

Freeze stress of deciduous trees induces attacks by opportunistic ambrosia beetles

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- Abstract**
- 1 A broad host range and the utilization of living but weakened trees contribute, in part, to the invasion success of ambrosia beetles (Curculionidae: Scolytinae). The present study assessed the capability of freeze stress to induce attacks by ambrosia beetles.
 - 2 Freeze stress predisposed *Cercis canadensis* L., *Cornus florida* L., *Malus pumila* Mill. and *Styrax japonicus* Sieb. to attack under field conditions, although no attacks occurred on untreated trees. More attacks occurred on freeze-stressed versus flood-stressed *M. pumila* in Virginia but not for *S. japonicus* in Ohio. Attacks on flooded trees were skewed towards the base of the trunk, whereas attacks on freeze-stressed trees mainly occurred around the upper regions of the trunk and into the branches. The predominant species recovered were *Anisandrus maiche* Stark and *Xylosandrus germanus* (Blandford) in Ohio, and *Xylosandrus crassiusculus* (Motschulsky) in Virginia.
 - 3 Ethanol emissions from trunks of *S. japonicus* were detected by solid phase microextraction-gas chromatography–mass spectrometry at 1 day after imposing freeze stress, peaking 4 days after injury.
 - 4 Trees with an intolerance of freeze stress are predicted to be vulnerable to attack, especially when subjected to mild winter temperatures followed by late-spring freezes. Freeze stress could thereby facilitate the destructiveness of exotic ambrosia beetles.

Keywords *Anisandrus maiche*, ethanol, freeze stress, *Xylosandrus germanus*, *Xylosandrus crassiusculus*.

Introduction

Ambrosia beetles (Curculionidae: Scolytinae) in the tribe Xyleborini are among the most successful and destructive wood-boring insects worldwide (Hulcr & Stelinski, 2017). Thirty non-native species of Xyleborini are currently established in North America (Gomez *et al.*, 2018) and the Xyleborini also represent half of the exotic Scolytinae introduced into Europe (Kirkendall & Faccoli, 2010). After dispersing from their natal galleries, adult females tunnel into host trees tissues, where they establish and cultivate gardens of their nutritional fungal symbiont (Hulcr & Stelinski, 2017). Adult females carry spores of their fungal symbiont within specialized structures called mycangia, which are then used to inoculate the tunnel walls

of host tissues. The fungal symbionts are usually *Ambrosiella* and *Raffaelea* spp., although examples also exist of mutualism with *Fusarium* spp. (Kasson *et al.*, 2013; Kostovcik *et al.*, 2015; O'Donnell *et al.*, 2015). Oviposition occurs after the fungal gardens are flourishing, with the larvae and adults consuming the fungal symbiont to properly develop and reproduce (French & Roeper, 1972; Biedermann & Taborsky, 2011). Xyleborini offspring are produced via haplodiploid reproduction and sibling mating subsequently occurs between the haploid males and diploid females. Their cryptic wood-boring behaviour, fungus farming lifestyle, haplodiploid reproduction and sibling mating likely contribute to the invasion success of ambrosia beetles (Smith & Hulcr, 2015; Gomez *et al.*, 2018). A broad host tree range and association with weakened trees also likely promotes the establishment and proliferation of ambrosia beetles introduced into new habitats, with this relationship probably being

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facilitated by extreme climatic events producing an availability of weakened host resources (Ranger *et al.*, 2013, 2015a, b).

Ambrosia beetles are often ranked among the most destructive insect pests of trees growing in ornamental nurseries because of their wood-boring behaviour and association with branch dieback and tree death (Oliver & Mannion, 2001; Fulcher *et al.*, 2012; Ranger *et al.*, 2016). They are also known to attack large stands of trees in forested systems (Grégoire *et al.*, 2001; Henin & Versteirt, 2004; La Spina *et al.*, 2013). In particular, *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) comprise two of the most destructive ambrosia beetles of trees growing in ornamental nurseries and tree fruit orchards (Agnello *et al.*, 2015; Ranger *et al.*, 2016). Both *X. crassiusculus* and *X. germanus* are native to southeast Asia and have become established around the world, including parts of North America, South America, Africa, Europe and Oceania (Rassati *et al.*, 2016; Gallego *et al.*, 2017; Landi, 2017; Gomez *et al.*, 2018; CABI, 2018a, b; Kavčič, 2018). Adult females of *X. crassiusculus* and *X. germanus* tunnel into the heartwood of host trees, where they cultivate fungal gardens of *Ambrosiella roeperi* T.C. Harr. & McNew and *Ambrosiella grosmanii* C. Mayers, McNew & T.C. Harr., respectively (Harrington *et al.*, 2014; Mayers *et al.*, 2015). Although *X. crassiusculus* and *X. germanus* attack a broad range of 100–200 trees (Schedl, 1963; Weber & McPherson, 1983), both species exhibit a narrow preference for living but weakened trees emitting stress-induced ethanol (Ranger *et al.*, 2013, 2015b).

Ethanol acts as a chemical indicator of weakened trees (Kelsey, 2001) and also benefits the colonization success of ambrosia beetles by promoting the growth of their fungal gardens (Ranger *et al.*, 2018). A variety of abiotic and biotic stressors can induce the production and emission of ethanol by trees, including flood and drought stress, frost injury, girdling, impaired root function, root and crown disturbance, pollutants, and pathogens (Kimmerer & Kozłowski, 1982; Kimmerer & MacDonald, 1987; Kelsey & Joseph, 2001; Kelsey *et al.*, 2013; Ranger *et al.*, 2015a). Previous studies have demonstrated that flood stress predisposes trees to attack, and that *Xylosandrus* spp. can distinguish among trees varying in the tolerance of flooding (Ott, 2007; Ranger *et al.*, 2013, 2015a; Reed *et al.*, 2015). Based on this knowledge, Frank and Ranger (2016) developed a moisture management threshold to help growers avoid overwatering their trees and thereby minimize the risk of ambrosia beetle attacks. Characterizing the role of tree health on host selection and utilization by Xyleborini ambrosia beetles is therefore useful for developing management tactics.

In addition to the role of flood-stress, a growing number of studies suggest that freeze-stressed trees are also preferentially attacked by ambrosia beetles. For example, oak (*Quercus rubra* L.) trees growing in Germany that showed signs of frost damage but otherwise appeared healthy were attacked by *X. germanus* (Heidenreich, 1960; Gauss, 1960). Young sycamore (*Acer pseudoplatanus* L.) trees severely injured or killed by frost were also attacked by *X. germanus* (Heidenreich, 1960; Gauss, 1960). More recently, La Spina *et al.* (2013) documented that extreme frost events preceded attacks by *X. germanus* and other ambrosia beetles on large stands of European beech trees, *Fagus sylvatica* L., in Western Europe. Bark tissues of *F. sylvatica*

experimentally-stressed using blocks of dry ice were also more attractive to *X. germanus* than untreated tissues (La Spina *et al.*, 2013). Freeze stress also preceded attacks on thin-barked deciduous trees growing in ornamental nurseries in northeastern Ohio, U.S.A. (Ranger *et al.*, 2015a).

Based on these previous studies, we hypothesized that deciduous trees subjected to freeze stress would be attacked by opportunistic ambrosia beetles targeting living but weakened trees, such as flood-stressed trees. To test this hypothesis, the present study aimed to: (i) assess the capability of freeze stress to induce attacks by ambrosia beetles; (ii) compare the preference behaviour of ambrosia beetles for freeze-stressed versus flood-stressed trees; and (iii) characterize the time course emission of ethanol from freeze-stressed trees.

