

Odor Preference, Feeding, Survival, and Reproductive Fitness of the Invasive Larger Grain Borer *Prostephanus truncatus* on Acorns of Three Native North America Oak Species

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Abstract

The larger grain borer, *Prostephanus truncatus* (Horn), is an invasive pest on stored grains but can also exploit non-grain food sources. Currently, this species has a limited distribution in North America but possesses the potential to further expand across the continent. Alternative non-grain foods could play a role in range expansion and previous research has shown that acorns can be a suitable food for the lesser grain borer *Rhyzopertha dominica* (F.), a closely related grain pest. Here, we assessed the odor preference of *P. truncatus* for acorns from black oak (*Quercus velutina*), red oak (*Quercus rubra*), and bur oak (*Quercus macrocarpa*) compared to maize and wheat, and analyzed their volatile organic compound (VOC) profiles. Further, we measured *P. truncatus* feeding and reproduction on these acorns. Results demonstrated that *P. truncatus* responded to volatiles of each acorn species but responded most positively to bur oak acorns. Interestingly, bur oak acorns were also more attractive than maize, its main host grain. VOC profiles showed that two compounds emitted from bur oak acorns, 3-hepten-2-one and 2-(5-methyl-furan-2-yl)-propionaldehyde, were unique compared to other treatments, which may be important for odor preference. *P. truncatus* also preferred to feed on bur oak acorns over other oak species. Bur oak was the only acorn species tested in which F₁ progeny were detected. These results suggest that *P. truncatus* exhibits evolutionary plasticity that enables it to survive on acorns in the absence of grains, highlighting an elevated risk of landscape-level invasion by this pest in North America.

Introduction

The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), is an invasive stored-product insect pest that is native to Mexico and Central America (Hodges 1986). The species was accidentally introduced into Tanzania in the late 1970s (Hodges et al. 1983) and subsequently spread across much of sub-Saharan Africa (Holst and Meikle 2003). It has also occasionally been intercepted in the southern USA, Asia, and Europe (Quellhorst et al. 2021) and interceptions are expected to increase moving forward (Harman et al. 2024). Although it is not established outside of Mexico, Central America, or Africa, Arthur et al. (2019) used species distribution models (SDMs) based on current climate data from locations with documented populations to predict that large areas of the tropics in Asia and the Western Hemisphere could support *P. truncatus* populations if the species was introduced. Moreover, Harman et al. (2024) demonstrated that the threat of range expansion by *P. truncatus* was most likely to worsen in the coming decades, and that future changes in environmental conditions could result in a higher risk to temperate regions. In a two-year study involving trapping at 19 locations along a latitudinal gradient across North America, Quellhorst et al. (2025) only found *P. truncatus* in Mexico. However, the continued range expansion of *P. truncatus* is a global concern due to climate change and globalization.

P. truncatus is hypothesized to have originally been a wood-boring insect and still retains this capability (Nang'ayo et al. 1993; Ramírez-Martínez et al. 1994; Scholz et al. 1997a), but it has expanded its host range to include maize and cassava (Hodges et al. 1985; Sadiku et al. 2019). Maize can become infested with *P. truncatus* while still on the cob in the field, and infestations continue at storage facilities (Golob and Hanks 1990). The ability of *P. truncatus* to infest root and tuber crops such as cassava, yam, and

sweet potato has also been documented (Nyakunga 1982; Osipitan et al. 2015; Ejiro et al. 2018; Sadiku et al. 2019). Interestingly, a variety of other commodities, including wheat, barley, oats, rice, and beans, are considered inferior food sources for *P. truncatus* and all fail to serve as reproductive hosts (Hodges 1986; Athanassiou et al. 2017). Thus, compared with other stored-product insect species that can exploit a wide range of commodities, *P. truncatus* is primarily found exploiting stored maize and is poorly adapted to the exploitation of most other stored commodities; as such, it may offer some insights into the early stages of host shifting that led to the development of stored-product pests (Quellhorst et al. 2021). Identifying original and alternative hosts of *P. truncatus* is important for preventing its establishment and spread, especially in non-native areas.

Dispersal of *P. truncatus* adults away from stored products often occurs at food facilities, especially when food sources are depleted or population density is high (Hodges 1986; Fadamiro and Wyatt 1995; Scholz et al. 1997b; Altwegg et al. 2013), during which they exploit non-grain resources while moving through the landscape. Borgemeister et al. (1998a) trapped *P. truncatus* in a primary forest, a peri-urban area, and a region with intensive agriculture in Benin and analyzed the gut contents of beetles. They found that *P. truncatus* individuals captured in wooded areas had more lignin in their guts, whereas those in intensive agriculture settings had both starch and lignin, indicating that woody hosts were being utilized. Nang'ayo (1996) conducted no-choice rearing tests in the laboratory and showed that branches of many tree species found in the Kenyan savanna were acceptable for reproduction by *P. truncatus*, although reproductive rates were generally low and inconsistent. Similarly, Nansen et al. (2004) evaluated *P. truncatus* performance on a variety of tree species native to Africa and observed heavy attack on fresh branches from eleven tree species and fresh roots from four tree species. They further reported successful reproduction on branches of *Delonix regia*, *Ceiba pentandra*, *Manihot esculenta*, and *Ficus exasperata*, as well as on roots of *Ficus capensis* and *F. exasperata*. In natural environments, the documented forest hosts for *P. truncatus* are branches of trees in the family Anacardiaceae that have been girdled by cerambycid beetles (Ramírez-Martínez et al. 1994; Nang'ayo 1996; Borgemeister et al. 1998b). These studies show the potential role of wooded habitats in the ecology of *P. truncatus* as reservoirs and breeding grounds, which may then contribute to its infestations in stored maize and cassava. Further research is needed to understand how *P. truncatus* selects hosts and reproduces in natural environments (Quellhorst et al. 2021).

Importantly, *P. truncatus* has been found to positively respond to host-produced volatiles from starchy commodities and woody species, including maize, wheat, and wood of *Commiphora africana* (A. Rich.) (Scholz et al. 1997a). Prior work has also determined that a single calling male *P. truncatus* on stored maize was sufficient to initiate a large infestation (Scholz et al. 1997b). Follow-up work demonstrated that like maize, wood of *Commiphora riparia* Engl. and *C. africana* caused arrestment of *P. truncatus* males (Scholz et al. 1998). In addition, other studies have also reported positive attraction in *P. truncatus* towards maize and cassava (Wright et al. 1993; Hodges 1994; Pike et al. 1994). Thus, it is likely that host odors can play an important role in attraction, colonization and establishment of *P. truncatus* populations in natural environments.

Oak trees (*Quercus* spp.) are widely distributed across the globe from northwestern South America, through Central America, North America, Europe, the northwestern edge of Africa, and much of eastern Asia (Steele et al. 2021). Particularly, oaks are a prevalent and dominant tree genus in North America (Frankel et al. 2022), especially in the southern United States, where *P. truncatus* poses a risk of establishment (Harman et al. 2024, 2025). Oak acorns are a significant resource for a range of animals, including insects. More relevantly, native oak acorn species in North America can be used for survival and reproduction by the stored-product pest, the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), a close relative of *P. truncatus* (Jia et al. 2008). It is interesting to note in that study that survival and reproduction were especially prominent on damaged acorns, but not undamaged acorns. Currently, it is not known whether *P. truncatus* is also attracted to volatiles produced by acorns or whether acorns can be used for survival and reproduction. Investigating whether *P. truncatus* can subsist on native resources in the landscape of North America will help enhance our understanding of population subsistence and risk posed by intercepted individuals. This is particularly important for predicting potential dispersal and immigration patterns in newly invaded natural environments.

The objective of this study was to evaluate the potential of acorns as sources of attractive odors, as well as the suitability of damaged and undamaged acorns as feeding and breeding substrates that could support *P. truncatus* host-range expansion. To that end, we first conducted *P. truncatus* odor preference assays with acorns of black oak (*Quercus velutina*), red oak (*Quercus rubra*) and bur oak (*Quercus macrocarpa*) that are all native, common, and widely distributed oak species in North America, but not native to Mexico and likely novel to *P. truncatus*. We then characterized the relative profiles of volatile organic compounds (VOCs) from these acorns and explored the semiochemical-mediated behavioral response between *P. truncatus* and acorn species. We finally determined *P. truncatus* feeding, survival and reproductive fitness on damaged or undamaged acorns in the laboratory. Considering the global distribution and abundance of oak trees and the impacts wrought by climate change and globalization on the geographical distribution and introduction of invasive insect pests, this study will help better assess the risk of *P. truncatus* to establish in North America by utilizing hosts already present in the landscape so that effective biosecurity measures can be implemented.

Materials and Methods

Insects

Prostephanus truncatus adults were obtained from an insect colony maintained at the United States Department of Agriculture (USDA), Agricultural Research Service, Center for Grain and Animal Health Research in Manhattan, Kansas, USA. The individuals were originally collected from Mexico, in native range of *P. truncatus*. Insects were reared on whole maize kernels in jars with screened lids and kept at 23°C and 45% relative humidity (RH) under continuous darkness.

Acorns and Grains

Acorns of black oak, red oak, and bur oak were collected from the ground beneath trees in Manhattan, Kansas, USA at two locations (39.18°N, -96.59°W and 39.20°N, -96.62°W) during the fall of 2022–2024. We removed the caps and discarded any acorns that had cracks or holes in the shells since these may already be infested with other insects. Intact acorns (without cracks or holes) from healthy oak trees were used in the experiments. Organic yellow maize (*Zea mays*) and hard red winter wheat (*Triticum aestivum*) (Heartland Mills, Marienthal, KS, USA) were also used in the experiments.

Odor Preference Assays with Acorns and Grains

Odor preference assays for adult *P. truncatus* were conducted in Petri dishes (100 × 15 mm, D × H, VWR, Radnor, PA, USA) serving as arenas, with one individual per dish. Two holes, spaced 5 cm apart and centered over each half of the dish, were carefully drilled on the bottom using a 7/64-inch (2.8-mm) drill bit. Holes were also drilled through a piece of filter paper (85 mm, Ahlstrom-Munksjö, Mt. Holly Springs, PA, USA) affixed with double-sided tape to the bottom of the arena to facilitate *P. truncatus* movement. The holes allowed the diffusion of host volatiles into the arena so that there were point source emissions of volatiles but no visual cues. The diffusion of volatiles was confirmed with a smoke test. For each pairwise comparison, two plastic cups (30 × 50 mm, D × H, CrystalWare, Lakewood, NJ, USA) were placed directly beneath the two arena holes, each containing one of the following food stimuli: a single intact acorn of black oak, red oak or bur oak; 1.5 g of maize or wheat kernels; or empty (i.e., blank control). *P. truncatus* adults were placed individually in the center of the arena, and they were given 16 h to respond to an odor source. At the end of the trial, the number of insects choosing each treatment was recorded. There were n = 74 replicates per treatment combination.

