY2017 Kyoto University Research Development Program (ISHIZUE) and Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 21H02234).

Abstract

Bark and ambrosia beetles use trees as hosts, and the preferred host condition—whether healthy, weakened, or dead—varies among beetle species. Though attraction to volatiles from trees under favorable conditions have been well studied in beetles, only a few studies have examined the repulsion of volatiles from trees under unfavorable conditions. We focused on this aspect by using the oak ambrosia beetle, *Platypus quercivorus*, as a model. This beetle attacks healthy oaks, causing Japanese oak wilt. Previous studies have suggested that leaf volatiles are important cues to detect the hosts of this beetle. Therefore, we used a Y-tube olfactometer to evaluate the beetle response to volatiles from fresh and dried leaves of their hosts and non-hosts. Volatile components were analyzed by gas chromatography-mass spectrometry (GC-MS). In the Y-tube, *P. quercivorus* preferred fresh leaf volatiles to clean air and did not generally prefer dried leaf volatiles. This preference was observed regardless of whether the source was a host or non-host. The degree of attraction to fresh leaf volatiles increased when the counterpart of the Y-tube was changed from clean air to dried leaf volatiles, suggesting a repelling effect of the dried leaf volatiles. The GC-MS results showed a clear difference in the chemical components of the volatiles from fresh and dried leaves. Common chemicals among the dried leaf volatiles of hosts and non-hosts, such as trans-2-hexenal, have been suggested as candidate repellents for *P. quercivorus*. The results suggest the possible use of dead branches to avoid beetle infestation.

Keywords

attractant, host selection, Japanese oak wilt, olfactometer, repellent

Statements and declarations

Competing Interests: The authors declare none.

Introduction

Herbivorous insects select their host plants in a heterogeneous environment by using visual, olfactory, gustatory, and tactile senses (Bernays and Chapman 1994). Herbivorous insects use plant volatiles to distinguish hosts from non-hosts (Visser 1986). Some volatiles from hosts are attractive for insects, while others from non-hosts might be repellents (Schoonhoven et al. 2005). Using a combination of attractants and repellents may increase the efficiency of host selection in heterogeneous environments (Bruce and Pickett 2011).

Bark and ambrosia beetles use plant volatiles for host selection. Although the resources they use are wood, they also use information from leaves for host selection. For example, conifer-inhabiting bark beetles are attracted to α -pinene, which is emitted from their hosts (Schroeder 1988; Erbilgin and Raffa 2000; Miller and Rabaglia 2009). These attraction responses to kairomones are disrupted by green leaf volatiles emitted from non-host broad-leaved trees (Schlyter et al. 2000), which is also observed for pheromone-based beetles attraction (Byers et al. 1998; Zhang et al. 2001; Zhang 2003; Byers et al. 2004; Fettig et al. 2005). These responses are evolved adaptively for discriminating between hosts and non-hosts in heterogeneous environments. Additionally, beetles must select trees under suitable conditions. The ambrosia beetle, *Xylosandrus germanus*, attacks stressed trees, and are attracted to the ethanol emitted from such trees (Ranger et al. 2010; Ranger et al. 2013). In contrast, the redbay ambrosia beetle, *Xyleborus glabratus*, attacks healthy trees (Johnson et al. 2014) and is attracted to host leaf volatiles (Martini et al. 2015). Healthy trees are thought to be unfavorable for beetles that attack stressed trees, and vice versa. Trees with unfavorable conditions for beetles may repulse insects by their volatiles; however, this topic is poorly studied (but see Martini et al. 2017).

The oak ambrosia beetle, *Platypus quercivorus*, attacks healthy oak trees (Kobayashi and Ueda 2005) and causes Japanese oak wilt by transporting a pathogenic fungus, *Raffaelea quercivora* (Kubono and Ito 2002). Discriminations between hosts and non-hosts are made by the beetles, and beetles wrongly land on non-host tree species only when they are located close to host tree species (Yamasaki et al. 2024). The reproductive success of *P. quercivorus* differs among several host tree species (Tarno et al. 2011), and the beetles attack the preferred host, *Quercus crispula*, in higher frequency compared to the less preferred host, *Castanea crenata* (Yamasaki et al. 2014). *P. quercivorus* beetles use leaf volatiles to distinguish its preferred host. In the laboratory, *P. quercivorus* is attracted to host leaf volatiles and repelled by non-host leaf volatiles, and the degree of attraction to volatiles from *Q. crispula* was higher than that from *Castanea crenata*, although the difference was not significant (Pham et al. 2020).

Furthermore, *P. quercivorus* distinguishes host tree conditions. When *P. quercivorus* beetles were made to choose between host leaf volatiles and clean air using a Y-tube olfactometer, host leaf volatiles were less attractive to the beetles as leaf deterioration progressed (Pham et al. 2019). If beetles prefer healthy hosts over weakened or dying hosts, as suggested by this result, their preference for fresh leaf volatiles will be clearer when the counterpart of the Y-tube is changed from clean air to volatiles from dried leaves. This

107 preference is achieved when the volatile compositions of fresh and dried leaves are different.
108 In addition, weakened or dying non-host tree species are also thought to be unsuitable for the
109 beetle; therefore, the beetle may avoid dried leaf volatiles in both the host and non-host
110 species. Avoidance of dried leaf volatiles including non-host can be achieved by avoiding
111 common chemicals for dried leaf volatiles if there are such chemicals.

112 In the present study, we evaluated the preference of *P. quercivorus* for fresh and dried
113 leaf volatiles from the host and non-host species. The chemical profiles of leaf volatiles were
114 analyzed and characterized by Gas Chromatography-Mass Spectrometry (GC-MS) to
115 compare the profiles of fresh and dried leaves and to explore the common chemicals in the
116 dried leaves of beetle hosts and non-hosts. These results are discussed from the viewpoint of
117 host selection by *P. quercivorus*.