Materials and methods

Imposing freeze stress

Experiments were conducted in Ohio, U.S.A., and Virginia, U.S.A., because *X. germanus* and *X. crassiusculus* are the predominant exotic ambrosia beetles attacking trees in ornamental nurseries in these two states, respectively. Trees used in the Ohio experiments were 3–4 years old, 2.54 cm in diameter, approximately 1.2 m in height and were grown in 18.9-L pots containing a mixture of 90 : 10 pine bark and sphagnum peat moss, along with lime and Micromax Micronutrients (Scotts Co., Marysville, Ohio). Trees used in the Virginia experiments were approximately 3–4 years old, 2.54 cm in diameter, approximately 1.2 m in height and were grown in 28-L pots containing a mixture of aged pine bark and coarse sand (92 : 8), as well as dolomitic lime to stabilize pH. The media was top dressed with Osmocote Plus 15–9–12 (Scotts Co., Marysville, Ohio) slow-release fertilizer.

For the Ohio experiments, freeze stress was imposed by holding four container-grown *Styrax japonicus* Sieb. trees overnight (approximately 12 h) in a freezer (internal dimensions, length × width × height: 76.2 × 69.9 × 160.0 cm; model NSPF331WWW/0; Norlake Scientific, Hudson, Wisconsin) programmed to –10 °C. For the Virginia experiments, freeze stress was imposed by holding container-grown *S. japonicus*, *Cornus florida* L. and *Cercis canadensis* L. trees overnight for three consecutive nights in a commercial cold storage facility maintained at –10 °C. Trees were relocated outside the cold room during the day and returned in the evening. *Malus pumila* trees deployed in Virginia were only held overnight in a freezer. After their removal from the freezer, freeze-stressed trees were used for assessing ambrosia beetle host selection (Ohio and Virginia) and the induction of ethanol emissions (Ohio). The deciduous trees *S. japonicus*, *C. florida* and *C. canadensis* were selected for our experiments because previous studies have indicated that freeze stress predisposes them to attack by ambrosia beetles (Ranger *et al.* 2015b). Similarly, *M. pumila* was selected because of an intolerance of freeze stress (Cannell & Smith, 1986; Khanizadehi *et al.*, 1989). We also incorporated these tree species into our experiments because *X. germanus* and *X. crassiusculus* are problematic in ornamental nurseries and tree fruit orchards.

Preference for freeze-stressed trees

Free-choice experiments were conducted under field conditions in Ohio and Virginia to assess the host selection behaviour of ambrosia beetles for freeze-stressed and untreated control trees.

Two separate experiments were conducted in Ohio. In both experiments, freeze-stressed *S. japonicus* trees ($n=4$) were removed from a laboratory freezer after overnight exposure and paired with an untreated control tree ($n=4$) in a deciduous woodlot in Wayne Co., Ohio ($40^{\circ}45'40.85''\text{N}$, $81^{\circ}51'14.71''\text{W}$). Freeze-stressed and control trees were spaced 1.5 m apart with 4 m between adjacent pairs of trees. Trees were held under field conditions for 8 and 10 days after imposing freeze stress in Experiments 1 and 2 conducted in Ohio, respectively. Attacks on trees were counted at 1, 4, 7, and 8 days after imposing freeze stress and field deployment during Experiment 1. Similarly, attacks were counted at 1, 4, 7, 8, and 10 days during Experiment 2. On the last day of field deployment, the trunks were cut at the soil line and transferred to a walk-in cooler stored at 5°C . Pruning shears were next used to dissect the tunnels/galleries and recover the ambrosia beetle foundresses. Scolytinae specimens were stored in 70% ethanol and identified to species. Experiment 1 was conducted in Ohio from 10 July 2014 to 18 July 2014 and Experiment 2 was conducted from 29 July 2014 to 11 August 2014.

Two separate field experiments were also conducted in Virginia. In Experiment 1, freeze-stressed *S. japonicus* ($n=6$) trees were freeze-stressed for three consecutive nights and then paired with a non-injured tree ($n=6$) along the edge of a deciduous woodlot in Virginia Beach, Virginia, U.S.A. ($36^{\circ}53'16''\text{N}$, $76^{\circ}10'22''\text{W}$). Freeze-stressed and untreated control trees were spaced 3 m apart with 6 m between adjacent pairs of trees. Trees were held under field conditions for 10 days after imposing freeze stress in Experiment 1. Attacks on trees were counted at 2, 4, 8, and 10 days after field deployment during Experiment 1. In Experiment 2, freeze-stressed *C. canadensis* ($n=6$), *C. florida* ($n=6$) and *S. japonicus* ($n=6$) trees were removed from a freezer and arranged in randomized complete blocks with untreated trees of each species ($n=6$ per species) along the edge of the aforementioned woodlot. A distance of 3 m was maintained between adjacent trees within a block and 6 m between adjacent blocks. Attacks on trees were counted at 4, 6, 12 and 14 days after field deployment during Experiment 2. On the last day of field deployment, the trunks were cut at the soil line, transferred to a walk-in cooler and stored at 5°C . Pruning shears were used to recover the ambrosia beetle foundresses, which were stored in 70% ethanol and identified to species. Experiment 1 was conducted in Virginia from 2 June 2014 to 12 June 2014 and Experiment 2 was conducted from 26 June 2014 to 10 July 2014.

Comparison between freeze-stressed and flooded trees

Because previous studies have demonstrated *Xylosandrus* spp. attack flood-stressed *S. japonicus* and other tree species (Ranger *et al.*, 2013, 2015b), we conducted additional experiments to compare their preference behaviour when given a choice between freeze-stressed versus flood-stressed *S. japonicus* and *M. pumila* Mill trees.

Container grown trees were freeze-stressed in accordance with the previously described methods for Ohio and Virginia, except that trees in Virginia were exposed to freeze stress for one night instead of three. Flood stress was imposed using a pot-in-pot system as described by Ranger *et al.* (2013). Freeze-stressed trees were deployed under field conditions the same day that they were removed from the freezer, whereas flood stress was imposed the same day trees were deployed under field conditions in Ohio and Virginia.

In Ohio, freeze-stressed ($n=12$), flood-stressed ($n=12$) and untreated control ($n=12$) *S. japonicus* trees were deployed in a randomized complete block design within the aforementioned deciduous woodlot in Ohio. A distance of 4 m was maintained between adjacent trees within a block and 8 m between adjacent blocks. Because only four container-grown *S. japonicus* trees could fit in the aforementioned freezer used in Ohio, three groups of four freeze-stressed trees were deployed along with flood-stressed and untreated control trees on three separate occasions. Trees were deployed on 1 June 2018 for the first group, on 5 June 2018 for the second group and on 15 June 2018 for the third group. Attacks per tree were counted every 2–3 days. Trees in the first and second groups were cut at the base on 14 June 2018, and trees in the third group were cut at the base on 3 July 2018. Cut trunks were immediately transferred to a walk-in cooler stored at 5°C .

To compare the host-selection behaviour of ambrosia beetles attacking the freeze-stressed versus flood-stressed trees, we measured the vertical distribution of attacks on the experimentally-stressed trees deployed in Ohio and Virginia. After returning the cut trunks to the laboratory, a ruler was used to measure the distance (cm) from the base of the trunk to each attack, along with noting whether the attacks occurred to the trunk or branches. Ambrosia beetle specimens dissected from the attacked trunks were identified to species. The presence of eggs, larvae and pupae within each gallery was also quantified.