HS-SPME/GC-MS Analysis of Acorns and Grains

VOCs from acorns and grains were analyzed using headspace solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS), based on previously developed methods (Buško et al. 2016; De Flaviis et al. 2021) with some modifications, as described below. Maize, wheat, and acorns of bur oak, black oak and red oak were ground separately with a dedicated coffee grinder (Hamilton Beach, Model 80335G, Glen Allen, VA, USA) into a fine whole flour. To prevent cross-contamination between samples, the coffee grinder was thoroughly rinsed with water, followed by repeated rinsing with hexane before use. A total of 2 g of the flour from each sample (n = 5 replicates per treatment) was transferred into a 20-mL headspace vial (Gerstel, Linthicum Heights, MD, USA) and a vial with no flour served as a negative control. The vials were then completely sealed with magnetic screw caps with a Teflon septum. SPME was performed using an automated program on an MPS autosampler (Gerstel, Linthicum Heights, MD, USA). The flour samples were incubated at 60°C on a heating block for 15 min in the pre-equilibration stage, and then a 50/30 µm DVB/Carboxen/PDMS fiber (Supelco 57298-U, St Louis, MO, USA) was exposed for 30 min at 60°C to extract the VOCs. After the extraction step, the SPME fiber was desorbed in the injection port of the GC-MS for 3 min at 260°C in a pulse splitless mode of 40 psi followed by a purge flow of 15 mL/min. VOCs were separated using an Agilent 7890B GC

(Agilent, Santa-Clara, CA, USA) coupled to a single quadrupole mass spectrometer (Agilent 5997B), using a Durabond HP-5 capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent). The GC oven ran initially 1 min at 40°C followed by ramping from 40°C to 80°C at a rate of 7°C/min, then from 80°C to 130°C at a rate of 1°C/min, and finally from 130°C to 260°C at a rate of 10°C/min. The He flow rate was held constant at 1.5 mL/min. Spectra were acquired at 50 spectra/s within a range of 40–380 amu. The transfer line temperature was 280°C and the ion source temperature was 200°C. Compounds were tentatively identified by comparison of spectral data with those from the NIST 17 library and by GC retention index.

Mobility in Close-range Food Preference Assays

Following the odor preference for bur oak acorns observed in the choice assays, insect mobility in close-range food preference assays was recorded over a 60-min period using a video-tracking apparatus combined with EthoVision software (v. 16.0, Noldus Information Technology Inc., Leesburg, VA, USA). The equipment was set up to track six arenas (100 × 15 mm, D × H, VWR, Radnor, PA, USA) simultaneously, and within each arena, a single adult was observed. The arenas were placed in cutouts on white foamboard to affix position that was 80 cm below a network camera (GigE, Basler AG, Ahrenburg, Germany) and backlit with a LED light box (42 × 30 cm, W × L, LPB3, Litup, Shenzhen, China). A piece of filter paper (85 mm, Ahlstrom-Munksjö, Mt. Holly Springs, PA, USA) was taped to the bottom of the arena to facilitate mobility. Two tiny notches exactly halfway on each side of the arena marked where food was to be placed (i.e., 2.1 cm from the edge of the arena). On either side of the arena, we left it empty (i.e., blank control), or placed a single kernel of maize, a single kernel of wheat, or a piece of bur oak acorn (approximately the same mass as a maize kernel). Stimuli were affixed in place with a 3 × 3 mm square of parafilm (Parafilm M, Bemis Co., Davisburg, MI, USA). In total, there were 6 food comparisons, including: maize vs wheat, maize vs bur oak, wheat vs bur oak, maize vs blank, wheat vs blank, and bur oak vs blank. For each individual trial, a new Petri dish and filter paper were used to prevent confounding stimuli. After tracking, the program calculated the frequency of visits and time spent in each zone adjacent to the food source (e.g., within a 1.6-cm diameter circle) for each adult. There were n = 18 replicates per treatment.

Prostephanus truncatus Feeding and Survival on Acorns

Acorns of black oak, red oak and bur oak were tested for their ability to support adult *P. truncatus* feeding and survival. We determined *P. truncatus* feeding, survival and reproductive fitness on both damaged and undamaged acorns. To simulate damage, three evenly spaced holes were drilled on the adaxial surface of each acorn shell using a Dremel multi-tool (Model 300, Racine, WI, USA) with a 5/64-inch (2-mm) drill bit. A piece of filter paper (60 mm, Whatman, GE Healthcare Life Sciences, Buckinghamshire, UK) was taped to the bottom of a glass jar (118 mL, Ball Quilted Crystal, Atlanta, GA, USA). A total of 20 adults were placed on a single damaged or undamaged acorn (red oak, black oak, or bur oak) in the jar with a screened lid in a no-choice assay. A jar with no food served as a negative control and a jar with 2.5 g of maize kernels served as a positive control. The jars were left undisturbed for 2 and 4 weeks to allow the adults to feed on the acorns at 23°C and 45% RH under continuous

darkness. Afterwards, each acorn was dissected to check for the presence of alive or dead adults, including those found inside or outside the acorn. The rates of adult survival and presence inside acorns were expressed as percentages, dividing the number of individuals that were alive or present inside acorns by the total number of adults in the original sample, respectively. At the end of each period, an X-ray imaging system (MX-20, Faxitron, Wheeling, IL, USA) was also used to examine the acorns for evidence of insect tunneling and feeding activity. Feeding damage was quantified based on the amount of fines produced, and the fines were weighed using a precision balance (B120S, Sartorius Basic, Göttingen, Germany). In total, there were 8 treatments, including: acorns (damaged or undamaged) from bur oak, black oak and red oak, maize as a positive control, and no food as a negative control. There were $n = 5$ replicates per treatment.

***Prostephanus truncatus* Reproductive Fitness on Acorns**

Damaged and undamaged acorns of black oak, red oak and bur oak were tested for their ability to support production of *P. truncatus* F_1 progeny. A piece of filter paper (60 mm, Whatman, GE Healthcare Life Sciences, Buckinghamshire, UK) was taped to the bottom of a glass jar (118 mL, Ball Quilted Crystal, Atlanta, GA, USA) and 20 adults were placed in the jar with a single damaged or undamaged acorn for a no-choice test. The resulting jars were left undisturbed under the same conditions as above for 4 weeks, after which the adults were removed. Subsequently, the acorns were maintained for 6 weeks. At the end of this period, the same X-ray imaging system mentioned above was used to examine the acorns for evidence of insect reproductive success. Then, the acorn was dissected to count the total number of *P. truncatus* F_1 progeny, including larvae, pupae and adults produced. There were 6 treatments, consisting of damaged or undamaged acorns of black oak, red oak, and bur oak, each with $n = 25$ replicates per treatment.

Data Analysis

A Chi-squared goodness of fit test was used to determine whether there were any odor preferences by *P. truncatus*. Except where otherwise noted, the significance level was $\alpha = 0.05$ for all analyses.

Percentages of final choices for each food were calculated to describe overall trends in odor preference.

To characterize the VOCs of each sample, raw peak areas were extracted from the GC and total ion chromatogram (TIC), and peaks were tentatively identified in profiles automatically using MassHunter Unknowns Analysis (Agilent Technologies, Inc., Santa Clara, CA, USA) with a minimum match factor of 70% to the NIST17 library. Peaks with a height less than 0.3% of the tallest peak were excluded from the exported peak table. Compounds were aligned across samples by pulling underlying m/z ratio data from public repositories and flexibly aligning compounds based on structural matches and retention time using the R package *uafR* (Stratton et al. 2024). Singleton compounds were deleted as they represented transient emissions that are not indicative of differences among treatments. Pairwise Bray-Curtis similarities were calculated among samples, and a non-metric multidimensional scaling (NMDS) plot was used to visualize the differences in VOC profiles among treatments. A total of $n = 1000$

permutations were used for the ordination procedure. Stress values for the NMDS procedure were < 0.10 , indicating that good interpretation was possible. Analysis of similarities (ANOSIM) was used to determine significant differences for VOC emissions among treatments, while similarity percentage (SIMPER) was used to determine the major compounds significantly contributing to differences among the groups. Permutations for both analyses were $n = 1000$. Based on the SIMPER analysis, differences in the emissions of key compounds were visualized using boxplots from the *ggplot2* package in R (Wickham 2016). For all multivariate statistics of VOCs, the R Package *vegan* was used (Oksanen et al. 2025). A table visualizing the VOC emissions of oak acorns and grains was created with the *gt* package in R (Iannone et al. 2025).

To analyze mobility in close-range food preference assays, two dependent variables, frequency and duration of visits to each food zone, were tested using a generalized linear model (GLM) with a negative binomial likelihood and a log link function. The fixed, explanatory variables included the food stimuli (blank control, maize, wheat, or bur oak acorn) and the main effects of each stimuli comparison were estimated. Means of frequency and duration of visits to each food zone were calculated to describe overall trends in food preference.

For *P. truncatus* survival and presence inside acorns, there were three factors, acorn species (black oak, red oak, or bur oak), acorn status (damaged or undamaged), and time (2 or 4 weeks). A Chi-square test of independence was used to determine whether there was a statistically significant association between the factors and the survival rates or percentages of *P. truncatus* inside acorns. When a significant association was found, post hoc pairwise comparisons with Bonferroni correction were then performed to examine if treatments were statistically different.

To analyze *P. truncatus* feeding and reproduction on acorns, a linear mixed model (LMM) with Bonferroni correction was used to compare the amounts of fines production and the numbers of F_1 progeny produced on damaged and undamaged acorns of three oak species. The fixed, explanatory variables included potential food sources (black oak, red oak, bur oak, or maize) and status of acorns (damaged or undamaged). Upon a significant result from the overall model, Tukey's HSD was used for multiple comparisons.

Results

Odor Preference of *Prostephanus truncatus*

In food odor preference tests, *P. truncatus* was significantly more attracted to acorns of black oak and bur oak, maize, and wheat than to the blank control (Table 1; Fig. 1A and B). By contrast, there was no difference between red oak and the control (Table 1; Fig. 1A). Bur oak was significantly more attractive than black oak, red oak (Table 1; Fig. 1A), and the maize host on which *P. truncatus* colony was reared (Table 1; Fig. 1B).