Materials and methods

Experimental beetles and leaves

Two *Q. serrata* trees attacked and killed by *P. quercivorus* in 2016 were felled in an urban forest in Hyogo Prefecture, Japan (34.912° N, 135.274° E), on November 24, 2016. Each tree consisted of two trunks, yielding four trunks of 36.5, 34.6, 46.4, and 90.5 cm diameter at breast height. The basal parts of the trees were transported to the Kitashirawa Experimental Station, Field Science Education and Research Center, Kyoto University (35.033° N, 135.786° E) on May 11, 2017. Spitz tubes with the cut bottom covered by nylon mesh to allow air exchange (16 mm in diameter and 6 cm in length, modified from Iidzuka et al. (2014)) were attached to each entry hole of the beetles on the trunk surface. Beetles that emerged in the traps in the morning, 6 to 7 AM, of each experimental days were immediately transferred to laboratory where they were maintained in the traps under the controlled temperature of 25 °C until being served for the olfactory bioassays.

Leaves of four species of beetle hosts, *Q. crispula*, *Q. serrata*, *Q. salicina* and *Castanea crenata*, and two species of non-hosts, *Fagus crenata* and *Cryptomeria japonica* were sampled from trees planted at the Kitashirawa Experimental Station described above for olfactory bioassays and analysis of leaf volatiles. The diameter at 130 cm above ground and height of these trees ranged between 9.2–45.8 cm and 2.1–15.7 m, respectively. On the morning of each experimental day, branches, including leaves, were cut to a length of 40–60 cm and immediately transported to the laboratory, where all leaves were visually inspected to ensure no visible signs of pathogen or pest infestation. Fresh leaves detached from branches were immediately used for olfactory bioassays or volatile analysis. Dried leaves were prepared by leaving cut branches in a laboratory at 25 °C for two days. This duration produced the greatest repellent effect of *Q. crispula* leaves on beetles (Pham et al. 2019).

In a previous study (Pham et al. 2020), leaves attached to seedlings were used as a source of leaf volatiles without detachment from the seedlings. Preparing seedlings with a similar declining status was difficult, therefore we used leaves dried for the same duration (two days) in this study.

Olfactory bioassays

Between June 22 and July 13, 2017, the olfactory responses of *P. quercivorus* adults to the leaf volatiles emitted by six tree species (*Q. crispula*, *Q. serrata*, *Q. salicina*, *Castanea crenata*, *F. crenata*, and *Cryptomeria japonica*) were examined. Processes were different based on the leaves prepared at the day of experiment. When fresh leaves of two species were prepared for the experiments, individual beetles were evaluated 20 times; 10 times to choose between fresh leaf volatiles of one species and clean air (control), and 10 times between fresh leaf volatiles of another species and control (Test 1). Ten trials of each test were conducted sequentially, not randomly, each time. For example, when fresh leaves of *Q. crispula* and *C. crenata* were prepared (June 25 in Supplementary table 1), beetles were first tested 10 times

to choose between the fresh leaf volatiles of *Q. crispula* and the control, and then tested 10 times to choose between the fresh leaf volatiles of *C. crenata* and the control. Several beetles were evaluated sequentially using the same pair of two tree species, and up to three pairs of tree species were evaluated per day by serving different beetles. When fresh and dried leaves of one tree species were prepared, individual beetles were evaluated 30 times; 10 times to choose between fresh leaf volatiles and control, 10 times between dried leaf volatiles and control, and 10 times between fresh and dried leaf volatiles (Test 2). Ten trials of each test were conducted sequentially, not randomly, each time. For example, when fresh and dried leaves of *Q. crispula* were prepared (June 27 in Supplementary table 1), beetles were first tested 10 times to choose between the fresh leaf volatiles of *Q. crispula* and the control, then tested 10 times to choose between the dried leaf volatiles of *Q. crispula* and the control, and finally tested 10 times to choose between the fresh and dried leaf volatiles of *Q. crispula*. Because the comparison of fresh leaf volatiles and the control was included in both Test 1 and Test 2, the number of beetles used for this comparison was larger than that for the other comparison pairs (Table 1). In addition, because of the unpredictability of the beetle emergence, the number of males and females served for these tests was different among pairs of comparison (Table 1). In total, 36 male beetles and 56 female beetles were evaluated in bioassays (Supplementary table 1).

A Y-tube olfactometer designed to evaluate the responses of *P. quercivorus* (Pham et al., 2019) was used for the bioassays. The device consisted of a Y-shaped transparent plastic tube with a stem 5 mm in internal diameter and 2.7 cm in length, and two side arms, each with an internal diameter of 5 mm and a length of 2.7 cm, forming a 60° angle. The ends of the two arms were connected to two transparent fluoroplastic gas wash bottles (250 ml). Each bottle had an inlet for clean air, supplied by an electric pump and filtered through activated charcoal and distilled water to remove non-volatile odor particulates before entering the Y-tube, and an outlet for air venting. Air circulation within the olfactometer was maintained by an electric pump (MP-2N, Code: 8086-2, Shibata Scientific Technology Ltd.), and the flow rate was regulated by a flow meter (RK-1350V, KOFLOC Co., Ltd.).

At the beginning of each olfactory bioassay, a single beetle of *P. quercivorus* was carefully moved from the emergence traps to a glass bottle (14 mm diameter, 4 cm long) using the tip of a paintbrush before releasing it into the entrance of the stem of the Y-tube. The sex of each individual was recorded to observe possible sexual differences in olfactory responses. Ten leaves were placed in the plastic chamber on the leaf-side of the olfactometer without measuring their weight. The leaf-side or clean air was randomly assigned to one arm before each bioassay. The position of the Y-tube arms was then rotated after every five out of 10 trials to minimize directional habit formation. In each trial, when a single beetle selected one of the two arms and traversed the length of the tubing, it was considered to have chosen the odor of the associated bait. It was immediately moved back to the base of the Y-tube, and a new trial was initiated. When a beetle remained in the basal area of the Y-tube and did not choose either arm within 5 min, the test was interrupted, and the data from these beetles were discarded from the statistical analysis. Practically no data were discarded because, in all cases where such inactivity was observed, the beetles did not move inside the Y-tube from the first

trial. All bioassays were conducted between 7 AM and 2 PM at the laboratory conditioned at 25 °C. The Y-tube olfactometer was illuminated by a 25 W fluorescent light, positioned on top of the room, 2 m above the arms. Airflow rates through the inlet to outlet of Y-tube arms were 55–99 ml/min, with an average value of 67 ml/min.