In Virginia, freeze-stressed ($n=8$), flood-stressed ($n=8$) and untreated control ($n=8$) *M. pumila* trees were used. Trees were deployed in a randomized complete block design within a deciduous woodlot in Virginia Beach, Virginia ($36^{\circ}52'3''\text{N}$, $76^{\circ}17'12''\text{W}$). A distance of 3 m was maintained between adjacent paired trees, and 6 m between adjacent pairs. Attacks per tree were counted every 2–3 days when trees were deployed from 18 April 2018 to 9 May 2018. The distance of attacks from the base of the trunk were measured (cm) as described above. Ambrosia beetle specimens recovered from the attacked trees were identified to species.

Ethanol emissions

Time course emissions of ethanol from the bark of freeze-stressed and uninjured *S. japonicus* trees were examined using solid phase microextraction-gas chromatography–mass spectrometry (SPME-GC–MS). *Styrax japonicus* trees were freeze-stressed in accordance with the method described above for the Ohio location, and then immediately transferred to a greenhouse on the campus of the Ohio Agricultural Research and Development Center. Sampling chambers (Fig. 1) were

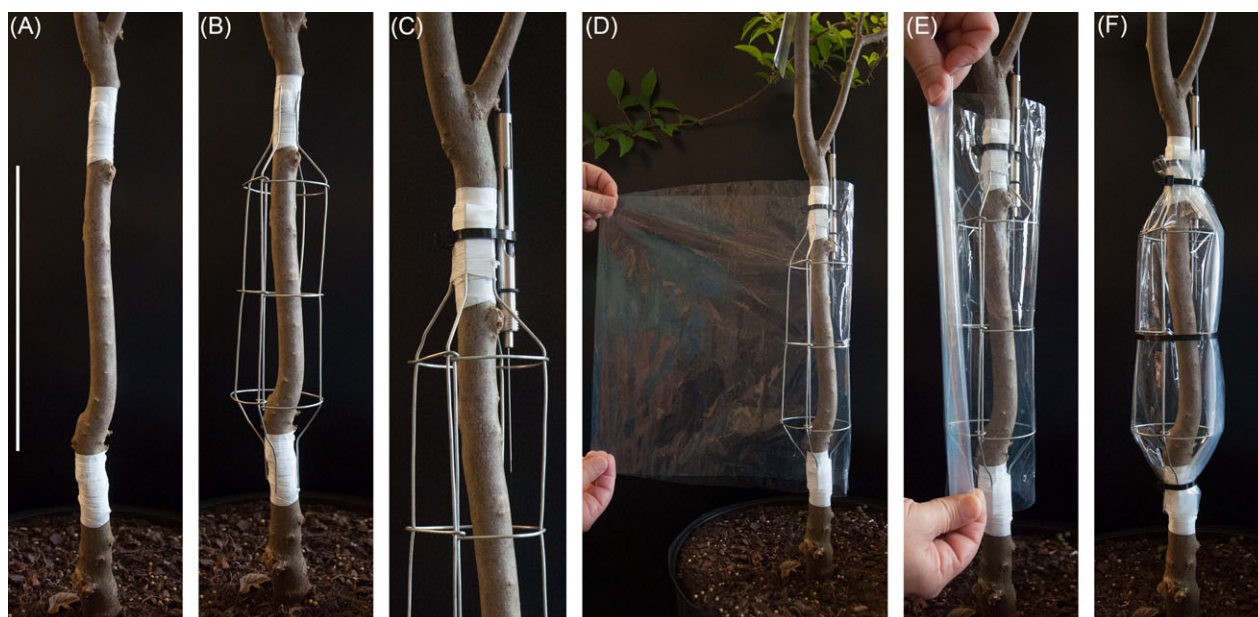


Figure 1 Chamber assembled using a metal frame and Teflon sheet for passively sampling volatile emissions from trunks of *Styrax japonicus* using solid phase microextraction (SPME)-gas chromatography-mass spectrometry. (A) Chambers were assembled by first wrapping two or three layers of Teflon tape around the trunk (vertical white scale bar = 20 cm). (B) A metal support frame was then secured to the trunk using an additional two or three layers of Teflon tape. (C) A syringe containing a retracted SPME fibre was then secured in parallel with the trunk using a zip tie. (D-E) A Teflon sheet was rolled tightly against the chamber. (F) Plastic zip ties were then tightened around the center and both ends of the chamber. For additional details, see Materials and methods. [Colour figure can be viewed at wileyonlinelibrary.com].

placed on the trunks of freeze-stressed and untreated *S. japonicus* trees to sample for ethanol emissions. Chambers were assembled by first wrapping two or three layers of Teflon tape around the trunk in a 4–5-cm band spaced approximately 21–22 cm apart (Fig. 1A). A metal support frame (diameter 6.5 cm; length 23 cm; volume 763.21 cm³) was then secured to the trunk using an additional two or three layers of Teflon tape (Fig. 1B). A syringe containing a retracted SPME fibre (CAR/PDMS; 75 µm coating; Sigma-Aldrich, St Louis, Missouri) was next secured in parallel with the trunk using a zip tie (Fig. 1C). The SPME fibre was positioned so the exposed fibre was in parallel with the trunk at a distance of 0.25–0.5 cm from the bark surface. A Teflon™ PFA sheet (length 60 cm, width 30 cm; Welch Fluorocarbon Inc., Dover, New Hampshire) was then placed around the chamber and the free ends were rolled together until snug against the metal frame (Fig. 1D, E). Plastic zip ties were tightened around the center and both ends of the chamber (Fig. 1F). Small gaps on either side of the SPME syringe were enclosed using a strip of activated charcoal filter (length 3 cm, width 1 cm; Hunter Fan Company, Tennessee). Because SPME is a passive sampling process, the chamber allowed for volatiles emitted from the bark epidermis (approximately 157 cm²) to accumulate in the headspace of the chamber. Assembling a chamber required 4–5 min and could be accomplished by one person. After assembly, the SPME fibre was immediately exposed within the chamber for 3 h to passively collect volatiles accumulating in the headspace. After sampling, the SPME fibres were retracted into the syringe holder and removed from the chamber. The syringe holders were then disassembled and the SPME fibre assembly was sealed

in a capped glass test tube and stored at –40 °C until analysis. Ethanol was sampled from freeze-stressed ($n = 3$) and untreated control ($n = 3$) trees on day 0, before imposing freeze stress on the same day, and then at 1, 4, 8, 11 and 19 days after imposing freeze stress.