Table 1
Statistical results of *Prostephanus truncates* odor preference

Treatment*	df [†]	χ^2	P
Maize vs Blank	1	6.82	< 0.01
Wheat vs Blank	1	5.23	< 0.05
Bur oak vs Blank	1	31.04	< 0.001
Black oak vs Blank	1	3.85	< 0.05
Red oak vs Blank	1	1.40	0.237
Maize vs Wheat	1	0.31	0.579
Maize vs Bur oak	1	23.20	< 0.001
Wheat vs Bur oak	1	1.00	0.317
Black oak vs Bur oak	1	16.33	< 0.001
Red oak vs Bur oak	1	8.65	< 0.01
Black oak vs Red oak	1	1.49	0.223
*Grains and acorns were used as odors in two-choice pitfall traps.			
[†] Chi-square goodness of fit test was used in data analysis.			

VOCs Emitted by Acorns and Grains

Out of 132 tentatively identified VOCs from oak acorns and grains through the HS-SPME/GC-MS analysis, 28 compounds were detected with a relative abundance greater than 1% in one treatment (Table 2). Black oak, red oak, bur oak, maize, and wheat had 22, 22, 24, 20, and 21 compounds, respectively. 2,4-dimethyl-1-heptene, 2-butyl-1-octanol, 4-methyl-decane and 2,6-dimethyl-nonane were the four most abundant compounds in each treatment. 2,4-dimethyl-1-heptene was the most abundant compound in acorns of black oak, red oak, and bur oak, whereas 2-butyl-1-octanol was the most abundant one in maize and wheat. Two compounds emitted from bur oak acorns, 3-hepten-2-one and 2-(5-methyl-furan-2-yl)-propionaldehyde, were unique compared to other treatments. There was some overlap in VOC compositions of maize and wheat grains, while there was significant differentiation among the three acorn species as well as the acorns and the grains (ANOSIM: R = 0.744, Permutations = 1000, $P < 0.001$, Fig. 2). The VOC composition of bur oak acorns was less variable than the red and black oak acorns (Fig. 2). Six compounds, including 2-butyl-octanol, 2,4-dimethyl-1-heptene, 2,6-dimethyl-nonane, 2,7-dimethyl-octanol, 4-methyl-decane, and heptanone, were shown to cumulatively contribute to a > 50% difference between bur oak and others including black oak, red oak, maize, and wheat, and there were significant differences among treatments in their emissions of these compounds (Fig. 3). Tukey's

HSD tests indicated that there were higher heptanone emissions by bur oak compared to black oak, red oak, maize, and wheat. Finally, bur oak emitted higher levels of 2,7-dimethyl-octanol than maize and wheat.

Table 2 Summary of volatiles tentatively identified by headspace GC-MS from oak acorns and grains potentially used by *Prostephanus truncatus*

COMPOUND ¹	RT (MIN)	Acorn									Grain					
		Black oak			Red oak			Bur oak			Maize			Wheat		
		MEAN ²	SE	% OF TOTAL	MEAN ²	SE	% OF TOTAL	MEAN ²	SE	% OF TOTAL	MEAN ²	SE	% OF TOTAL	MEAN ²	SE	% OF TOTAL
2,4-Dimethyl-1-heptene	3.34	20	2	24.7	30	3	35.8	10	0.2	15.7	20	2	16.5	10	1	12.4
1-Hexanol	4.91	0.3	0.3	0.300	0	0	0	0	0	0	0.2	0.2	0.300	1	0.07	1.40
4-Heptanone	5.07	0	0	0	1	0.1	1.20	1	0.04	1.40	0	0	0	0	0	0
2-Heptanone	5.94	0.1	0.1	0.100	3	0.5	3.60	4	0.09	4.70	0	0	0	0	0	0
2,5-Dimethylhexane-2,5-dihydroperoxide	7.60	1	0.03	1.60	0.8	0.2	0.900	0.06	0.06	0.100	1	0.04	1.40	1	0.02	1.30
3-Hepten-2-one	7.88	0	0	0	0	0	0	1	0.02	1.50	0	0	0	0	0	0
Nonane, 4-methyl-	9.00	3	0.1	3.80	4	0.2	4.50	4	0.03	4.00	4	0.1	3.90	3	0.1	3.20
iso-Butyl aldehyde propylene glycol acetal 1	9.40	0.8	0.09	0.900	1	0.03	1.50	0	0	0	0.6	0.04	0.700	0.5	0.01	0.500
2-[5-Methyl-furan-2-yl]-propionaldehyde	10.38	0	0	0	0	0	0	2	0.04	1.80	0	0	0	0	0	0
5-Methyl-1R,3-trans-cyclohexanediol	10.55	4	0.9	4.00	1	1	1.30	0	0	0	0	0	0	0	0	0
Decane	10.79	2	0.06	2.70	2	0.8	2.10	3	0.05	3.30	3	0.06	2.80	2	0.05	2.40
Orthoformic acid, trisobutyl ester	10.98	0.3	0.3	0.300	0	0	0	0	0	0	0.3	0.3	0.300	1	0.3	1.40
Orthoformic acid, tri-sec-butyl ester	10.99	1	0.3	1.40	0	0	0	0.6	0.3	0.600	1	0.3	1.30	0.6	0.4	0.700
Decane, 4-methyl-	11.30	10	6	14.0	5	3	5.90	10	0.1	13.9	10	0.3	15.6	10	0.1	14.6
4-Decene, 7-methyl-, (E)-	11.88	3	0.08	3.50	3	0.1	3.20	3	0.04	3.70	4	0.07	4.00	3	0.03	3.70
Nonane, 2,6-dimethyl-	12.00	10	6	13.8	20	3	22.3	10	0.1	13.8	10	0.3	15.0	10	0.1	13.7
2-Undecene, (Z)-	12.58	1	0.04	1.30	0.9	0.06	1.00	1	0.02	1.40	1	0.03	1.50	1	0.01	1.50
2-Undecene, (E)-	12.58	1	0.04	1.30	0.9	0.06	1.00	1	0.02	1.40	1	0.03	1.50	1	0.01	1.50
1-Decene, 2,4-dimethyl-	13.15	0.02	0.02	0	0.7	0.7	0.800	0	0	0	0	0	0	2	2	1.80
Nonane, 4,5-dimethyl-	15.13	2	0.3	2.20	0.4	0.2	0.400	2	0.3	2.40	3	0.1	3.00	3	0.07	3.20
1-Octanol, 2,7-dimethyl-	15.48	3	2	2.80	2	2	2.70	3	2	3.30	0	0	0	0	0	0
1-Octanol, 2-butyl-	18.46	10	2	10.6	4	2	4.80	10	2	13.1	20	0.7	17.7	20	2	16.6
1-Octanol, 3,7-dimethyl-	21.94	0	0	0	1	1	1.30	0	0	0	0.06	0.06	0.100	0	0	0
1-Dodecanol, 3,7,11-trimethyl-	22.05	1	1	1.40	2	1	2.00	0.06	0.06	0.100	0	0	0	0.1	0.08	0.100
Dodecane	24.24	0.9	0.08	1.00	0.4	0.2	0.400	0.8	0.06	0.900	1	0.05	1.30	1	0.06	1.40
Dodecane, 4,6-dimethyl-	28.28	1	0.2	1.20	0.2	0.2	0.200	1	0.05	1.50	2	0.2	1.80	2	0.1	2.30
Benzene, 1,3-bis[1,1-dimethylethyl]-	29.08	6	0.9	6.20	3	0.7	3.00	8	0.2	8.40	8	0.8	8.80	10	0.5	11.8
1-Decanol, 2-methyl-	35.28	0.8	0.4	0.900	0	0	0	3	0.1	2.90	2	0.4	2.60	4	0.4	4.60

¹ Tentative identifications based on NIST17 library spectra and uafR structural references to public repositories; all compounds with >=1% abundance in a sample were listed here.
² Means represent normalized emissions of volatiles; zero emissions represent cases where volatiles were below the detection threshold.
³ Shaded from low values in dark blues to high values in warmer colors.

Close-range Food Preference by *Prostephanus truncatus*

In the close-range food preference mobility assays, *P. truncatus* visited the zone with maize significantly more than the no-food zone (blank control), but no significant differences were observed in the frequencies of visits to wheat or bur oak acorns (Table 3; Fig. 4A). Conspecifics spent significantly more time in the zone with either maize, wheat, or bur oak compared to the no-food zone (Table 3; Fig. 4B). Moreover, bur oak elicited a greater frequency of visits (Table 3; Fig. 4A) and longer visit duration (Table 3; Fig. 4B) spent adjacent to the acorns than to maize kernels.

Table 3
Statistical results of *Prostephanus truncatus* mobility in food preference

Treatment*	df [†]	Frequency of visits		Duration	
		Wald χ^2	P	Wald χ^2	P
Maize vs Blank	1	7.58	< 0.01	13.43	< 0.001
Wheat vs Blank	1	1.35	0.245	51.16	< 0.001
Bur oak vs Blank	1	3.45	0.063	25.93	< 0.001
Maize vs Wheat	1	6.49	< 0.01	3.11	0.078
Maize vs Bur oak	1	13.84	< 0.001	20.98	< 0.001
Wheat vs Bur oak	1	0.45	0.501	0.73	0.394
*Grains and acorns were used in food preference mobility assays.					
[†] A negative binomial generalized linear model was used in data analysis.					

Boring Activity of *Prostephanus truncatus* in Acorns

After 2 or 4 weeks of foraging by *P. truncatus* adults in the vials with host material, no individuals were found inside the undamaged acorns from any of the oak species (Fig. 5A and B). By contrast, after foraging for 2 weeks, 72%, 76%, and 90% of the released adults were found present inside the damaged acorns of black oak, red oak, and bur oak, respectively. Despite detecting significant differences among acorns species ($\chi^2 = 10.90$, $df = 2$, $P < 0.01$), pairwise comparisons showed that infestation rates did not differ between them (Fig. 5A). After foraging for 4 weeks, 47%, 50%, and 60% of the adults were found inside the damaged acorns of black oak, red oak, and bur oak, respectively. Again, there were no differences among acorn species ($\chi^2 = 3.72$, $df = 2$, $P = 0.156$, Fig. 5B).

Survival of *Prostephanus truncatus* on Acorns

After foraging for 2 weeks, 91%, 79%, 90%, and 92% of the adults survived on the damaged acorns of black oak, red oak, bur oak, and maize, respectively, with an overall difference among treatments ($\chi^2 = 10.42$, $df = 3$, $P < 0.05$). However, there were no differences in adult survival between acorns species (Fig. 6A). After foraging for 4 weeks, 15%, 27%, 62%, and 85% of the adults survived on the damaged acorns of black oak, red oak, bur oak, and maize, respectively. Survival was higher on bur oak than black oak and red oak but was significantly lower than survival on maize ($\chi^2 = 124.09$, $df = 3$, $P < 0.001$, Fig. 6B). By contrast, after 2 or 4 weeks, there were few or no survivors found on the undamaged acorns of any of the oak species. There was also no significant difference in survival among undamaged acorn species and the blank control ($\chi^2 = 7.55$, $df = 3$, $P = 0.056$, Fig. 6A; no statistics were computed due to a constant variable, Fig. 6B).