Analysis of leaf volatiles

Fresh leaves were prepared by cutting branches with leaves from each of the six trees between 6 and 10 AM. Dried leaves were prepared by keeping the branches with fresh leaves under controlled conditions of 25 °C for 48 h at the laboratory. Ten leaves were placed in the plastic chamber of the olfactometer without measuring their weight. A volatile collection trap, 75 mm long and 6 mm in outer diameter, with 30 mg HayeSep Q (80/100 mesh; Hayes Separations, Bandera, TX, USA) packed inside a Teflon tube, was connected directly to the outlet of the fluoroplastic gas wash bottle to capture leaf volatiles. In addition to the activated charcoal and distilled water already in place, another volatile collection trap was set up at the inlet of the fluoroplastic gas wash bottle as a filter to draw clean air into the bottle. This supplemental filter was washed with dichloromethane/hexane (50:50), and water was exchanged between collection days. Leaf volatile compounds were collected once from the six tree species on different days. Airflow was passed through the plants at a rate of 70 L/min. The headspace of leaf volatiles was collected for 24 h at room temperature of 25 °C.

The captured leaf volatiles were removed from the collection traps by rinsing with 150 µl of dichloromethane/hexane (1:1) under a stream of nitrogen. Nonyl acetate (5 µl) was added as an internal standard to the extracts at a concentration of 0.5 ng µl⁻¹. A 1-µl portion of each sample was manually subjected to an Agilent Technologies 5975 gas chromatograph/mass spectrometer (GC-MS) equipped with an Agilent 6890N chromatograph operated at 69.9 eV in the split-less mode, using an HP-5MS (Agilent) capillary column (30 m × 0.25 mm ID, with 0.25 µm thickness) and flame ionization detector. Helium was used as the carrier gas. The injector was kept at 250 °C and the detector at 290 °C. The column oven temperature was programmed at 60 °C for 2 min and then increased at a rate of 8 °C/min to a final temperature of 290 °C, held for 5 min. Gas-liquid chromatography (GC) was performed with an Agilent 5975 Inert/N MSD ChemStation using the same column and conditions as those used for GC/MS analysis. Quantization of the GC/MS profile was based on comparing the peak areas of nonyl acetate (5 µl) with the peak areas of compounds extracted from the samples. Compounds were tentatively identified by matching the experimental mass spectra to those of the National Institute of Standards and Technology (NIST). Compounds with > 90% mass spectral similarity to those in the NIST library were considered candidate compounds.

Statistical analysis

All data analyses were performed using R 4.2.3 (R Core Team 2023). We constructed generalized linear mixed models to predict the probabilities of the behavioral responses of *P*.

quercivorus to leaf volatiles. The side of the Y-tube olfactometer selected by the beetle in each trial was regarded as the response variable. Selection of the leaf side was regarded as a value of “1,” and selection of clean air was regarded as “0.” In the case when the beetle selection was tested between fresh and dried leaf volatiles, selection of the fresh leaf side was regarded as a value of “1,” and selection of dried leaf side was regarded as “0.” The selection variable followed a binomial distribution; therefore, a logit link function was used. The candidate explanatory variable was the tree species (*Q. crispula*, *Q. serrata*, *Q. salicina*, *Castanea crenata*, *Fagus crenata*, and *Cryptomeria japonica*). Though enough replicates were not prepared for both sexes in some comparison pairs (Table 1), the sexual difference in the response to volatiles cannot be ignored. Additionally, the data were not independent because 20 or 30 trials were performed for each beetle. To address these issues, we used a mixed model with random intercepts for the individual number of the beetle nested within beetle sex (male or female), which assumes a dependent structure for the data. We compared the Akaike Information Criterion (AIC) values of the model that includes tree species as an explanatory variable with those of the null model. When an increase in AIC of more than 2 was observed upon the deletion of the explanatory variable, we regarded tree species as an influential factor. The proportion of trials in which an individual selected a specific side of a Y-tube olfactometer was predicted by the selected models, and its 95% confidence intervals (CIs) were calculated. When lower CIs were higher than 0.5, we considered that the leaf volatiles significantly attracted the beetle. In contrast, when higher CIs were lower than 0.5, we considered that the leaf volatiles significantly repelled the beetles. When the CIs crossed 0.5, we considered that the beetles responded randomly to the leaf volatiles. Additionally, multiple comparison with Tukey’s correction was performed when tree species was selected as an explanatory variable of the models. The models were constructed using *lme4* package 1.1-35.3 (Bates et al. 2015), the model selections were performed using *MuMIn* package 1.47.5 (Bartoń 2023), and the predictions and multiple comparisons were done using *emmeans* package 1.10.1 (Lenth 2024) of R (R Core Team 2023), respectively.

Results

Olfactory bioassays

Tree species was selected for the model to predict the proportion of trials in which *P. quercivorus* beetles were forced to select fresh leaf volatiles or clean air in the Y-tube (Supplementary table 2). A significant preference for leaf volatiles was observed irrespective of the tree species evaluated (Fig. 1). Significant difference in the proportion of leaf side selection was observed between *Q. crispula* and *Q. salicina*, and they significantly preferred *Q. crispula* to *Q. salicina* (Fig. 1).

In contrast, beetles generally selected the side with clean air when they were forced to select dried leaf volatiles or clean air (Fig. 2), and tree species was selected for the model (Supplementary table 3). Significant repulsion of dried leaf volatiles was observed for *Q. crispula*, *Q. serrata*, and *F. crenata* (Fig. 2). Significant difference in the proportion of leaf side selection was observed between *Cryptomeria japonica* and other three species, *Q. crispula*, *Q. serrata*, and *F. crenata*, and they were significantly repelled by latter three species (Fig. 2).

Tree species was also selected for the model to predict the proportion of trials in which *P. quercivorus* beetles were forced to select fresh leaf volatiles or dried leaf volatiles in the Y-tube (Supplementary table 4). The preference of the beetle for fresh leaf volatiles became clear when the counterpart of the Y-tube was changed from clean air to dried leaf volatiles (Fig. 3). A significant preference for fresh over dried leaf volatiles was observed for all tree species (Fig. 3). Multiple comparisons showed significant difference in this preference only in the case between *Q. crispula* and *Cryptomeria japonica* (Fig. 3).