For analysis by GC-MS, SPME fibres were thermally desorbed for 2 min at 250 °C in the injection port of an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, California) operated in splitless mode from 0–2 min and then a 1 : 20 split for the remainder of the run. A DB-5MS column (0.25 µm × 30 m × 0.25 µm; cross-linked/surface bonded 5% phenyl, 95% methylpolysiloxane; Agilent J&W, Santa Clara, California) was used for analysis: 40–60 °C at 3 °C/min and 60–230 °C at 20 °C/min. An Agilent 5977A mass spectrometer was operated in electron impact mode with a scan range of 40–415 amu. The external standard method was used for determining concentrations of ethanol present within the headspace of the chambers (Bartelt, 1997; Romeo, 2009). Serial dilutions containing known amounts of ethanol were applied using a pipette to filter paper discs (diameter 2.5 cm; qualitative P5, medium porosity; Fisher Scientific, Fair Lawn, New Jersey) placed inside the chamber and sampled using SPME as described above. A glass rod (diameter 2.54 cm) was used in replacement of a tree stem within the sampling chamber. Peak areas associated with the ethanol standards were measured and a standard concentration curve was used to determine concentrations of ethanol associated with the trunk tissues. Fibres were conditioned before sampling by exposure within

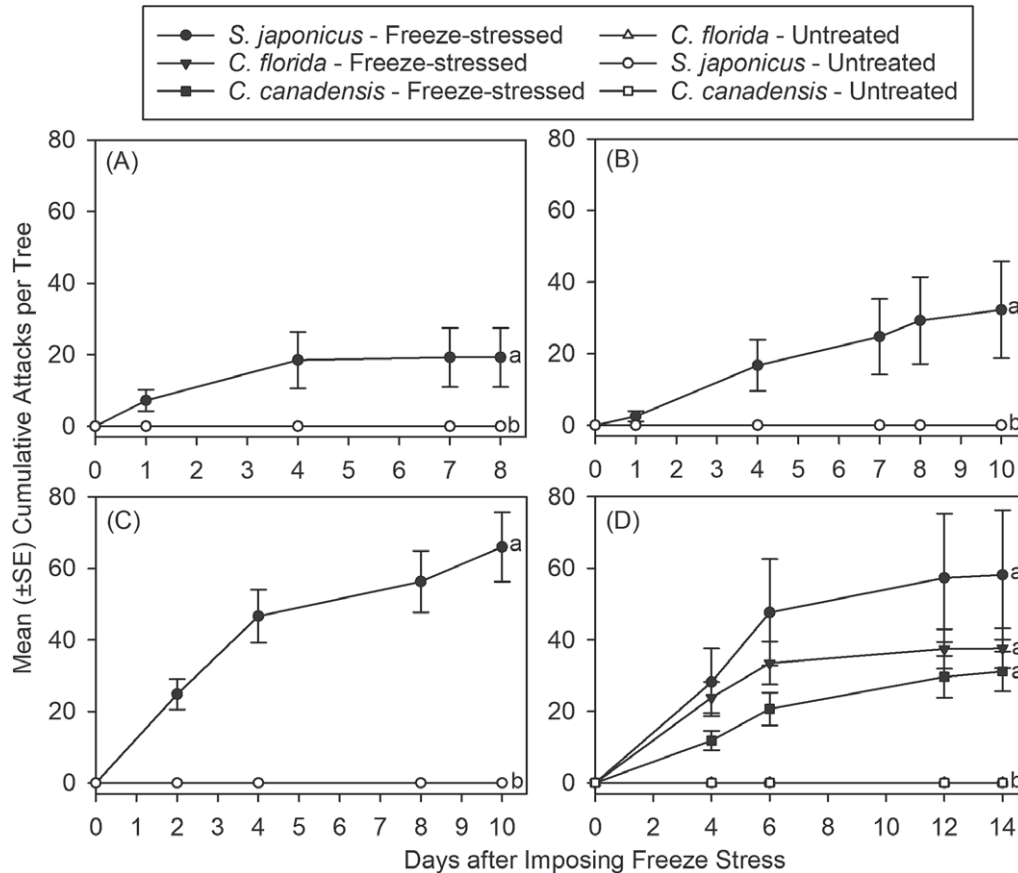


Figure 2 Cumulative ambrosia beetle attacks on freeze-stressed and untreated control trees. (A, B) *Styrax japonicus* deployed in Ohio. (C, D) *Styrax japonicus*, *Cornus florida* and *Cercis canadensis* deployed in Virginia. Different lowercase letters indicate significant differences in mean \pm SE total attacks per tree. (A) $t = 4.93$; d.f. = 6; $P = 0.003$; (B) $t = 4.45$; d.f. = 6; $P = 0.004$; (C) $t = 30.74$; d.f. = 10; $P < 0.0001$; and (D) $F = 154.66$; d.f. = 5, 30; $P < 0.0001$.

the injection port for 20 min at 250 °C. In all cases prior to sampling, Teflon sheets and steel chambers were heated in an oven for 2 h at 37 °C.

Statistical analysis

For the experiments concerning the preference for freeze-stressed trees, an unpaired t -test ($\alpha = 0.05$) was used to compare the number of attacks per tree on freeze-stressed versus untreated control *S. japonicus* trees as part of both experiments conducted in Ohio (SAS Institute, 2001). An unpaired t -test was also used to compare attacks to trunks versus branches on freeze-stressed *S. japonicus* trees deployed in Ohio, along with the number of *X. germanus* and *Anisandrus maiche* specimens recovered from the trunks versus branches. Similarly, an unpaired t -test was used to compare the number of attacks on freeze-stressed versus untreated control *S. japonicus* trees deployed as part of the first experiment conducted in Virginia. A one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) was used to compare the number of attacks on freeze-stressed and uninjured *C. canadensis*, *C. florida* and *S. japonicus* trees deployed as part of the second experiment conducted in Virginia. An unpaired t -test was used to compare the number of

X. crassiusculus versus *X. germanus* specimens recovered from freeze-stressed *C. canadensis* as part of Experiment 1 and *C. canadensis*, *C. florida* and *S. japonicus* trees deployed as part of Experiment 2 in Virginia.

For the experiments concerning the comparison between freeze-stressed and flooded trees, an unpaired t -test ($\alpha = 0.05$) was used to compare the number of attacks on freeze-stressed versus flood-stressed *S. japonicus* trees deployed in Ohio, and also Virginia. A correlation analysis was used to assess the relationship between freeze stress and flood stress on the vertical distribution of attacks (SAS Institute, 2001). Furthermore, the Kolmogorov–Smirnov two-sample test was used to compare the vertical distribution of ambrosia beetle attacks on freeze-stressed versus flood-stressed trees (SAS Institute, 2001). An unpaired t -test was used to separately compare the number of *A. maiche* Stark versus *X. germanus* adults, eggs and larvae recovered from freeze-stressed and flood-stressed *S. japonicus* trees deployed in Ohio. According to the species of ambrosia beetles, an unpaired t -test was also used to compare the number *A. maiche* or *X. germanus* adults, eggs and larvae recovered from freeze-stressed versus flood-stressed trees deployed in Ohio. A one-way ANOVA and Tukey's HSD were used to separately compare the number of ambrosia beetle adults recovered from freeze-stressed and flood-stressed *M. pumila* trees deployed in Virginia. According

Table 1 Ambrosia beetle attacks and specimens dissected from freeze-stressed *Styrax japonicus* trees deployed in Ohio

Experiment	Mean \pm SE per tree								
	Total attacks ^a			<i>Xylosandrus germanus</i>			<i>Anisandrus maiche</i>		
	Trunk	Branches	<i>t, P</i>	Trunk	Branches	<i>t, P</i>	Trunk	Branches	<i>t, P</i>
1 ^b	9.5 \pm 2.9 a	12.0 \pm 7.0 a	2.44, 0.55	2.8 \pm 1.8 a	11.3 \pm 7.0 a	0.63, 0.55	0.0 \pm 0.0	0.0 \pm 0.0	–
2 ^b	12.5 \pm 12.5 a	23.0 \pm 9.4 a	2.45, 0.53	4.8 \pm 4.8 a	16.8 \pm 6.4 a	1.78, 0.13	0.3 \pm 0.3 a	1.0 \pm 0.4 a	1.56, 0.17

^aNo attacks occurred on untreated *S. japonicus* (data not included).