Fines Production of *Prostephanus truncatus* on Acorns

Overall, there were significant differences in fines production at 2 ($F_{5,12} = 21.24$, $P < 0.001$) and 4 weeks ($F_{5,12} = 26.13$, $P < 0.001$) among treatments. At 2 weeks, *P. truncatus* feeding on the damaged bur oak produced the highest fines, which were 2.3- and 1.9-fold greater than black oak and red oak, respectively (Fig. 7A). At 4 weeks, *P. truncatus* feeding on the damaged bur oak produced 2.1-fold more fines than on black oak, but not significantly more than on red oak (Fig. 7B). The weight of fines production was higher for damaged acorns than undamaged acorns across oak species (Fig. 7A and B).

Reproductive Fitness of *Prostephanus truncatus* on Acorns

After 10 weeks, *P. truncatus* F_1 progeny were found on damaged acorns of bur oak (Fig. 8A), but not on undamaged acorns of bur oak (Fig. 8B) or on the acorns of any other oaks regardless of condition (damaged or undamaged). Overall, acorn species and their condition had a significant effect on *P. truncatus* reproductive fitness ($F_{5,12} = 6.49$, $P < 0.01$). Conspecifics produced an average of $19.0 (\pm 2.1)$ F_1 offsprings on damaged acorns of bur oak, which was greater than that on undamaged acorns of bur oak and on damaged acorns of black oak and red oak (Fig. 9).

Discussion

Laboratory studies have shown that *P. truncatus* can reproduce on a wide range of woody plant species from its invaded range in Africa (Helbig et al. 1990; Nang'ayo et al. 1993, 1996, 2002; Ramirez-Martinez et al. 1994; Hill et al. 2002; Nansen et al. 2002, 2004; Sadiku et al. 2019; Mlambo et al. 2024), and a closely related species, *R. dominica*, has been reported to survive and reproduce on a variety of oak acorns in North America (Wright et al. 1990; Edde and Phillips 2006; Jia et al. 2008). Thus, natural habitats in North America landscapes, especially those including oak acorns, may function as reservoirs for the invasive *P. truncatus* and influence its movement into and out of grain storage, potentially facilitating its dispersal across the landscape. To assess the risk posed by North America natural habitats in spreading *P. truncatus*, this study determined the attractiveness of black oak, red oak, and bur oak acorns to conspecifics, compared the profiles of VOC emissions from these acorns, measured food preference with mobility assays, and investigated if *P. truncatus* can feed, survive, and reproduce on them.

P. truncatus adults responded positively to volatiles from all three species of oak acorns and maize compared to the blank control in a short-distance choice bioassay, which suggests that they can use odor-mediated orientation at least over short distances rather than just encountering the food substrate by chance. We focused on short-range attractions in this study because *P. truncatus* was previously shown to arrest and exhibit attraction to two of its preferred stored product hosts (maize and cassava) at short ranges (Wright et al. 1993; Hodges 1994; Scholz et al. 1997a), but not at long ranges (Wright et al. 1993; Tigar et al. 1994). In addition, Scholz et al. (1997a) demonstrated that *P. truncatus* responded positively to native woody species from Africa at short ranges. Arnold et al. (2015) reported no evidence

of color-mediated host selection in *P. truncatus* adults, suggesting that host preferences might predominantly be mediated by VOCs. Additional studies are needed to evaluate the potential use of these cues in long range orientation, which might play a role in landscape utilization. Furthermore, *P. truncatus* was more attracted to bur oak acorns rather than those from black oak, red oak, or its primary host, maize. This preference for bur oak acorns indicates that *P. truncatus* can perceive the difference in acorns among oak species, suggesting that this pest may use VOCs as chemical cues to locate its preferred food sources during the host-selection process as we effectively excluded visual and other cues during these bioassays. In a subsequent close-range assay, we allowed *P. truncatus* to use a combination of olfactory, visual, tactile and gustatory cues to differentiate between potential food sources. We found that *P. truncatus* visited bur oak acorns significantly more frequently and for longer durations than maize, indicating a stronger preference for bur oak acorns. Interestingly, this preference suggests that *P. truncatus* may still be in the early processes of shifting its dietary niche to include stored commodities and retains an ability to use resources from woody plants, which are thought to be its ancestral food sources (Quellhorst et al. 2021). It is likely that oaks constitute at least a part of the endemic vegetation in the native range of *P. truncatus* in Central America (Nixon 2006). Further research using oak species in *P. truncatus* native range is needed to evaluate the role of acorns and if VOCs from oak acorns within the native range are similar to those found in bur oak acorns.

Because odors likely play a prominent role in host selection by *P. truncatus*, we analyzed and compared the volatile profiles of acorns and grains to look for qualitative and/or quantitative differences in profiles on which conspecifics could act. VOC profiles from black oak, red oak and bur oak acorns, as well as maize and wheat, share some compounds, but also exhibit distinct differences in their overall composition (Table 2). Interestingly, black oak, red oak, and bur oak acorns shared 2,4-dimethyl-1-heptene as the most abundant compound, whereas maize and wheat shared 2-butyl-1-octanol as the most abundant compound. 4-methyl-decane and 2,6-dimethyl-nonane were also abundant in each treatment of acorns and grains. The initial attraction of *P. truncatus* to black oak, red oak and bur oak, maize and wheat may be due to these shared volatile cues emitted by acorns and grains. Compounds uniquely emitted by or emitted in higher ratios by bur oak acorns in comparison to other treatments (Table 2) may be important for the odor preference by *P. truncatus*. For example, both 3-hepten-2-one and 2-(5-methyl-furan-2-yl)-propionaldehyde were only present in bur oak acorns and not in any other treatment. There were also higher emissions of heptanone and 2,7-dimethyl-octanol from bur oak acorns compared to other treatments. It has been reported that the compound 2,4-dimethyl-1-heptene is emitted by *Gentiana asclepiadea* leaves (Georgieva et al. 2005), *Citrus grandis* fruits (Chung et al. 2012), *Anoplophora glabripennis* infested *Acer* trees (Makarow et al. 2020), *Aspergillus flavus* or *Fusarium verticillioides* inoculated/uninoculated wheats (Van Winkle et al. 2022; Ponce et al. 2022, 2023), *Amorphophallus titanum* flowers (Kang et al. 2023), spruce trees (*Picea abies*) under drought stress (Huber et al. 2024), and *Cnaphalocrocis medinalis* egg-treated rice plants (Tian et al. 2025). Although host plant-derived and stress-related compounds can enhance the efficiency of pheromone traps in some insect species (Birkinshaw 1998; Likhayo and Hodges 2000; Bashir et al. 2001; Morrison et al. 2018), maize volatiles did not improve *P. truncatus* trap captures or upwind flight responses (Hodges et

al. 1998; Fadamiro et al. 1998). Our study demonstrated significant short-range attraction to bur oak acorns, exceeding that of maize. Further research is needed to determine whether the compounds associated with bur oak acorns can elicit attraction over longer ranges and synergize pheromone traps.

In the final experiment, we investigated the ability of *P. truncatus* adults to bore into, feed on, survive, and reproduce in acorns of different oak species. The results showed that *P. truncatus* adults were unable to colonize undamaged acorns, indicating a preference for damaged acorns and suggesting that damage is necessary for colonization in the field. The acorn weevil *Curculio glandium* Marsham (Coleoptera: Curculionidae) (Reut et al. 2022), the acorn moth *Valentinia glandulella* (Riley) (Lepidoptera: Blastobasidae) (Galford and Weiss-Cottrill 1991), and the lesser grain borer *R. dominica* (Jia et al. 2008) also prefer damaged acorns. Surprisingly, other acorn weevils such as *Curculio venosus*, *Curculio pellitus*, and *Curculio elephas* do not exhibit this preference and they can infest undamaged acorns (Reut et al. 2021, 2022). Natural or mechanical damage to acorns that have fallen to the ground is also common, providing another route of entry. Thus, *P. truncatus* may infest naturally cracked or mammal-damaged acorns or follow infestation by some acorn weevils. Together, this acorn insect community may make oaks a riskier tree in the landscape that can facilitate invasion by *P. truncatus* in North America.

After *P. truncatus* adults bored into damaged black oak, red oak, and bur oak acorns, they produced dust or fines as a byproduct of their tunneling and feeding activities in all three species. However, the quantity of fines produced from bur oak was far greater than those produced from black oak and red oak. It is possible that the physical and chemical properties of bur oak acorns facilitated more tunnelling and feeding by *P. truncatus* compared to other oak species. According to previous studies (Von Berg and Biliwa 1990; Popoola et al. 2015), the quantity of dust produced by *P. truncatus* during tunneling and feeding activities is a reliable indicator of both the degree of damage and the suitability of the host. For example, *P. truncatus* tended to produce large amounts of dust while boring, tunneling, and feeding on maize (Quellhorst et al. 2021). Ramírez-Martínez and Silver (1983) reported that maize phenotypes with soft pericarp and endosperm are most vulnerable to attack by *P. truncatus* because conspecifics can tunnel and feed easily. Due to more extensive tunnelling and feeding activities, *P. truncatus* produced more dust in maize than in sorghum and larger quantities of dust were generated in the high-yielding sorghum varieties (Framida and Naga-White) compared to the local low yielding variety (Mankaraga) (Mailafiya 2003).

As an internal feeder, *P. truncatus* completes its entire life cycle within its host, which provides sites for both feeding and reproduction (Sadiku et al. 2019). The undamaged acorns of all tested oak species were unsuitable for *P. truncatus* adults to feed and reproduce, presumably because it was difficult for them to gain entry. Despite being able to feed on the damaged acorns of all species, *P. truncatus* adults could only successfully reproduce on bur oak, suggesting that it is the only acorn species tested in this study capable of serving as a suitable host in the field. It is possible that the black and red oak acorns lack sufficient nutrients needed to support *P. truncatus* larval development. Alternatively, it is possible that the black and red oak acorns contain antifeedant or antinutritive compounds that make it difficult

for developing beetles to sufficiently feed to complete their development. It has been reported that tannins can act as antinutritional factors and feeding deterrents for many insects by binding to proteins, reducing the efficacy of digestion and nutrient absorption, and causing midgut lesions (El-Aswad et al. 2023). Acorns from the red oak group (e.g., red or black oak) contain two to four times more tannins than those from the white oak group (e.g., bur oak) (Wood 2005). Consequently, the higher tannin levels in black and red oak acorns are likely to inhibit *P. truncatus* feeding, development, and reproduction due to their toxic and anti-nutritional effects.