Analysis of leaf volatiles

Quercus crispula and *Q. serrata* shared many common chemical components in their fresh leaf volatiles, including 3-hexen-1-ol acetate; (E)-4,8-dimethyl-1,3,7-nonatriene; dodecane; tridecane; α -copaene; caryophyllene; aromadendrene; germacrene; δ -cadinene; nerolidol; hexadecane; heptadecane; octadecane; and kaur-16-ene, 10 of these 14 chemicals were unique to these two *Quercus* species (Table 2). *Quercus salicina*, *Castanea crenata* and *F. crenata* were characterized by a few chemical components in their fresh leaf volatiles (Table 2). Ten chemicals were detected in the fresh leaf volatiles of *Cryptomeria japonica*, and 3-carene, d-limonene, 4-terpinenol, and β -pinene were uniquely detected in this species (Table 2).

Similar to fresh leaf volatiles, *Q. crispula* and *Q. serrata* shared many common chemical components with their dried leaf volatiles, although individual components differed between fresh and dried leaves. Trans-2-hexenal, benzaldehyde, β -cyclocitral, and α -humulene were common chemicals detected in these two *Quercus* species and were uniquely observed in the dried leaf volatiles (Table 3). In addition, trans-2-hexenal, benzaldehyde, β -cyclocitral, and α -farnesene were detected in the dried leaf volatiles of four or five of the six

tested species (Table 3). In the case of *Quercus salicina*, *Castanea crenata* and *F. crenata*, the number of chemicals detected in dried leaf volatiles increased compared to fresh leaf volatiles, and the components differed between fresh and dried leaves (Table 3). Eight chemicals were detected in the dried leaf volatiles of *Cryptomeria japonica*, and α -pinene, sabinene, 3-carene, D-limonene, and α -terpinene were common to both fresh and dried leaf volatiles of this species (Tables 2, 3).

Discussion

Selecting trees with favorable conditions is critical for bark and ambrosia beetles, and dying or dead trees should be avoided by *P. quercivorus*, which attacks healthy trees. We simulated the dying status of trees using dried leaves and evaluated the responses of *P. quercivorus* to fresh and dried leaf volatiles. The results showed that the beetles avoided dried leaf volatiles, regardless of whether their source was host or non-host (Figs. 2, 3).

Chemicals commonly observed in the dried leaf volatiles of host and non-host plants, such as trans-2-hexenal, benzaldehyde, and β -cyclocitralne (Table 3), are candidate repellents for *P. quercivorus* though it is necessary to confirm these chemicals by comparing their mass spectra and retention times with those of the authentic compounds. Trans-2-hexenal is a green leaf volatile, and its relationship to insect borers has been suggested. Electroantennographic responses to this chemical have been confirmed in the black-banded oak borer *Coroebus florentinus*, which attacks *Quercus* trees (Fürstenau et al. 2012). Benzaldehyde is a major component of the essential oil extracted from the dried leaves of *Q. dentata* (Kameoka et al. 1983), a host of *P. quercivorus*. β -cyclocitral has been recognized as a leaf volatile of strawberries, and its attractive effect on the drosophilid fly *Drosophila suzukii* has been confirmed (Bolton et al. 2019). It is crucial to examine further the behavioral and antennal responses of *P. quercivorus* to these chemicals. Antennal responses of *P. quercivorus* to trans-2-hexenal and benzaldehyde has been confirmed, and the response to trans-2-hexenal was stronger than that to benzaldehyde (Okada et al., unpublished data). In addition, it should be noted that the beetle's response is most likely driven by a mix of different compounds rather than a single one (Bruce and Pickett 2011).

The chemical components of leaf volatiles, except *Cryptomeria japonica*, were generally different between fresh and dried leaves (Tables 2, 3). In this context, leaf volatiles can be a good indicator for *P. quercivorus* whether its target tree is healthy. Volatiles from the bark are more likely to be the key to detecting host trees and assessing their condition because beetles bore holes in the bark surface. However, captures of *P. quercivorus* inside canopies of their host trees at the time the beetles actively attack the basal part of trees (Yamasaki et al. 2023) and the frequent incorrect landing of the beetles on non-hosts under the host canopy (Yamasaki et al. 2024) suggested an approach path from the top of the canopy to the basal trunk and their detection of volatiles from the host canopy. Beetles may fly above the canopy and select host tree species under favorable conditions by detecting specific leaf volatiles.

Regarding *Cryptomeria japonica*, which did not show clear differences in the chemical components of fresh and dried needle volatiles, the fresh and dried needles prepared for the experiment were not visually different (Pham, personal observation). Treatment with two days of drying might not be sufficient for this evergreen conifer species to simulate its dying status.

In the field, *P. quercivorus* is presumed to detect and select host trees when flying. In this study, the behavioral responses of *P. quercivorus* to leaf volatiles were measured using a Y-tube olfactometer, where the beetle walked on the short platform of the tube during rapid observation, which may be different from natural conditions. It is also possible that in the

narrow field of Y-tube, the beetles may select fresh air because they perceive no obstacles to their escape. Using a wind tunnel and allowing the beetles to fly is one of the alternative options to test their response. Another option is to use a flight mill. Although a flight mill to measure the flying capacity of *P. quercivorus* has been developed (Okada et al. 2018), assessing the behavioral response of beetles attached to this flight mill is difficult. Improvements in the mill structure will enable future studies to assess the responses of flying beetles.

Another limitation of this study is that we used cut leaves as a source of leaf volatiles, and the chemicals detected by GC-MS were likely different from the volatiles emitted from intact leaves attached to branches. This is also supported by the difference in the chemical profiles of fresh leaf volatiles between the present study and a previous study that used seedlings (Pham et al. 2020). Pham et al. (2020) observed a clear difference in beetle response to leaf volatiles between hosts and non-hosts. Furthermore, tree species were selected as an explanatory variable of the best-fit model to predict the beetle preference; however, the results of multiple comparisons revealed that the difference among tree species was limited in several cases.

Regarding the results of dried leaf volatiles, however, clear avoidance of volatiles irrespective of the host and non-host, and the presence of several common chemicals in volatiles indicate the general avoidance of dried leaf volatiles as information of trees under non-favorable conditions. Applying candidate repellents in the field may decrease the attack frequency of *P. quercivorus* on oak trees. Cut branches of broad-leaved trees may be sufficient to keep the beetles away from oak trees. Because avoidance was generally observed for the dried leaf volatiles of both hosts and non-hosts, the source of the branches is not necessarily the beetle host.