^bDifferent lowercase letters within a row indicate significantly different means for total attacks and excavated specimens between trunks versus branches in Experiments 1 and 2 (d.f. = 6 for all comparisons).

Table 2 Ambrosia beetles dissected from freeze-stressed *Styrax japonicus*, *Cornus florida* and *Cercis canadensis* trees deployed in Virginia

Experiment	Mean \pm SE per tree								
	<i>Styrax japonicus</i>			<i>Cornus florida</i> ^a			<i>Cercis canadensis</i> ^a		
	<i>X. crassiusculus</i>	<i>X. germanus</i>	<i>t, P</i>	<i>X. crassiusculus</i>	<i>X. germanus</i>	<i>t, P</i>	<i>X. crassiusculus</i>	<i>X. germanus</i>	<i>t, P</i>
1 ^b	27.0 \pm 3.6 a	0.3 \pm 0.2 b	15.1, <0.0001	–	–	–	–	–	–
2 ^b	22.2 \pm 6.0 a	0.2 \pm 0.2 b	8.97, <0.0001	18.7 \pm 6.3 a	0.0 \pm 0.0b	6.35, <0.0001	5.4 \pm 1.2 a	0.0 \pm 0.0 b	9.35, <0.0001

^a*Cornus florida* and *C. canadensis* were not tested in Experiment 1, as indicated by '–'.

^bDifferent lowercase letters indicate significantly different means between *Xylosandrus crassiusculus* and *Xylosandrus germanus* recovered from *S. japonicus* within an experiment (*t*-test; d.f. = 10 for all comparisons).

to the species of ambrosia beetles, an unpaired *t*-test was also used to compare the number adults, eggs and larvae recovered from freeze-stressed versus flood-stressed trees deployed in Virginia.

Repeated measures ANOVA was used to compare the emission of ethanol from freeze-stressed *S. japonicus* trees over time (SAS Institute, 2001). Count data were $\log(x + 1)$ transformed prior to analysis but untransformed data are presented.

Results

Preference for freeze-stressed trees

Ambrosia beetles preferentially attacked freeze-stressed *S. japonicus* trees over untreated control trees during two experiments conducted in Ohio (Fig. 2A, B). In Experiment 1, ambrosia beetle attacks on *S. japonicus* were detected 1 day after imposing freeze stress (Fig. 2A). By day 8, a mean \pm SE of 22.5 \pm 9.6 attacks per tree was recorded on freeze-stressed *S. japonicus*, whereas no attacks occurred on the untreated control trees. There was no difference in the mean number of attacks that occurred to the trunks versus branches of freeze-stressed *S. japonicus* (Table 1). In Experiment 2, ambrosia beetle attacks on *S. japonicus* were also initiated 1 day after imposing freeze stress (Fig. 2B). A mean of 37.3 \pm 17.7 attacks per tree occurred by day 10, whereas no attacks occurred on the untreated control *S. japonicus* trees. No difference was detected in the number of attacks that occurred to the trunks versus branches of freeze-stressed *S. japonicus* trees (Table 1).

During the two free-choice experiments conducted in Virginia ambrosia beetles preferentially attacked freeze-stressed deciduous trees deployed under field conditions (Fig. 2C, D). In Experiment 1, a mean \pm SE of 31.5 \pm 13.6 attacks per tree occurred by

day 10 to freeze-stressed *S. japonicus* trees, whereas no attacks occurred on the untreated control trees (Fig. 2C). In Experiment 2, a mean of 58.2 \pm 18.1 attacks per *S. japonicus* tree, 37.7 \pm 5.6 attacks per *C. florida* tree and 31.2 \pm 5.6 attacks per *C. canadensis* tree were recorded at 14 days after imposing freeze stress (Fig. 2D). There was no difference in cumulative attacks among freeze-stressed *C. canadensis*, *C. florida* and *S. japonicus* trees, although there was a significant difference compared with the 0.0 \pm 0.0 attacks on the untreated control trees (Fig. 2D).

In total, 54 *X. germanus* specimens were recovered from the freeze-stressed *S. japonicus* trees attacked during Experiment 1 in Ohio; no other Scolytinae species were recovered (Table 1). A mean \pm SE of 13.5 \pm 6.4 *X. germanus* were recovered per freeze-stressed *S. japonicus*. There was no difference in the number of *X. germanus* specimens recovered from trunks versus branches during Experiment 1 (Table 1). In total, 86 *X. germanus* and five *Anisandrus maiche* Stark were recovered from the freeze-stressed *S. japonicus* trees deployed during Experiment 2 in Ohio (Table 1). A mean of 21.5 \pm 9.5 *X. germanus* specimens was recovered per freeze-stressed *S. japonicus* compared with 1.3 \pm 0.5 *A. maiche* per tree (*t* = 2.57; d.f. = 6; *P* = 0.043). There was no difference in the number of *X. germanus* or *A. maiche* specimens recovered from trunks versus branches during Experiment 2 (Table 1).

In total, 162 *X. crassiusculus* and 2 *X. germanus* were recovered from freeze-stressed *S. japonicus* trees deployed during Experiment 1 in Virginia (Table 2). Similarly, 133 *X. crassiusculus* and 1 *X. germanus* were recovered from freeze-stressed *S. japonicus* deployed during Experiment 2 (Table 2). In total, 112 and 40 *X. crassiusculus* specimens were recovered from

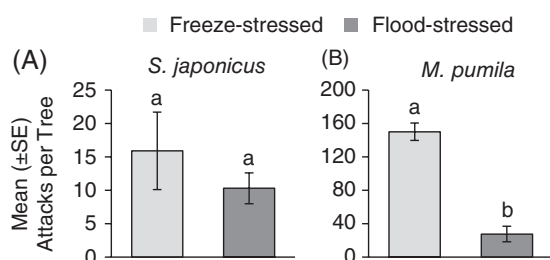


Figure 3 Mean \pm SE cumulative attacks on freeze-stressed versus flood-stressed (A) *Styrax japonicus* deployed in Ohio and (B) *Malus pumila* deployed in Virginia. No attacks occurred on untreated control trees (data not shown). Different lowercase letters indicate significantly different means within a trees species. (A) $t=0.31$; d.f. = 22; $P=0.76$ and (B) $t=3.99$; d.f. = 14; $P=0.0013$.

freeze-stressed *C. florida* and *C. canadensis* trees deployed during Experiment 2 in Virginia, respectively (Table 2). No *X. germanus* were recovered from freeze-stressed *C. florida* or *C. canadensis* trees.

Comparison between freeze-stressed and flooded trees

Ambrosia beetle host-selection and preference behaviour were compared when given a choice between freeze-stressed versus flood-stressed trees deployed in Ohio and Virginia. Under free-choice conditions, ambrosia beetles attacked both freeze-stressed and flood-stressed trees deployed in Ohio and Virginia. In Ohio, there was no difference in the mean \pm SE of 17.1 ± 6.2 attacks per freeze-stressed *S. japonicus* tree compared with the 10.3 ± 2.3 attacks per flood-stressed tree (Fig. 3A). In Virginia, a mean of 154.6 ± 10.9 attacks occurred per freeze-stressed *M. pumila* tree, which was higher than the 28.8 ± 6.7 attacks per flood-stressed tree (Fig. 3B).