The range of bur oaks begins in southern Texas, where *P. truncatus* has been intercepted in the past (Arthur et al. 2019), and the range continues northward into Southern Canada (Gucker 2011). As a result, this species may act in providing an invasion corridor for *P. truncatus* into the Great Plains where the majority of maize production occurs in the United States of America. Jia et al. (2008) found that *R. dominica*, a closely related bostrichid, can feed and reproduce on damaged acorns of six oak species, including chinkapin oak *Quercus muehlenbergii*, pin oak *Quercus palustris*, red oak *Quercus rubra*, Shumard oak *Quercus shumardii*, bur oak *Quercus macrocarpa*, and English oak *Quercus robur*, but not on their undamaged acorns. Earlier studies have also shown that *R. dominica* can reproduce on acorns of chinkapin oak *Q. muehlenbergii* (Wright et al. 1990) and post oak *Quercus stellata* (Edde and Phillips 2006). Moreover, acorn shells are naturally broken with seasonal freezing and thawing cycles or commonly damaged by other acorn predators, so damaged acorns are abundant due to both natural processes, small mammal predation, and primary insect infestation (Jia et al. 2008). Thus, the lacking capacity of *P. truncatus* to penetrate undamaged acorns may not be a real limiting factor, and bur oak acorns in natural habitats could potentially serve as temporary reservoirs to sustain *P. truncatus* if there was no other food available, thereby facilitating long-range dispersal.

Studies have been conducted to understand the potential hosts of *P. truncatus* in its endemic and invaded range. For example, of the 81 local tree species tested in Kenya, 23 were found favorable to *P. truncatus* for reproduction (Nang'ayo et al. 1993). *P. truncatus* adults and larvae were found in branches of *Spondias purpurea* L. (Anacardiaceae), *Lannea nigritana* (Scott Elliot) Keay (Anacardiaceae), *Bursera fagaroides* (Kunth) Engl. (Burseraceae), *Commiphora riparia* Engl. (Burseraceae), and *Commiphora africana* Engl. (Burseraceae) that have been girdled by other insects, especially cerambycids, suggesting that natural populations of *P. truncatus* may target already damaged woody plants as part of its realized niche to feed and reproduce (Nang'ayo et al. 1993; Ramírez-Martínez et al. 1994; Borgemeister et al. 1998b). Nansen et al. (2004) examined the performance of *P. truncatus* on a variety of tree species native to Africa and found that it can reproduce on the branches from four species of trees or shrubs (*Delonix regia*, *Ceiba pentandra*, *Manihot esculenta*, and *Ficus exasperata*) and on the roots from two species (*Ficus capensis*, and *F. exasperata*). They also demonstrated that *P. truncatus* can reproduce on the seeds of teak *Tectona grandis* (L.), highlighting an interaction between this pest and tree seeds. However, there has been no assessment of the seeds or flora of North America for use as a potential host by *P. truncatus*, which is of interest given its invasion potential. While we have confirmed the acorns of one oak species that can be used by *P. truncatus*, more studies are needed to fully understand the

extent to which tree seeds in natural environments serve as a crucial food source and breeding substrate for this invasive species.

In conclusion, this study demonstrates that *P. truncatus* prefers bur oak acorns and volatiles emitted by bur oak acorns are more attractive to this beetle than maize, its main common host commodity. *P. truncatus* can feed and reproduce on damaged bur oak acorns. Bur oak acorns provide a potential food source and natural habitat where *P. truncatus* may be attracted to survive and spread across the landscape, alluding to a heightened risk for this invasive pest in North America.

Declarations

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Author Contributions QLM, WRM, JFC, KYZ and EDS conceived the ideas and designed the experiments; QLM, BM and MKM performed the experiments and collected the data. MH, WRM, MKM, BM and QLM performed GC-MS analyses. QLM, WRM, MH, MKM and BM analyzed the data. QLM, WRM, JFC, KYZ and EDS led the writing of the manuscript. MH, MKM, BM, MT and DHW edited the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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Data Availability Datasets can be accessed through the Ag Data Commons (DOI will be inserted upon approval).

Competing Interests The authors declare no competing interests.

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Figures

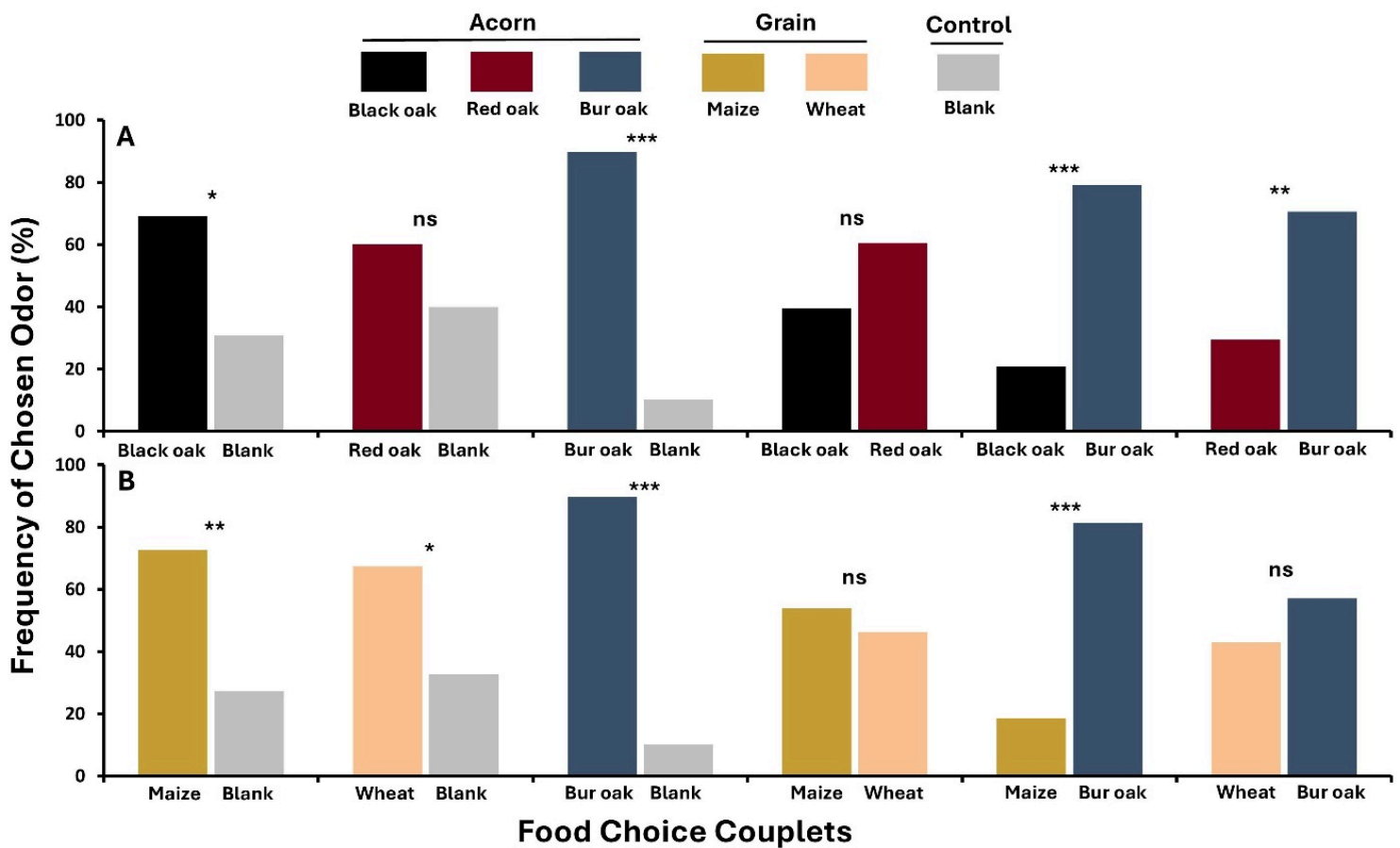


Figure 1

Response of adult *Prostephanus truncatus* in two-choice pitfall bioassay to odors from A) different oak acorns and B) grains and bur oak acorns. Each treatment included n = 74 replicates, with one adult individual per replicate. For each paired treatment, symbols *, **, and *** represent a significant difference between treatments at the $P < 0.05$, 0.01 , and 0.001 levels in a chi-square goodness of fit test, respectively, and ns denotes a non-significant difference.

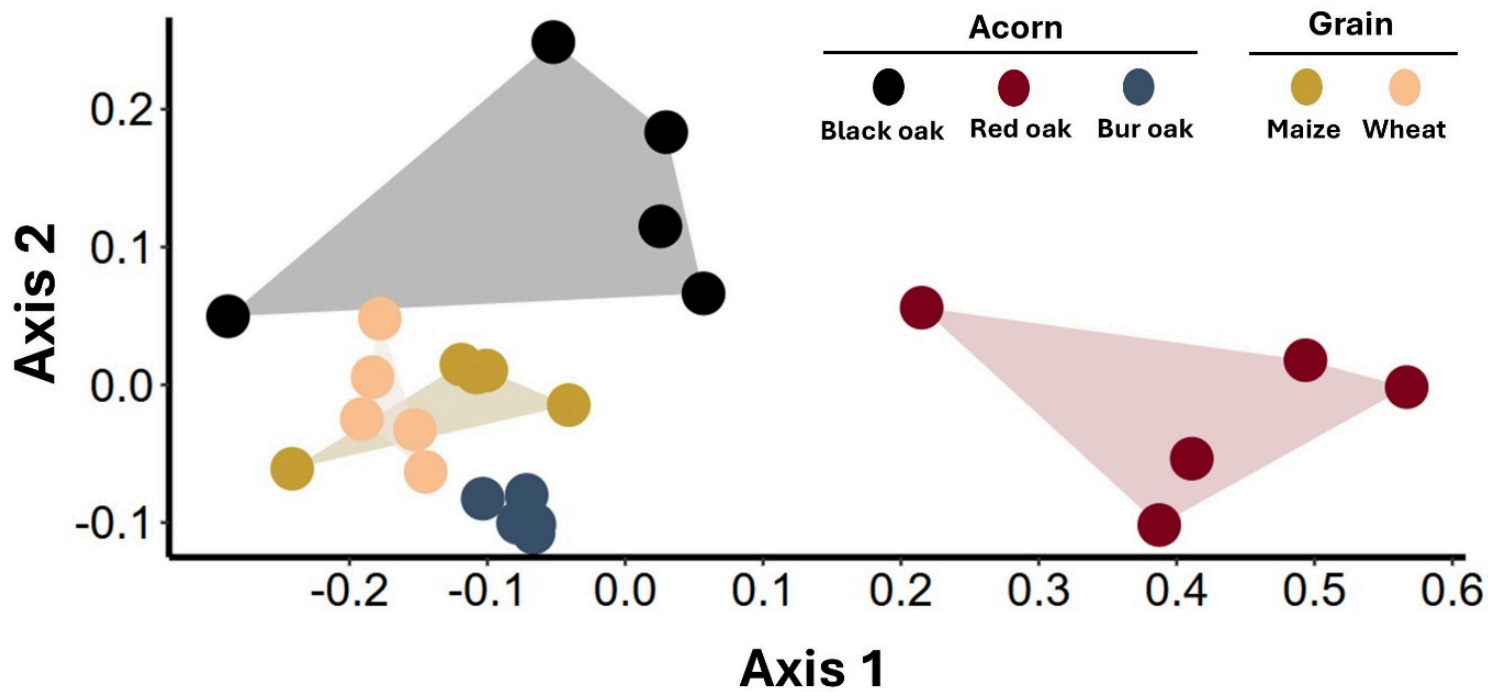


Figure 2

Non-metric multidimensional scaling (NMDS) ordination plot of volatile compound profiles from acorns and grains. Each treatment included $n = 5$ replicates. NMDS analysis was conducted based on the pairwise Bray–Curtis similarity between samples. Polygons represent convex hulls drawn between samples and are color coded similarly to treatments. There were a total of $n = 1000$ permutations, and stress was < 0.10 , enabling adequate interpretation.