References

- Bartoń K (2023) MuMIn: Multi-Model Inference. R package version 1.47.5.
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1-48. <https://doi.org/10.18637/jss.v067.i01>
- Bernays EA, Chapman RF (1994) Host-Plant Selection by Phytophagous Insects. Chapman & Hall, New York
- Bolton LG, Piñero JC, Barrett BA (2019) Electrophysiological and behavioral responses of *Drosophila suzukii* (Diptera: Drosophilidae) towards the leaf volatile β -cyclocitral and selected fruit-ripening volatiles. Environ Entomol 48:1049-1055. <https://doi.org/10.1093/ee/nvz092>
- Bruce TJA, Pickett JA (2011) Perception of plant volatile blends by herbivorous insects – Finding the right mix. Phytochemistry 72:1605-1611. <https://doi.org/doi:10.1016/j.phytochem.2011.04.011>
- Byers JA, Zhang Q-H, Birgersson G (2004) Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften 91:215-219. <https://doi.org/10.1007/s00114-004-0520-1>
- Byers JA, Zhang QH, Schlyter F (1998) Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles. Naturwissenschaften 85:557-561. <https://doi.org/10.1007/s001140050551>
- Erbilgin N, Raffa KF (2000) Opposing effects of host monoterpenes on responses by two sympatric species of bark beetles to their aggregation pheromones. J Chem Ecol 26:2527-2548. <https://doi.org/10.1023/A:1005532612117>
- Fettig CJ, McKelvey SR, Huber DPW (2005) Nonhost angiosperm volatiles and verbenone disrupt response of western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Scolytidae), to attractant-baited traps. J Econ Entomol 98:2041-2048. <https://doi.org/10.1093/jee/98.6.2041>
- Fürstenau B, Rosell G, Guerrero A, Quero C (2012) Electrophysiological and behavioral responses of the black-banded oak borer, *Coroebus florentinus*, to conspecific and host-plant volatiles. J Chem Ecol 38:378-388. <https://doi.org/10.1007/s10886-012-0110-1>
- Iidzuka H, Goto H, Yamasaki M, Osawa N (2014) Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) on *Fagus crenata* Blume: community structure, seasonal population trends and resource utilization patterns. Entomol Sci 17:167-180. <https://doi.org/10.1111/ens.12042>
- Johnson CW, Cameron RS, Hanula JL, Bates C (2014) The attractiveness of manuka oil and ethanol, alone and in combination, to *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae) and other Curculionidae. Florida Ent 97:861-864. <https://doi.org/10.1653/024.097.0281>
- Kameoka H, Tsujino H, Aso Y (1983) Constituents of essential oil of leaves of *Quercus dentata* Thunb. Nippon Nôgeikagaku Kaishi 57:135-138. <https://doi.org/10.1271/nogeikagaku1924.57.135>

- Kobayashi M, Ueda A (2005) Wilt disease of Fagaceae trees caused by *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) and the associated fungus: Aim is to clarify the damage factor (in Japanese with English summary). J Jpn For Soc 87:435-450. <https://doi.org/10.4005/jjfs.87.435>
- Kubono T, Ito S (2002) *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*). Mycoscience 43:255-260. <https://doi.org/10.1007/S102670200037>
- Lenth R (2024) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.1.
- Martini X, Hughes MA, Killiny N, George J, Lapointe SL, Smith JA, Stelinski LL (2017) The fungus *Raffaelea lauricola* modifies behavior of its symbiont and vector, the redbay ambrosia beetle (*Xyleborus glabratus*), by altering host plant volatile production. J Chem Ecol 43:519-531. <https://doi.org/10.1007/s10886-017-0843-y>
- Martini X, Hughes MA, Smith JA, Stelinski LL (2015) Attraction of redbay ambrosia beetle, *Xyleborus glabratus*, to leaf volatiles of its host plants in North America. J Chem Ecol 41:613-621. <https://doi.org/10.1007/s10886-015-0595-5>
- Miller DR, Rabaglia RJ (2009) Ethanol and (-)-alpha-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. J Chem Ecol 35:435-448. <https://doi.org/10.1007/s10886-009-9613-9>
- Okada R, Pham DL, Ito Y, Yamasaki M, Ikeno H (2018) Measuring the flight ability of the ambrosia beetle, *Platypus quercivorus* (Murayama), using a low-cost, small, and easily constructed flight mill. J Vis Exp 2018. <https://doi.org/10.3791/57468>
- Pham DL, Ito Y, Okada R, Ikeno H, Isagi Y, Yamasaki M (2019) Effects of leaf conditions and flight activity on the behaviour of *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). J Appl Entomol 143:1000-1010. <https://doi.org/10.1111/jen.12671>
- Pham DL, Ito Y, Okada R, Ikeno H, Kazama H, Mori N, Yamasaki M (2020) *Platypus quercivorus* ambrosia beetles use leaf volatiles in host selection. Entomol Exp Appl 168:928-939. <https://doi.org/10.1111/eea.12993>
- R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Ranger CM, Reding ME, Persad AB, Herms DA (2010) Ability of stress-related volatiles to attract and induce attacks by *Xylosandrus germanus* and other ambrosia beetles. Agric For Entomol 12:177-185. <https://doi.org/10.1111/j.1461-9563.2009.00469.x>
- Ranger CM, Reding ME, Schultz PB, Oliver JB (2013) Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate. Agric For Entomol 15:56-64. <https://doi.org/10.1111/j.1461-9563.2012.00591.x>
- Schlyter F, Zhang Q-H, Anderson P, Byers JA, Wadhams LJ, Löfqvist J, Birgersson G (2000) Electrophysiological and behavioural responses of *Tomicus piniperda* and *Tomicus minor* (Coleoptera: Scolytidae) to non-host leaf and bark volatiles. Can Entomol 132:965-981. <https://doi.org/10.4039/Ent132965-6>
- Schoonhoven LM, Van Loon JJA, Dicke M (2005) Insect-Plant Biology. Second edn. Oxford University Press, New York