Because ambrosia beetles were observed to attack the branches of *S. japonicus* during the previously described experiments concerning the preference for freeze-stressed trees, we compared the vertical distribution of attacks on freeze-stressed versus flood-stressed trees. A significant difference was detected in the vertical distribution of ambrosia beetle attacks on freeze-stressed versus flood-stressed *S. japonicus* trees deployed in Ohio (Kolmogorov–Smirnov two-sample test; $P < 0.0001$) (Fig. 4A, B). Attacks on flood-stressed *S. japonicus* trees were skewed towards the lower portion of the trunk, whereas attacks on freeze-stressed *S. japonicus* mainly occurred around the middle to upper regions of the trunk and extended into the branches (Fig. 4A, B).

A significant difference was also detected in the vertical distribution of ambrosia beetle attacks on freeze-stressed versus flood-stressed *M. pumila* trees deployed in Virginia (Kolmogorov–Smirnov two-sample test; $P < 0.0001$) (Fig. 4C, D). Attacks on flood-stressed *M. pumila* trees were skewed towards the lower portion of the trunk, whereas attacks on freeze-stressed *M. pumila* trees mainly occurred around the middle to upper regions of the trunk and extended into the branches (Fig. 4C, D).

In Ohio, *A. maiche* and *X. germanus* followed by *Xyleborinus saxesenii* were the most abundant Scolytinae dissected from

freeze-stressed *S. japonicus* trees (Table 3). In particular, 65 *A. maiche*, 66 *X. germanus* and one *X. saxesenii* were recovered from the freeze-stressed trees. Similarly, *A. maiche* and *X. germanus* were the two most abundant species dissected from the flood-stressed *S. japonicus* trees (Table 3). In total, 47 *A. maiche*, 34 *X. germanus*, two *Monarthrum mali* (Fitch) and one *Anisandrus sayi* (Hopkins) were recovered from the flood-stressed trees. Eggs and larvae of *A. maiche* and *X. germanus* were present in galleries created in the freeze-stressed and flood-stressed *S. japonicus* trees (Table 3). No pupae were recovered from the freeze-stressed or flood-stressed trees. There was no difference in the number of *A. maiche* versus *X. germanus* adults, eggs and larvae recovered from freeze-stressed *S. japonicus* or from flood-stressed *S. japonicus* (Table 3). Significantly more *A. maiche* larvae were recovered from freeze-stressed versus flood-stressed *S. japonicus* trees ($t=2.220$; d.f. = 22; $P=0.039$), although there was no difference in the number of *A. maiche* adults ($t=0.21$; d.f. = 22; $P=0.84$) and eggs ($t=0.59$; d.f. = 22; $P=0.57$). There was no difference in the number of *X. germanus* adults ($t=0.74$; d.f. = 22; $P=0.47$), eggs ($t=0.21$; d.f. = 22; $P=0.84$) and larvae ($t=0.75$; d.f. = 22; $P=0.46$) recovered from freeze-stressed versus flood-stressed *S. japonicus* trees deployed in Ohio (Table 3). There was no difference in the mean \pm SE proportion of galleries per tree containing pooled adult ambrosia beetle specimens for freeze-stressed (0.68 ± 0.07) versus flood-stressed trees (0.53 ± 0.11) ($t=1.56$; d.f. = 22; $P=0.13$), the proportion of galleries containing eggs for freeze-stressed (0.04 ± 0.02) versus flood-stressed trees (0.07 ± 0.02) ($t=0.56$; d.f. = 22; $P=0.58$) or the proportion of galleries containing larvae for freeze-stressed (0.06 ± 0.03) versus flood-stressed trees (0.01 ± 0.01) ($t=1.70$; d.f. = 22; $P=0.10$).

In Virginia, *X. crassiusculus* was the most abundant Scolytinae dissected from freeze-stressed *M. pumila* trees, followed by seven other species, namely *X. germanus*, *Cnestus mutilatus* (Blandford), *Hypothenemus dissimilis* Zimmermann, *Ambrosiodmus rubricolis* (Eichhoff), *Xylosandrus compactus* Eichhoff, *X. saxesenii* (Ratzeburg) and *Hypothenemus eruditus* Westwood (Table 4). Similarly, *X. crassiusculus* was the most abundant Scolytinae species recovered from flood-stressed *M. pumila* trees, followed by *X. germanus*, *A. rubricolis* and *C. mutilatus* (Table 4). Significantly more *X. crassiusculus* ($t=6.02$; d.f. = 14; $P < 0.0001$), *X. saxesenii* ($t=4.08$; d.f. = 14; $P=0.002$) and *H. dissimilis* ($t=3.18$; d.f. = 14; $P=0.008$) were recovered from the freeze-stressed versus flood-stressed *M. pumila* trees deployed in Virginia, although no difference was detected for *X. germanus* ($t=1.36$; d.f. = 14; $P=0.20$), *C. mutilatus* ($t=0.93$; d.f. = 14; $P=0.37$), *H. eruditus* ($t=0.83$; d.f. = 14; $P=0.42$), *A. rubricolis* ($t=1.41$; d.f. = 14; $P=0.19$) and *X. compactus* ($t=0.97$; d.f. = 14; $P=0.42$) (Table 4). A significantly higher proportion of galleries per tree created in freeze-stressed *M. pumila* trees (0.73 ± 0.03) contained ambrosia beetle foundresses (pooled across species) compared with the flood-stressed trees (0.44 ± 0.11) ($t=2.61$; d.f. = 14; $P=0.021$).

Stress-induced ethanol emissions

Time course emissions of ethanol from the trunks of freeze-stressed and untreated control *S. japonicus* were

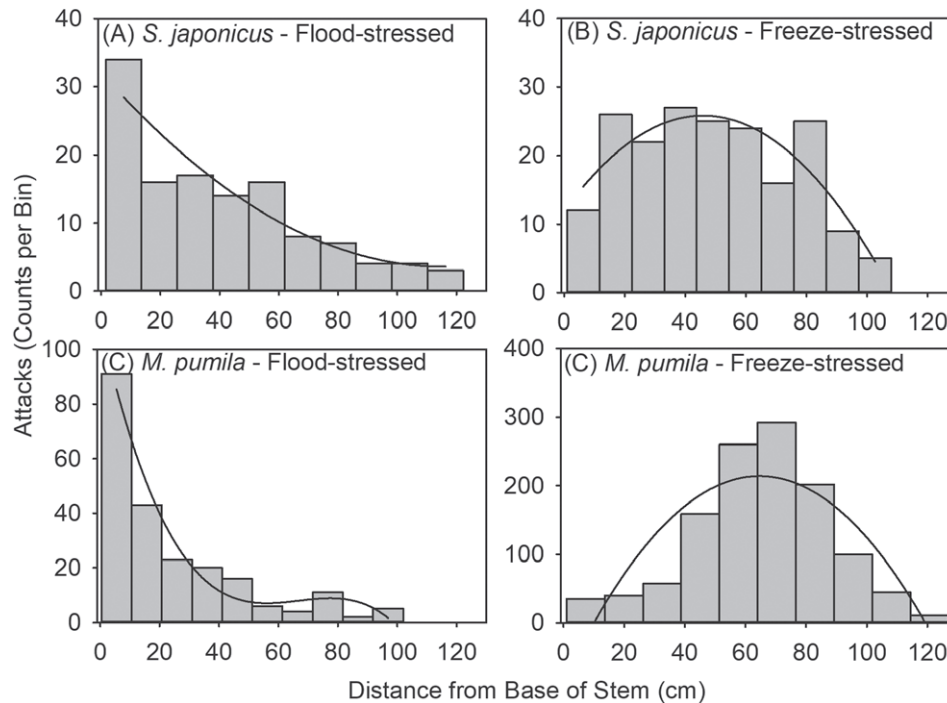


Figure 4 Histogram analysis of the vertical distribution of attacks on freeze-stressed and flood-stressed (A, B) *Styrax japonicus* deployed in Ohio and (C, D) *Malus pumila* deployed in Virginia. Ten bins were used to group attacks for each tree species and treatment. (A) $r^2 = 0.86$; $F = 21.70$; d.f. = 2, 7; $P = 0.001$; (B) $r^2 = 0.74$; $F = 10.10$; d.f. = 2, 7; $P = 0.009$; (C) $r^2 = 0.96$; $F = 47.92$; d.f. = 2, 7; $P = 0.0001$; and (D) $r^2 = 0.74$; $F = 9.75$; d.f. = 2, 7; $P = 0.01$.