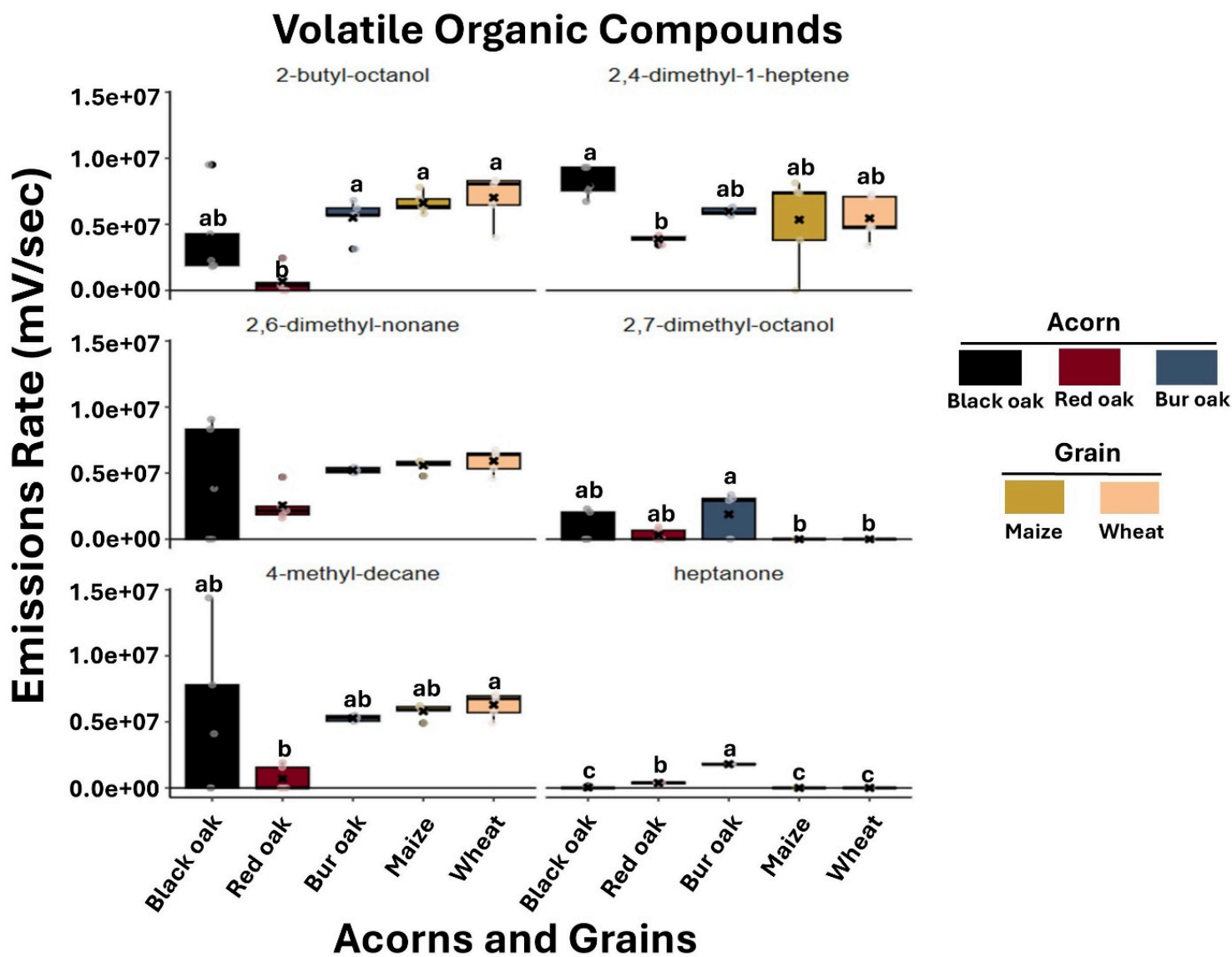


Figure 3

Normalized emissions rate of volatile compounds from oak acorns and grains. Within a graph, different letters represent a significant difference between treatments (Tukey HSD, $\alpha = 0.05$). In the box plots, the whiskers represent the range of data points from the 25th quartile to the minimum value and from the 75th quartile to the maximum value, and the horizontal line and symbol $\times$ within the box indicates the median and mean, respectively. There were $n = 5$ replicates per treatment.

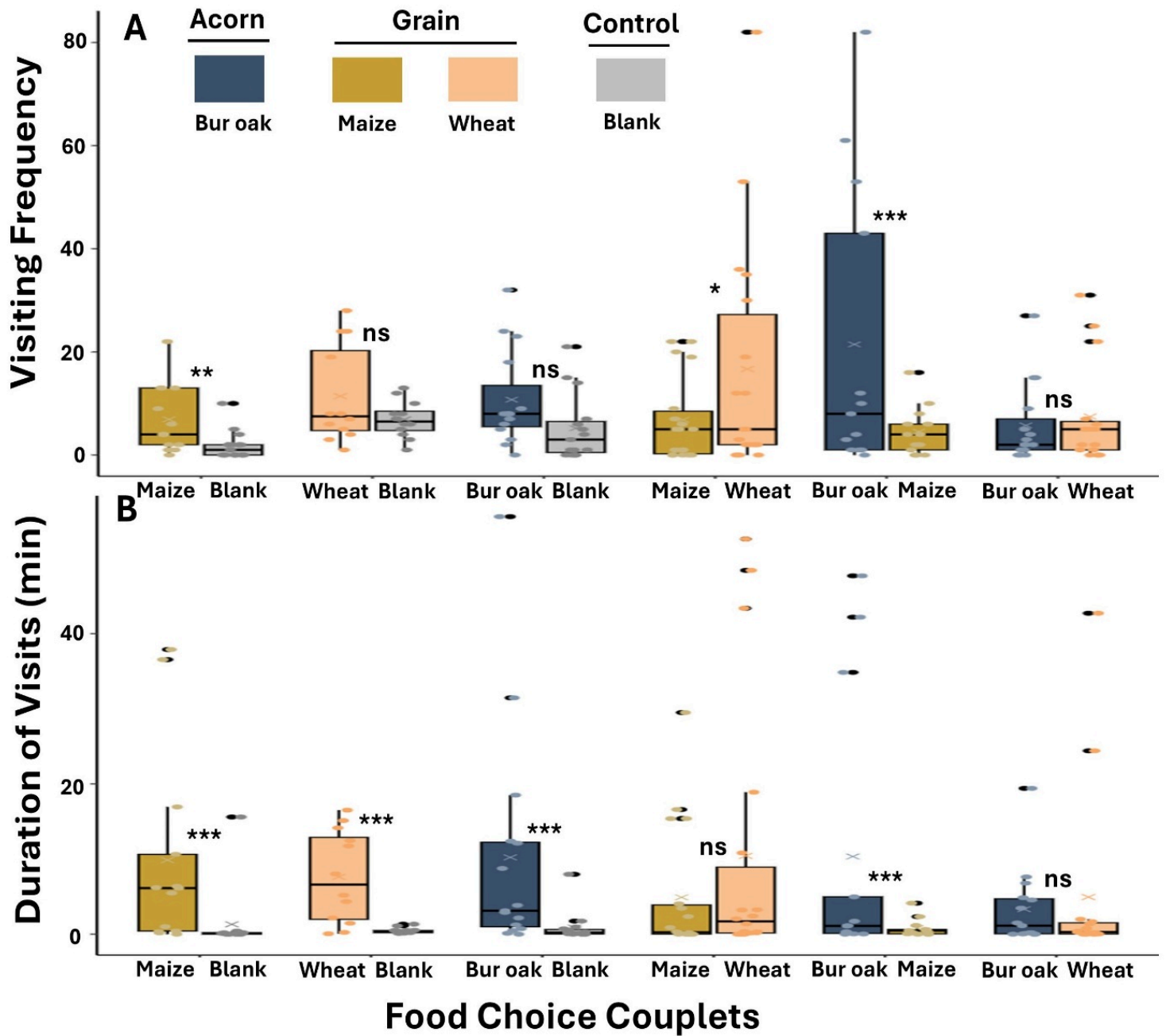


Figure 4

Visitation A) frequency and B) duration of adult *Prostephanus truncatus* in food zones containing grains and bur oak acorns. Each treatment included $n = 18$ individual adult replicates. For each paired treatment, symbols *, **, and *** represent a significant difference between treatments at the $P < 0.05$, 0.01 , and 0.001 levels in a negative binomial GLM test, respectively, and ns denotes a non-significant difference. In the box plots, the whiskers represent the range of data points from the 25th quartile to the minimum value and from the 75th quartile to the maximum value, outliers noted beyond the whiskers, and the horizontal line and symbol $\times$ within the box indicates the median and mean, respectively.