- Schroeder LM (1988) Attraction of the bark beetle *Tomicus piniperda* and some other bark- and wood-living beetles to the host volatiles α -pinene and ethanol. Entomol Exp Appl 46:203-210. <https://doi.org/10.1111/j.1570-7458.1988.tb01113.x>
- Tarno H, Qi HY, Endoh R, Kobayashi M, Goto H, Futai K (2011) Types of frass produced by the ambrosia beetle *Platypus quercivorus* during gallery construction, and host suitability of five tree species for the beetle. J For Res 16:68-75. <https://doi.org/10.1007/s10310-010-0211-z>
- Visser JH (1986) Host odor perception in phytophagous insects. Annu Rev Entomol 31:121-144. <https://doi.org/10.1146/annurev.en.31.010186.001005>
- Yamasaki M, Ito Y, Ando M (2014) The effect of stem density on the probability of attack by the ambrosia beetle *Platypus quercivorus* varies with spatial scale. Agric For Entomol 16:54-62. <https://doi.org/10.1111/afe.12033>
- Yamasaki M, Tatsumi K, Ito Y (2023) Capture of the oak ambrosia beetle within the host tree canopy. Can Entomol 155:E28. <https://doi.org/10.4039/tce.2023.20>
- Yamasaki M, Tatsumi K, Kobayashi T, Ito Y (2024) Wrong landing on a non-host occurs under the canopy of a host: the presumed path of the ambrosia beetle *Platypus quercivorus* to approach its host. Agric For Entomol 26:135-147. <https://doi.org/10.1111/afe.12601>
- Yasui H et al. (2007) Host plant chemicals serve intraspecific communication in the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae). Appl Entomol Zool 42:255-268. <https://doi.org/10.1303/aez.2007.255>
- Zhang Q-H (2003) Interruption of aggregation pheromone in *Ips typographus* (L.) (Col. Scolytidae) by non-host bark volatiles. Agric For Entomol 5:145-153. <https://doi.org/10.1046/j.1461-9563.2003.00173.x>
- Zhang Q-H, Liu GT, Schlyter F, Birgersson G, Anderson P, Valeur P (2001) Olfactory responses of *Ips duplicatus* from Inner Mongolia, China to nonhost leaf and bark volatiles. J Chem Ecol 27:995-1009. <https://doi.org/10.1023/A:1010395221953>

Table 1 Number of males and females of the oak ambrosia beetle, *Platypus quercivorus*, used to evaluate the olfactory response to leaf volatiles of their host and non-host. Beetles were evaluated their response in Y-tube olfactometer to select between fresh leaf volatiles and clean air, dried leaf volatiles and clean air or fresh and dried leaf volatiles. Same beetles were used for multiple tests of comparison and each test was repeated 10 times for one individual beetle. See Supplementary table 1 for details.

Pair of comparison		Number of beetles	
		Males	Females
Fresh leaves of <i>Quercus crispula</i>	- Clean air	18	27
Fresh leaves of <i>Quercus serrata</i>	- Clean air	12	12
Fresh leaves of <i>Quercus salicina</i>	- Clean air	5	18
Fresh leaves of <i>Castanea crenata</i>	- Clean air	8	14
Fresh leaves of <i>Fagus crenata</i>	- Clean air	6	11
Fresh leaves of <i>Cryptomeria japonica</i>	- Clean air	10	9
Dried leaves of <i>Quercus crispula</i>	- Clean air	1	4
Dried leaves of <i>Quercus serrata</i>	- Clean air	3	3
Dried leaves of <i>Quercus salicina</i>	- Clean air	-	6
Dried leaves of <i>Castanea crenata</i>	- Clean air	3	3
Dried leaves of <i>Fagus crenata</i>	- Clean air	2	3
Dried leaves of <i>Cryptomeria japonica</i>	- Clean air	4	2
Fresh leaves of <i>Quercus crispula</i>	- Dried leaves of <i>Quercus crispula</i>	1	4
Fresh leaves of <i>Quercus serrata</i>	- Dried leaves of <i>Quercus serrata</i>	3	3
Fresh leaves of <i>Quercus salicina</i>	- Dried leaves of <i>Quercus salicina</i>	-	6
Fresh leaves of <i>Castanea crenata</i>	- Dried leaves of <i>Castanea crenata</i>	3	3
Fresh leaves of <i>Fagus crenata</i>	- Dried leaves of <i>Fagus crenata</i>	2	3
Fresh leaves of <i>Cryptomeria japonica</i>	- Dried leaves of <i>Cryptomeria japonica</i>	4	2

Table 2 Chemical profiles of fresh leaf volatiles of the six tree species identified by gas chromatography–mass spectrometry. Identification was performed by matching the experimental mass spectra to those in the National Institute of Standards and Technology (NIST) database. The numbers under the names of the tree species indicate the ratios between compound peak areas and nonyl acetate peak areas. Only compounds with more than 90% mass-spectrum similarity to the NIST database are shown

Compound name	Retention time	<i>Quercus crispula</i>	<i>Quercus serrata</i>	<i>Quercus salicina</i>	<i>Castanea crenata</i>	<i>Fagus crenata</i>	<i>Cryptomeria japonica</i>
3-Hexen-1-ol	3.2	0.75			0.31	1.11	
α -Pinene	4.4			0.22	0.17		3.31
Sabinene	5.1			0.47	0.36		9.68
3-Hexen-1-ol, acetate	5.6	6.23	17.44			7.85	
3-Carene	5.7						0.22
D-Limonene	5.9						3.05
α -Terpinene	6.9		1.08				0.34
(E)-4,8-Dimethyl-1,3,7-nonatriene	7.3	6.50	15.97		2.63	1.37	
4-Terpinenol	8.3						0.10
Dodecane	8.6	0.41	0.43				
Decanal	8.7	0.29				0.03	
β -Pinene	9.4						0.06
Tridecane	10.0	0.33	0.32				
α -Cubebene	10.8	0.35					
α -Copaene	11.1	0.20	0.20				
β -Bourbonene	11.2	1.76					0.01
β -Elemene	11.3		2.08				
Tetradecane	11.4	0.08			0.02		
Caryophyllene	11.7	3.39	2.60				
β -Cubebene	11.9	0.14					
Aromadendrene	12.2	1.98	0.98				
Germacrene	12.5	1.12	10.08				
β -Selinene	12.6		0.90				
α -Farnesene	12.7		18.50				
δ -Cadinene	13.0	0.35	0.76				0.03
Nerolidol	13.4	0.47	1.21				
Hexadecane	13.7	0.10	0.11				
Heptadecane	14.9	0.26	0.30				
Octadecane	16.0	0.16	0.17				
Kaur-16-ene	18.4	0.21	0.43				0.64