Table 3 Ambrosia beetles recovered from *Styrax japonicus* trees deployed in Ohio to compare preference for freeze-stressed versus flood-stressed trees

Treatment	Life stage	Mean \pm SE specimens per tree			t ; P^a
		<i>Anisandrus maiche</i>	<i>Xylosandrus germanus</i>		
Freeze	Adults	5.4 \pm 2.7 a	5.5 \pm 2.9 a	0.01; 0.99	
	Eggs	5.1 \pm 4.0 a	10.8 \pm 6.5 a	0.42; 0.68	
	Larvae	19.7 \pm 10.4 a	4.0 \pm 2.7 a	1.05; 0.30	
	Pupae	0.0 \pm 0.0	0.0 \pm 0.0	–	
Flood	Adults	3.9 \pm 1.3 a	2.8 \pm 1.1 a	0.59; 0.56	
	Eggs	1.8 \pm 1.4 a	5.3 \pm 2.8 a	0.82; 0.43	
	Larvae	0.0 \pm 0.0 a	1.1 \pm 1.1 a	1.00; 0.33	
	Pupae	0.0 \pm 0.0	0.0 \pm 0.0	–	

^aDifferent lowercase letters within a row indicate significantly different means (t -test; d.f. = 22 for all comparisons). No attacks occurred on the uninjured trees and they were not included in the analysis.

characterized using SPME-GC–MS (Figs 1 and 5). A significant repeated measures within-treatment effect was detected in the emission of ethanol from the freeze-stressed trees ($F = 22.49$; d.f. = 5, 10; $P = 0.0087$). Ethanol emissions were not detected on day 0 before inducing freeze stress, although a mean \pm SE of 4.31 ± 2.36 ng of ethanol/cm²/h was detected 1 day after freeze stress (Fig. 5). Ethanol emissions increased to 570.52 ± 198.76 ng of ethanol/cm²/h and 395.66 ± 148.51 ng of ethanol/cm²/h by days 4 and 8, respectively. By day 11, ethanol emissions decreased to 42.41 ± 13.56 ng of ethanol/cm²/h and, by day 19, ethanol emissions decreased to 13.07 ± 11.18 ng of ethanol/cm²/h (Fig. 5). Overall, ethanol emissions were significantly higher on day 4 after imposing freeze stress compared with day 1 and days 11 and 19 (Fig. 5). No ethanol emissions

were detected from the bark of untreated control *S. japonicus* (data not included).

Discussion

Low temperature stress is one of the most important factors affecting the production of horticultural crops (Ashworth, 1992). Freeze injury is generally the result of a rapid drop in temperature below freezing after warm or mild weather during autumn, winter or spring months (Schoeneweiss, 1975; Burke *et al.*, 1976). This scenario can occur at relatively small scales affecting a few individual trees (Motzkin *et al.*, 2002; Ranger *et al.*, 2015a) or at a regional scale affecting large stands of trees

Table 4 Ambrosia beetles recovered from freeze-stressed and flood-stressed *Malus pumila* trees deployed in Virginia

Treatment	Mean \pm SE Scolytinae adults ^a per tree							
	Xcr	Xg	Xs	Hd	Cm	He	Ar	Xco
Freeze ^b	108.6 \pm 8.9 a	3.9 \pm 0.7 b	1.8 \pm 0.5 bc	1.5 \pm 0.5 cd	0.5 \pm 0.3 cd	0.3 \pm 0.3 d	0.3 \pm 0.2 d	0.3 \pm 0.3 d
Flood ^b	18.8 \pm 8.1 a	2.5 \pm 1.0 b	0.0 \pm 0.0 c	0.0 \pm 0.0 c	0.2 \pm 0.2c	0.0 \pm 0.0 c	1.0 \pm 0.5 bc	0.0 \pm 0.0 c

^aXcr = *Xylosandrus crassiusculus*, Xg = *Xylosandrus germanus*, Xs = *Xyleborinus saxesenii*, Hd = *Hypothenemus dissimilis*, Cm = *Cnestus mutilatus*, He = *Hypothenemus eruditus*, Ar = *Ambrosiodmus rubricolis* and Xco = *Xylosandrus compactus*. –, specimens not recovered from flood-stressed trees.

^bDifferent lowercase letters within a row indicate significant differences by one-way analysis of variance and Tukey's honestly significant difference (Freeze: $F = 93.78$; d.f. = 7, 56; $P < 0.0001$; Flood: $F = 20.84$; d.f. = 7, 56; $P < 0.0001$).

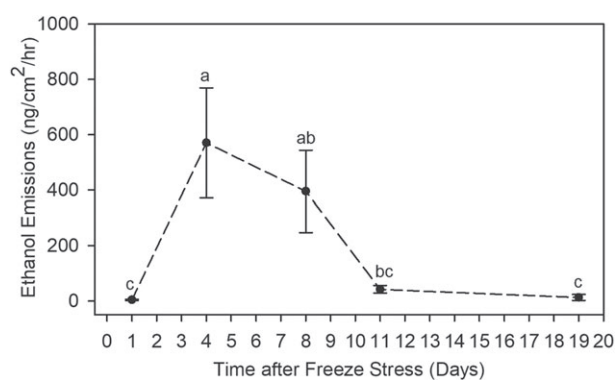


Figure 5 Mean \pm SE time course emissions of ethanol by solid phase microextraction-gas chromatography–mass spectrometry from trunks of freeze-stressed *Styrax japonicus*. No ethanol was detected in emissions from untreated control trees or from *S. japonicus* prior to initiating freeze stress (data not included). Different lowercase letters indicate significant within-treatment differences in ethanol emissions by freeze-stressed trees (repeated measures analysis of variance: $F = 11.32$; d.f. = 4, 8; $P = 0.0022$; followed by one-way analysis of variance and Tukey's honestly significant difference: $F = 12.22$; d.f. = 4, 10; $P = 0.0007$).

(La Spina *et al.*, 2013; Kosiba *et al.*, 2018; Vitasse & Rebetez, 2018). Trees injured by freezing can then be predisposed to attack by secondary wood-boring insects (Solomon, 1995; Moraal & Hilszczanski, 2000; Vansteenkiste *et al.*, 2004). The present study provides one of the few examples of ambrosia beetles attacking experimentally freeze-stressed deciduous trees, in addition to demonstrating the emission of stress-induced ethanol from trunks of injured trees. Most attacks were by non-native Xyleborini species that rapidly attacked the freeze-stressed trees. Targeting trees in the early stages of physiological stress might contribute to the invasion success of Xyleborini ambrosia beetles that utilize living but weakened trees (Kühnholz *et al.*, 2001; Ranger *et al.*, 2015a).