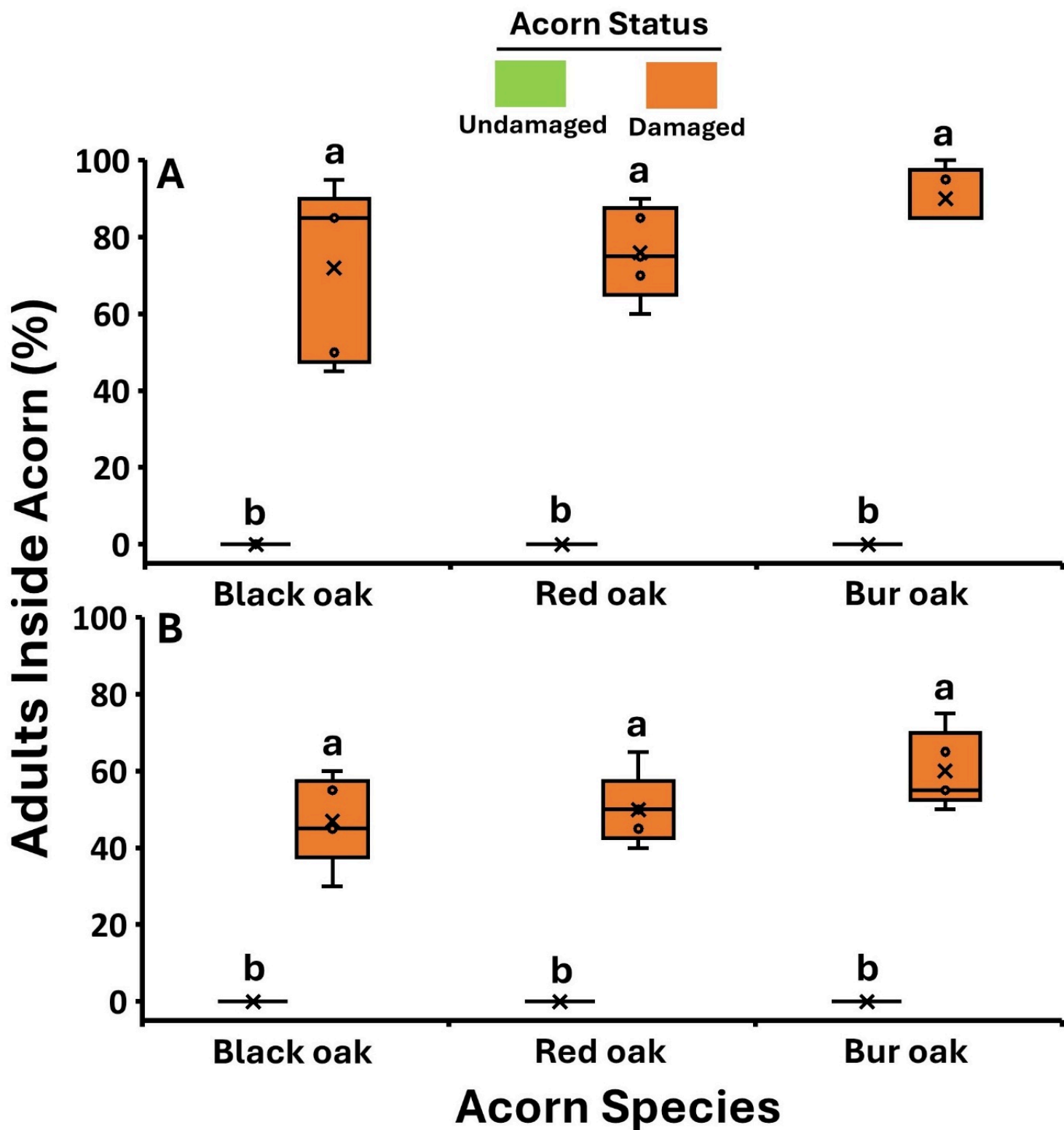


Figure 5

Percentage of adult *Prostephanus truncatus* present inside undamaged and damaged acorns of different oak species at A) 2 and B) 4 weeks. Undamaged acorns consisted of a whole intact acorn, whereas damaged acorns had three 2 mm holes drilled in the adaxial surface of the shell of each acorn. Each treatment included n = 5 replicates, with 20 adults per replicate. Within a graph, different letters represent a significant difference between treatments (chi-square test of independence with Bonferroni

correction, $\alpha = 0.05$). In the box plots, the whiskers represent the range of data points from the 25th quartile to the minimum value and from the 75th quartile to the maximum value, and the horizontal line and symbol $\times$ within the box indicates the median and mean, respectively.

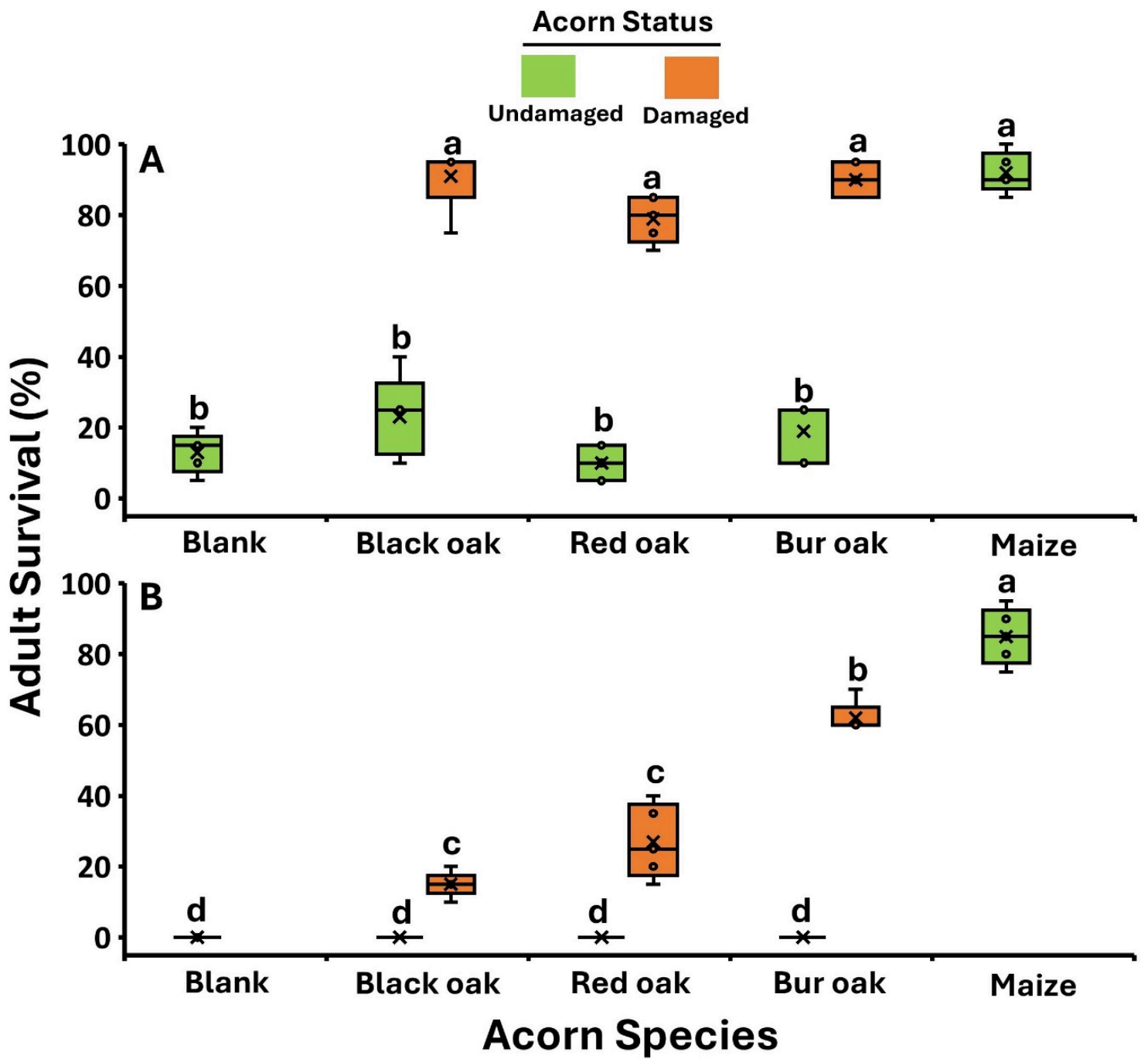


Figure 6

Survival of adult *Prostephanus truncatus* on undamaged and damaged acorns of different oak species at A) 2 and B) 4 weeks. Undamaged acorns consisted of a whole intact acorn, whereas damaged acorns had three 2 mm holes drilled in the adaxial surface of the shell of each acorn. Each treatment included $n = 5$ replicates, with 20 adults per replicate. Within a graph, different letters represent a significant difference between treatments (chi-square test of independence with Bonferroni correction, $\alpha = 0.05$). In the box plots, the whiskers represent the range of data points from the 25th quartile to the minimum

value and from the 75th quartile to the maximum value, and the horizontal line and symbol × within the box indicates the median and mean, respectively.