Table 3 Chemical profiles of dried leaf volatiles of the six tree species identified by gas chromatography–mass spectrometry. Identification was performed by matching the experimental mass spectra to those in the National Institute of Standards and Technology (NIST) database. The numbers under the names of the tree species indicate the ratios between compound peak areas and nonyl acetate peak areas. Only compounds with more than 90% mass-spectrum similarity to the NIST database are shown

Compound name	Retention time	<i>Quercus crispula</i>	<i>Quercus serrata</i>	<i>Quercus salicina</i>	<i>Castanea crenata</i>	<i>Fagus crenata</i>	<i>Cryptomeria japonica</i>
Trans-2-hexenal	3.2	2.54	1.89	27.37	10.13	11.12	
α -Pinene	4.4			4.04			6.20
Benzaldehyde	4.9	0.07	0.16		0.51	1.36	
Sabinene	5.1			7.98			18.25
3-Carene	5.7						0.73
D-Limonene	5.9						5.51
γ -Caprolactone	6.3				1.94	4.30	
γ -Terpinene	6.4			4.36			0.61
α -Terpinene	6.9						0.68
Nonanal	7.1			4.97			
(E)-4,8-Dimethyl-1,3,7-nonatriene	7.3				5.70	3.22	0.44
4-Terpinenol	8.3			3.72			
Decanal	8.7			2.95	0.28	0.39	
β -Cyclocitral	9.0	0.03	0.06		0.49	0.37	
2,6-Octadienal,3,7-dimethyl	9.2				0.39		
Geraniol	9.4				0.25	2.77	
Linalyl acetate	9.5						0.50
Citral	9.6			2.34	0.83	0.49	
Tridecane	10.0			1.64			
α -Cubebene	10.8					0.46	
Eugenol	10.9					0.42	
α -Copaene	11.1	0.03	0.04				
Tetradecane	11.4	0.03	0.05			0.56	
Caryophyllene	11.7	0.03	0.05				
α -Ionone	11.9				0.35	0.09	
α -Humulene	12.2	0.02	0.03				
Germacrene	12.5	0.06					
β -Ionone	12.6				0.13	0.22	
α -Farnesene	12.7	0.22	0.29	70.16	0.81	1.81	
δ -Cadinene	13.0	0.02	0.02	1.55			
Hexadecane	13.7			0.79			
Diethyl phthalate	13.8					0.41	
Kaur-16-ene	18.4					0.10	

Fig. 1 Predicted proportion and 95% confidence interval (CI) of trials in which *Platypus quercivorus* beetles selected fresh leaf volatiles (F) of six tree species compared to clean air (Cont). Qcr, *Quercus crispula*; Qse, *Q. serrata*; Qsa, *Q. salicina*; Cas, *Castanea crenata*; Fag, *Fagus crenata*; Cry, *Cryptomeria japonica*. The predicted proportions attached with the same small letters are not significantly different

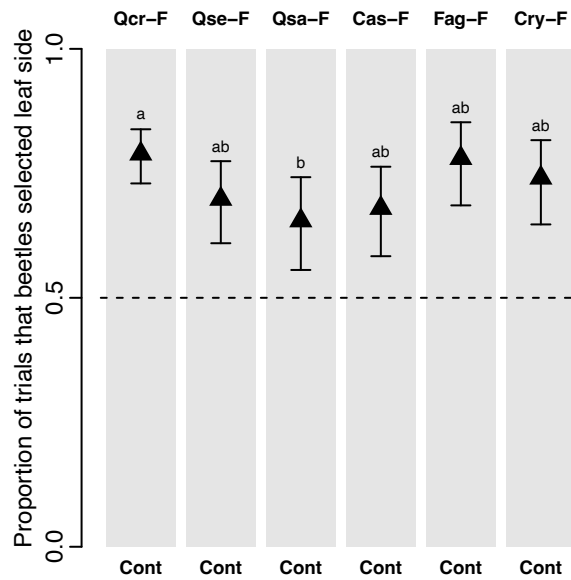


Fig. 2 Predicted proportion and 95% confidence interval (CI) of trials in which *Platyous quercivorus* beetles selected dried leaf volatiles (D) of six tree species compared to clean air (Cont). Qcr, *Quercus crispula*; Qse, *Q. serrata*; Qsa, *Q. salicina*; Cas, *Castanea crenata*; Fag, *Fagus crenata*; Cry, *Cryptomeria japonica*. The predicted proportions attached with the same small letters are not significantly different

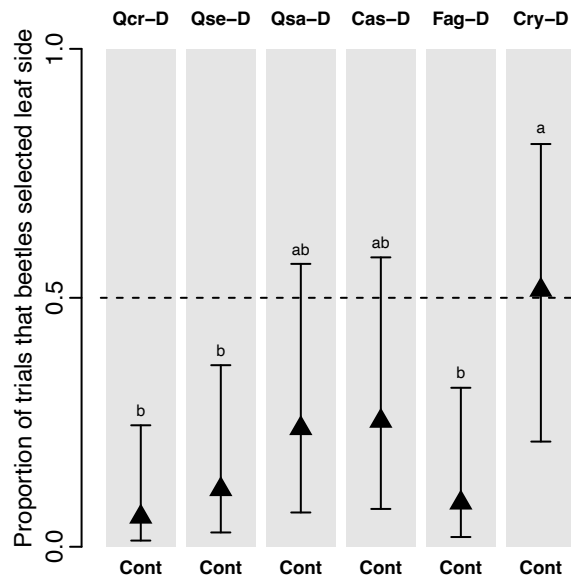
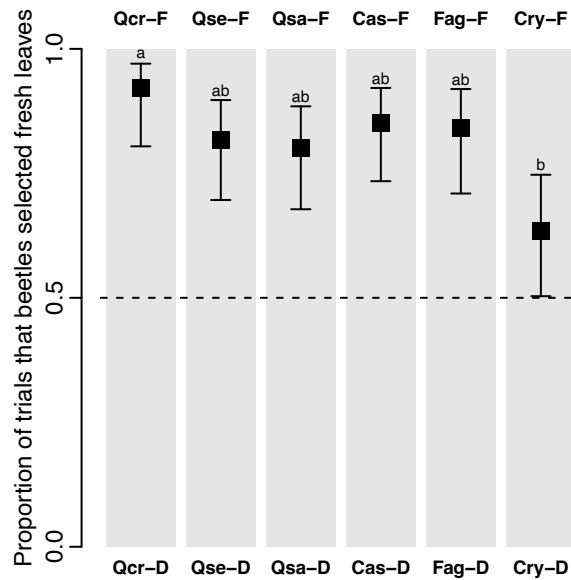