Freeze-stressed trees were rapidly attacked during the present study, with attacks often beginning within 1–2 days after injury. Ambrosia beetles have also been observed to begin attacking flood-stressed trees within 1–2 days after flooding (Ranger *et al.*, 2013, 2015b). *Xylosandrus germanus* was one of the predominant species excavated from freeze-stressed trees deployed in Ohio, whereas *X. crassiusculus* was the predominant species recovered in Virginia. The present study also documents the recovery of *A. maiche* from stressed trees deployed in Ohio. Notably, *A. maiche* was one of the predominant species recovered along with *X. germanus* when beetles were allowed to freely

choose between freeze-stressed and flood-stressed trees. After first being reported from North America in 2009, *A. maiche* now appears to be established in eight states within the Midwestern and Eastern U.S.A. (Rabaglia *et al.*, 2009; Atkinson, 2018). Successful colonization of freeze-stressed and flood-stressed trees, as made evident by the presence of eggs and larvae within galleries, was documented for *A. maiche* and *X. germanus* during the present study. These results provide additional insight into the utilization of trees in the early stages of physiological stress by certain Xyleborini ambrosia beetles.

By measuring the vertical distribution of attacks, ambrosia beetles were demonstrated to preferentially attack the lower trunk region on flood-stressed trees, whereas attacks on freeze-stressed trees mainly occurred around the middle and upper trunk regions and branches. Heidenreich (1960) also observed that trunks and branches of freeze-stressed trees were attacked by *X. germanus*, whereas trunks are mainly attacked on flood-stressed trees (Ranger *et al.*, 2016). Understanding how physiological stressors influence the vertical distribution of ambrosia beetle attacks could aid tree care professionals in determining what stressors potentially predisposed the trees to attack, thereby helping to predict and mitigate the risk of future attacks. Although not confirmed, we hypothesize the difference in distribution of attacks on freeze-stressed versus flood-stressed trees corresponds to the presence of ethanol within these tissues. Kelsey *et al.* (2013) demonstrated that more than four-fold ambrosia beetle attacks occurred above ethanol-infused sapwood tissues compared with the opposite side of the same log that did not any receive ethanol treatment.

Gara *et al.* (1993) used a stainless steel chamber to detect ethanol emissions from the bark of lodgepole pine [*Pinus contorta murrayana* (Grev. and Balf) Engelm]. trees infected with heartwood decay fungi. The wire frame and Teflon-base chambers used as part of the present study allowed for the confirmation and measurement of ethanol emissions from the bark of freeze-stressed trees.

When cellular oxygen levels decline and are depleted, anaerobic respiration results in pyruvic acid being converted into acetaldehyde, which is then reduced to ethanol (Fall, 1999). Alcoholic fermentation typically occurs in the roots of plants or trees in response to flooding (Fall, 1999). Ethanol and acetaldehyde are not only then secreted by the roots, but also enter the transpiration stream and are subsequently emitted from the bark epidermis and the leaves (Kimmerer & Kozlowski, 1982; MacDonald & Kimmerer, 1993; Ranger *et al.*, 2013, 2015b). By contrast, an inability to uptake O_2 by cells within trunks injured by freeze stress would also lead to the production and emission of

ethanol from these tissues (Kimmerer & Kozlowski, 1982; Forney *et al.*, 2000; Obenland *et al.*, 2003).

As noted previously, the presence of ethanol within host tree tissues benefits fungiculture by *X. germanus* and other ambrosia beetles by promoting the growth of the fungal symbiont and suppressing the growth of other fungal competitors (Ranger *et al.*, 2018). Because foundress ambrosia beetles do not begin laying eggs until the fungal gardens are established, the presence of ethanol within the host tree tissues indirectly benefits the colonization success of these opportunistic ambrosia beetles. Healthy trees baited with an ethanol lure were not included as a positive control as part of the present study. However, Ranger *et al.* (2018) previously demonstrated that healthy *C. canadensis* and *C. florida* trees baited with ethanol lures were attacked but no fungal gardens or offspring were produced in the absence of *in vivo* ethanol within the host tree tissues. Still, additional studies are warranted to determine whether other compounds besides ethanol within the host tree tissues are beneficial to ambrosia beetle fungiculture and their colonization success.

A warming climate with a continued risk of freeze stress could result in increased attacks by Xyleborini ambrosia beetles, particularly for intolerant trees growing in ornamental nurseries and tree fruit orchards. Unusually warm periods followed by wide-spread freeze stress of trees have recently been reported for the U.S.A. and Europe (Ningre & Colin, 2007; Gu *et al.*, 2008; Augspurger, 2009; Hufkens *et al.*, 2012; Timlin *et al.*, 2012; Kosiba *et al.*, 2018; Vitasse & Rebetez, 2018). An increase in global temperatures is anticipated to promote earlier plant development, although an increased risk of spring freeze injury has also been predicted even if the frequency and severity of late spring frosts remains the same (Hänninen, 1991; Arora, 2018; Vitasse *et al.*, 2018). Wild trees growing within their natural distribution ranges rarely experience severe frost injury (Vitasse *et al.*, 2018), although trees growing in ornamental nurseries and tree fruit orchards will likely be vulnerable to attack. Trees being grown outside their normal distribution or the loss of freeze tolerance traits resulting from selective breeding programmes would contribute to the risk of injury and subsequent ambrosia beetle attacks.

For example, a mild winter that occurred in northeastern Ohio in 2011–2012 resulted in trees budding up to 1 month earlier than normal (Timlin *et al.*, 2012; NOAA, 2012). Vulnerable root and vegetative tissues were then exposed to six below freezing events that occurred in the region in April 2012. Ambrosia beetle attacks on trees growing in ornamental nurseries were subsequently documented in May 2012 after the spring flight of overwintering adults had commenced (Ranger *et al.*, 2015a). Thin-barked deciduous trees with known intolerance of freeze stress were attacked, namely *Acer palmatum* Thunb., *C. canadensis*, *Cladrastis kentukea* (Dum.-Cours.) Rudd, *S. japonicus* and *Zelkova serrata* (Thunb.) Makino (Cappiello & Littlefield, 1994; Boerner *et al.*, 1988; Brand, 2002; Reed, 2005; Ranger *et al.*, 2015a). In support of these field observations, the present study demonstrated that experimentally-stressed *C. canadensis*, *C. florida*, *M. pumila* and *S. japonicus* were attacked by non-native ambrosia beetles. La Spina *et al.* (2013) previously reported that experimentally freeze-stressed tissues of *F. sylvatica* also attracted and induced attacks by *X. germanus* and other ambrosia beetles.

In summary, in the present study, we have demonstrated that freeze stress predisposes thin-barked deciduous trees to attack by opportunistic ambrosia beetles, especially *X. crassiusculus* and *X. germanus*. Both of these species are strongly attracted to trees emitting stress-induced ethanol, and freeze stress of *S. japonicus* was demonstrated to induce the emission of ethanol. The results of the present study indicate that trees intolerant of freeze stress growing in ornamental nurseries and tree fruit orchards could be at risk of attack by ambrosia beetles, especially when freeze stress is preceded by a mild winter. Additional studies are therefore warranted to characterize the freeze stress intolerance of trees commonly planted in ornamental nurseries and tree fruit orchards that are attacked by ambrosia beetles. The incidence of freeze stress and ambrosia beetle attacks on trees within nurseries and orchards should also be characterized and modelled to assist in developing management strategies.

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