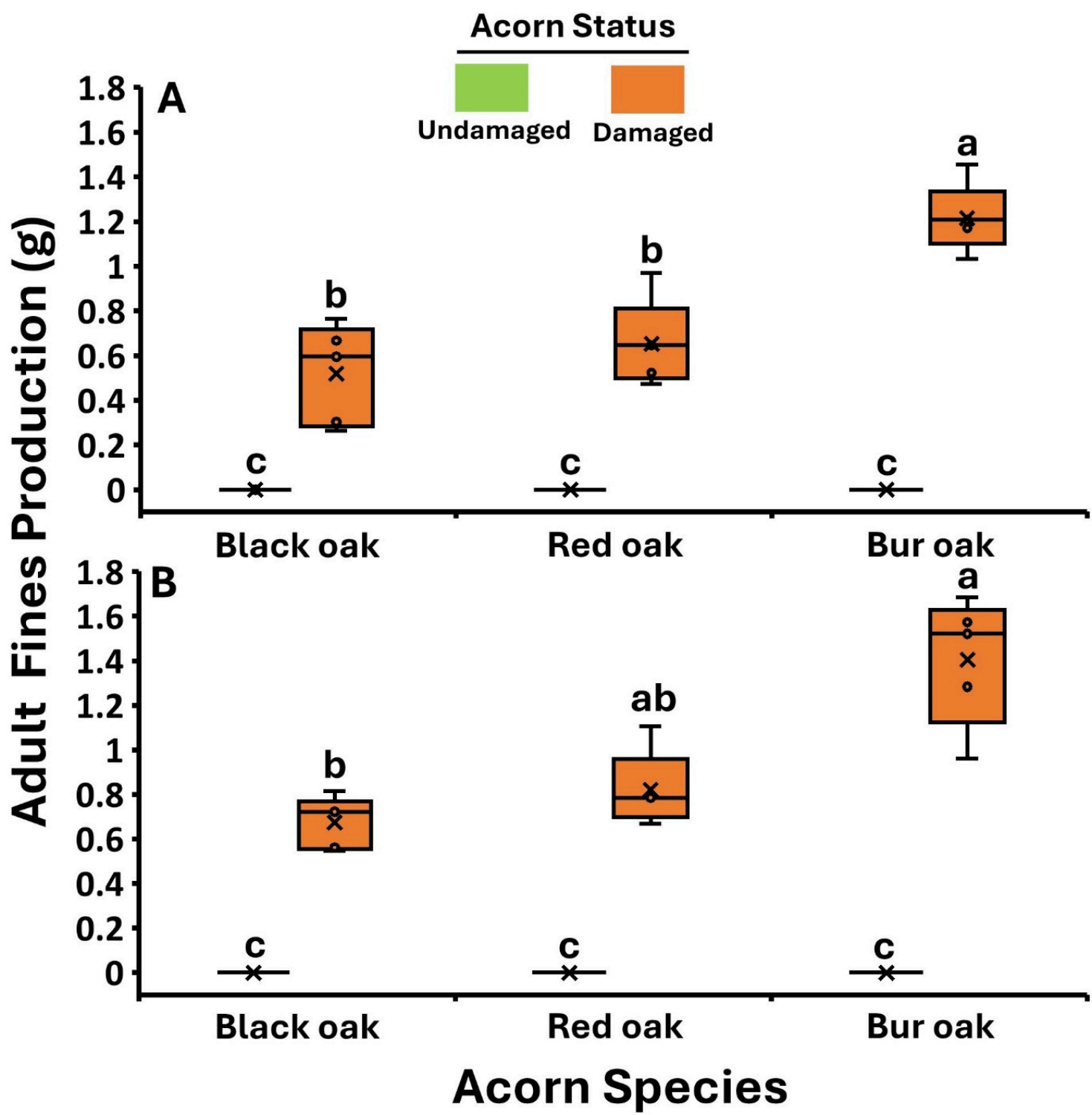


Figure 7

Fines produced by adult *Prostephanus truncatus* on undamaged and damaged acorns of different oak species at A) 2 and B) 4 weeks. Undamaged acorns consisted of a whole intact acorn, whereas damaged acorns had three 2 mm holes drilled in the adaxial surface of the shell of each acorn. Each treatment included n = 5 replicates, with 20 adults per replicate. Within a graph, different letters

represent a significant difference between treatments (linear mixed model test with Bonferroni correction, $\alpha = 0.05$). In the box plots, the whiskers represent the range of data points from the 25th quartile to the minimum value and from the 75th quartile to the maximum value, and the horizontal line and symbol $\times$ within the box indicates the median and mean, respectively.

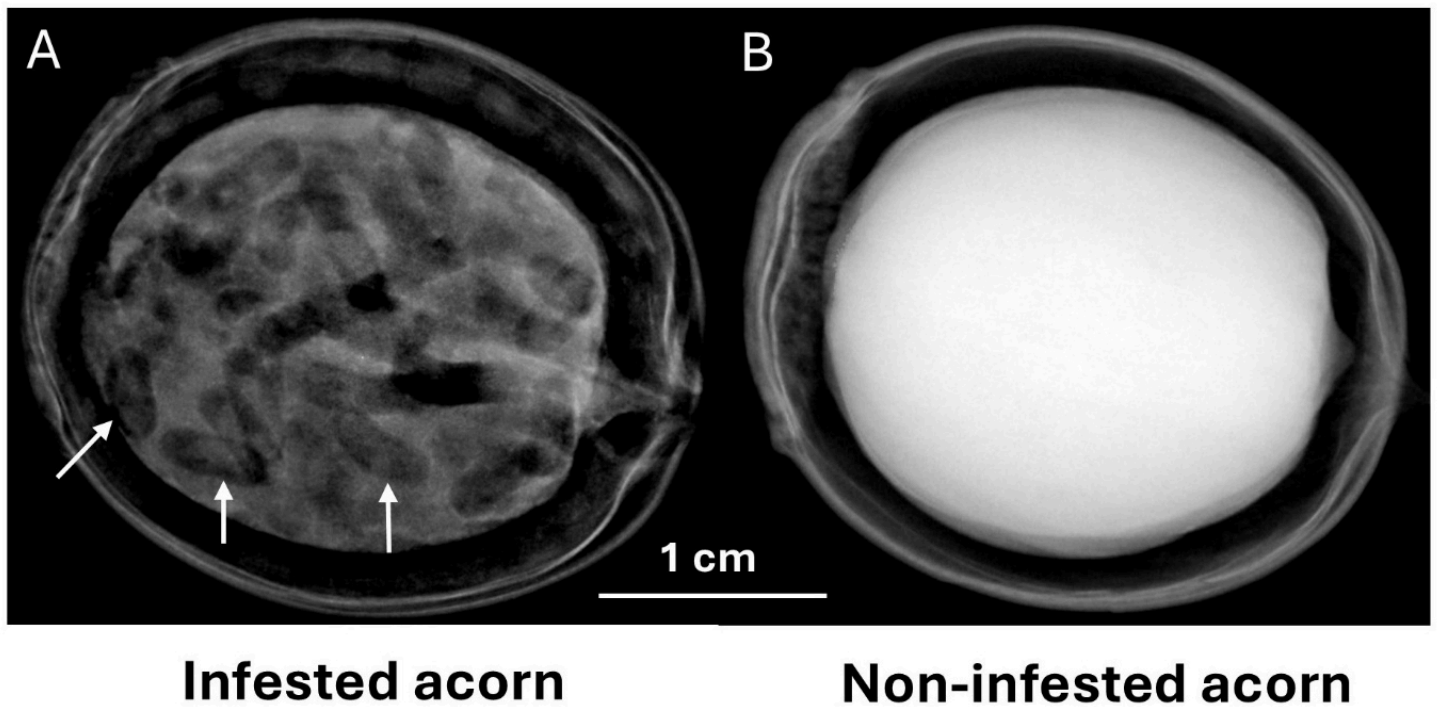


Figure 8

X-ray images of A) infested acorns and B) non-infested of bur oak caused by adult *Prostephanus truncatus*. Arrows indicate the presence of *Prostephanus* F₁ progeny in tunnels.

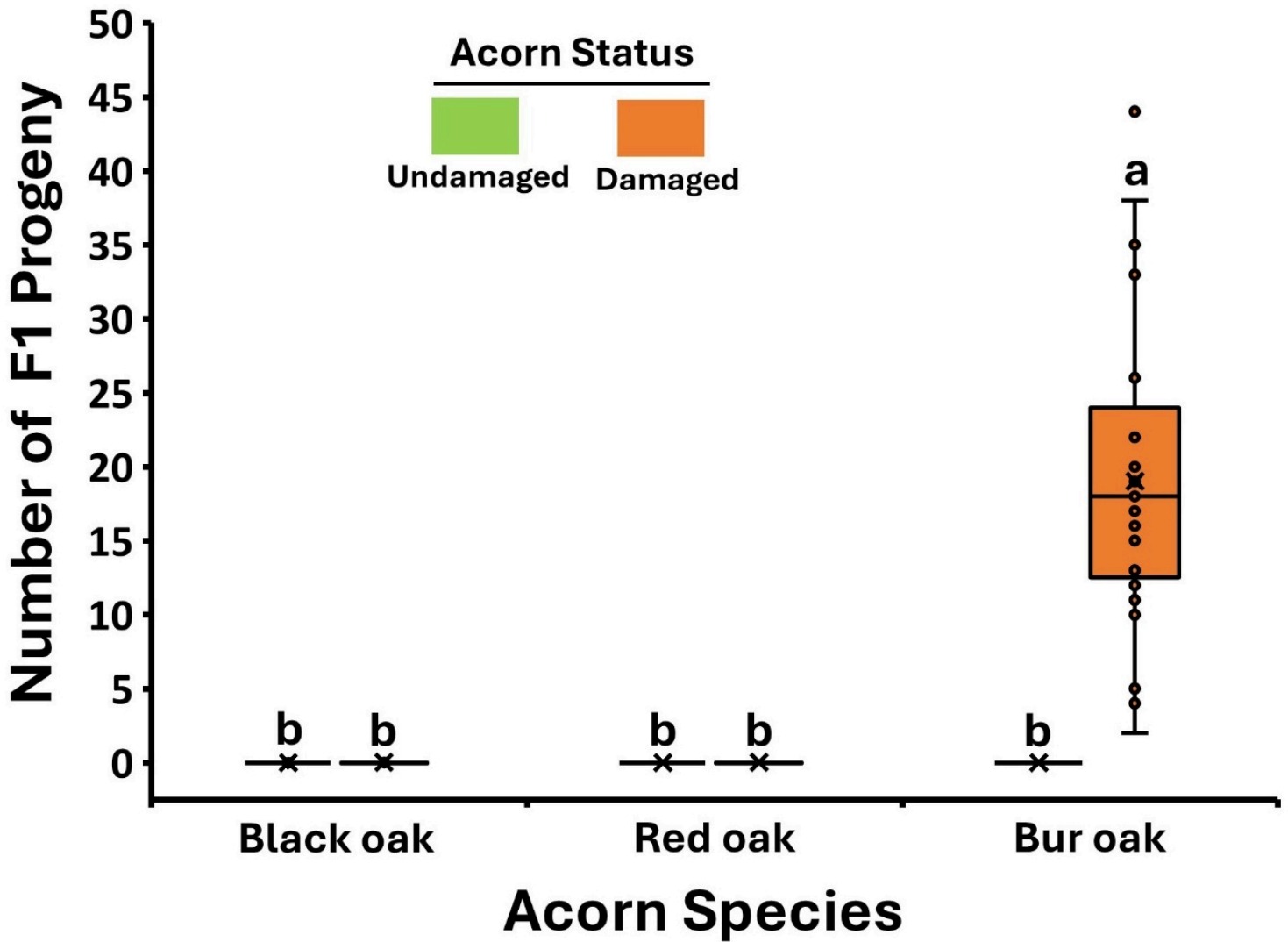


Figure 9

Total number of F_1 progeny produced by adult *Prostephanus truncatus* on undamaged and damaged acorns at 10 weeks. Undamaged acorns consisted of a whole intact acorn, whereas damaged acorns had three 2 mm holes drilled in the adaxial surface of the shell of each acorn. Each treatment included $n = 25$ replicates, with 20 parent adults per replicate. Within a graph, different letters represent a significant difference between treatments (linear mixed model test with Bonferroni correction, $\alpha = 0.05$). In the box plots, whiskers represent the range of data points from the 25th quartile to the minimum value and from the 75th quartile to the maximum value, with one outlier beyond the whiskers, and the median and mean are represented by horizontal line and $\times$ within the box, respectively.