Fig. 3 Predicted proportion and 95% confidence interval (CI) of trials in which *Platypus quercivorus* beetles selected fresh leaf volatiles (F) compared to dried leaf volatiles (D) of six tree species. Qcr, *Quercus crispula*; Qse, *Q. serrata*; Qsa, *Q. salicina*; Cas, *Castanea crenata*; Fag, *Fagus crenata*; Cry, *Cryptomeria japonica*. The predicted proportions attached with the same small letters are not significantly different



Supplementary table 1 Number of males and females of the oak ambrosia beetle, *Platypus quercivorus*, for the tests on each day of the experiment. Beetles were evaluated their response in Y-tube olfactometer to select between fresh leaf volatiles (Fresh) and clean air (Cont), dried leaf volatiles (Dried) and clean air or fresh and dried leaf volatiles. Same beetles were used for multiple tests of comparison and each test was repeated 10 times for one individual beetle.

Day of experiment	Sources of leaf volatiles						Pair of comparison			Number of tested beetles	
	<i>Quercus crispula</i>	<i>Quercus serrata</i>	<i>Quercus salicina</i>	<i>Castanea crenata</i>	<i>Fagus crenata</i>	<i>Cryptomeria japonica</i>	Fresh - Cont	Dried - Cont	Fresh - Dried	Males	Females
June	22	v				v	v			1	1
	23	v	v				v			2	2
	24	v	v				v			3	1
	25	v		v			v			1	2
	26	v				v	v			4	2
	27	v					v	v	v	1	4
	29	v			v		v			3	4
	30		v	v			v			1	4
	2	v		v	v		v			1	3
	3	v		v			v			2	3
July	4	v	v				v			0	4
		v				v	v			0	2
	5	v			v		v			0	1
		v	v				v			2	2
	6		v				v	v	v	0	6
	7				v		v	v	v	2	3
	10		v			v	v			1	2
			v	v			v			1	2
		v	v				v			1	0
	11	v					v	v	v	3	3
	12					v	v	v	v	4	2
	13			v			v	v	v	3	3
										36	56

Supplementary table 2 Results of a generalized linear mixed model with a logit link function to predict the proportion of trials in which *Platypus quercivorus* beetles selected a side with fresh leaf volatiles over clean air in a Y-tube olfactometer. Estimated coefficients, their standard errors, *Z* statistics, and significance of differences from 0 (**P* < 0.05, ***P* < 0.01, ****P* < 0.001) are shown. Because *Quercus crispula* is set as a baseline, estimate for *Q. serrata* shows the difference between *Q. serrata* and *Q. crispula* on the logit scale, for example. Statistical tests were conducted individually for each estimate; therefore, the results differ from those of multiple comparisons among the six species shown in Fig. 1. The individual beetles nested within beetle sex were included in the model as random intercepts.

	Estimate	Std. Error	<i>Z</i>	<i>P</i>	
Intercept (<i>Quercus crispula</i>)	1.320	0.167	7.914	< 0.001	***
<i>Q. serrata</i>	-0.481	0.221	-2.174	0.030	*
<i>Q. salicina</i>	-0.679	0.225	-3.014	0.003	**
<i>Castanea crenata</i>	-0.566	0.229	-2.476	0.013	*
<i>Fagus crenata</i>	-0.053	0.257	-0.205	0.837	
<i>Cryptomeria japonica</i>	-0.269	0.239	-1.129	0.259	

Supplementary table 3 Results of a generalized linear mixed model with alogit link function to predict the proportion of trials in which *Platypus quercivorus* beetles selected a side with dried leaf volatiles over clean air in a Y-tube olfactometer. Estimated coefficients, their standard errors, *Z* statistics, and significance of differences from 0 (**P* < 0.05, ***P* < 0.01, ****P* < 0.001) are shown. Because *Quercus crispula* is set as a baseline, estimate for *Q. serrata* shows difference between *Q. serrata* and *Q. crispula* on the logit scale, for example. Statistical tests were conducted individually for each estimate; therefore, the results differ from those of multiple comparisons among the six species shown in Fig. 2. The individual beetles nested within beetle sex were included in the model as random intercepts.

	Estimate	Std. Error	<i>Z</i>	<i>P</i>	
Intercept (<i>Quercus crispula</i>)	-2.752	0.827	-3.328	< 0.001	***
<i>Q. serrata</i>	0.714	0.698	1.023	0.306	
<i>Q. salicina</i>	1.587	0.633	2.508	0.012	*
<i>Castanea crenata</i>	1.666	0.668	2.496	0.013	*
<i>Fagus crenata</i>	0.417	0.726	0.574	0.566	
<i>Cryptomeria japonica</i>	2.814	0.707	3.979	< 0.001	***

Supplementary table 4 Results of a generalized linear mixed model with a logit link function to predict the proportion of trials in which *Platypus quercivorus* beetles selected a side with fresh leaf volatiles over dried leaf volatiles in a Y-tube olfactometer. Estimated coefficients, their standard errors, Z statistics, and significance of differences from 0 ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) are shown. Because *Quercus crispula* is set as a baseline, estimate for *Q. serrata* shows difference between *Q. serrata* and *Q. crispula* on the logit scale, for example. Statistical tests were conducted individually for each estimate; therefore, the results differ from those of multiple comparisons among the six species shown in Fig. 3. The individual beetles nested within beetle sex were included in the model as random intercepts.

	Estimate	Std. Error	Z	P	
Intercept (<i>Quercus crispula</i>)	2.448	0.528	4.636	< 0.001	***
<i>Q. serrata</i>	-0.950	0.623	-1.524	0.128	
<i>Q. salicina</i>	-1.058	0.618	-1.713	0.087	
<i>Castanea crenata</i>	-0.709	0.639	-1.110	0.267	
<i>Fagus crenata</i>	-0.785	0.653	-1.203	0.229	
<i>Cryptomeria japonica</i>	-1.899	0.592	-3.208	0.001	**