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Survival of elongate hemlock scale (Hemiptera: Diaspididae) with prolonged cold exposure: overwintering mortality risk across North America

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Elongate hemlock scale, *Fiorinia externa* Ferris (Hemiptera: Diaspididae), is an invasive pest of eastern hemlock, *Tsuga canadensis*, and other Pinaceae such as Fraser fir, *Abies fraseri*. Cut Fraser firs (ie for Christmas trees and other holiday greenery) with *F. externa* have been intercepted in US states beyond where the insect is known to be established and, in some cases, where exposure to freezing temperatures might be prolonged. This study measures the effect of constant low temperatures (3, −10, or −20 °C) on survival of overwintering *F. externa* females from eastern hemlock in Michigan and from eastern hemlock and Fraser fir in North Carolina. Survival was determined with biochemical viability testing, which assesses the presence of metabolically active cells, and changes in survivorship through time were quantified with Kaplan–Meier methods and beta regression. Collection date and host occasionally affected survival rate but not in a consistent pattern. Survival rates generally decreased as temperature decreased. At 3 °C, *F. externa* maintained high survival (>75%). At −10 °C, survival was projected to fall to 50% within 22 to 92 d and 10% by 45 to 195 d. At −20 °C, survival typically declined to 50% by 1 to 27 d and 10% by 6 to 52 d. We used survival durations at −20 °C to map how often *F. externa* might experience 50 or 90% mortality from prolonged cold exposure. Temperatures in recent winters have not been below −20 °C for long enough to stop *F. externa* from spreading throughout the ranges of eastern hemlock or other hosts in Canada and the continental United States.

Keywords: cold tolerance, invasive species, lower lethal time, pest risk mapping, range limits

Introduction

The environmental effects that govern the establishment, spread, and impact of nonnative invasive insects in a changing climate remain understudied. For example, winter temperatures can be a key limiting factor of insect ranges, and as winters trend shorter and warmer (IPCC 2023), these limits will shift and enable range expansion to higher elevations and poleward latitudes (Williams et al. 2015). The capacity of invasive insects to survive low temperatures can be estimated by measuring their responses to laboratory-simulated winter conditions in carefully designed studies (eg Jing and Kang 2003, Ayrinhac et al. 2004, Liu et al. 2007, Cira et al. 2016, Morey et al. 2016, Elkinton et al. 2017, Hefty et al. 2017, Chandler et al. 2020, Vétek et al. 2020, Hafker et al. 2024). These cold hardiness metrics, taken together with information on the species' behavior and microhabitat, can be used to explain or project reductions in pest population density after realized or forecasted cold exposures. This information is particularly important for invasive insects that may have recently arrived in a new geographic area. Areas with winter temperatures that annually cause severe mortality are unlikely to be suitable for local establishment of a spreading pest (Koch and Smith 2008, Hefty et al. 2017).

The elongate hemlock scale, *Fiorinia externa* Ferris (Hemiptera: Diaspididae), is native to eastern Asia (Takagi 1963) and invasive in the United States. Its primary hosts in the United States are eastern hemlock, *Tsuga canadensis* (L.) Carr. and Carolina hemlock, *Tsuga caroliniana* Engelm. (Sasscer 1912, Davidson and McComb 1958), although several other Pinaceae are hosts (reviewed in Venette et al. 2024). The insect can be

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problematic on Fraser fir, *Abies fraseri* (Pursh) Poir, which is often commercially grown for Christmas trees and holiday greenery (Darr et al. 2022). This insect was introduced inadvertently near New York, New York in 1908 (Sasscer 1912, Ferris 1942). Its current range in the eastern United States includes 14 states from Maine to Georgia (USDA 2019) with a disjunct population in Michigan (Petrice et al. 2022). However, cut Fraser firs with *F. externa* have been detected in California (Leathers 2016), Florida (Stocks 2016), Oregon (Oregon Department of Forestry 2019), Minnesota (Petrice et al. 2022), and Wisconsin (Wisconsin Department of Agriculture Trade and Consumer Protection 2023). In Minnesota, interceptions have recurred over multiple years and have led to increased phytosanitary inspections, destruction of infested products, and lost profits for the Christmas tree industry (Angie Ambourn, personal communication). Whether *F. externa* has established in these states is unknown.

The potential spread of *F. externa* is a concern to regulators, foresters, and Christmas tree growers because feeding by *F. externa* causes photosynthate loss, which intensifies as densities of the insect increase (Manz et al. 2025). Early literature reported this feeding to cause needle yellowing, needle dropping, and mortality of eastern hemlock trees at high densities (Wallner 1962, McClure and Fergione 1977). However, *F. externa* rarely causes mortality of eastern hemlock (Preisser et al. 2008b, Mech et al. 2019, McAvoy et al. 2025); damage is localized at feeding sites (Miller-Pierce et al. 2010, Gonda-King et al. 2012, Soltis et al. 2015). *Fiorinia externa* is less harmful than the co-occurring hemlock woolly adelgid, *Adelges tsugae* Annand, and may reduce damage from hemlock woolly adelgid through competition (Preisser and Elkinton 2008, Gomez et al. 2014). Nevertheless, *F. externa* might exacerbate the effects of other stressors such as heat, drought, and pathogens that may reduce survival or growth rates of host trees.

Fiorinia externa disperse as crawlers (ie mobile first instars) passively via wind, typically < 15 m, and actively by crawling short distances (McClure 1977). Crawlers settle on the underside of needles and insert their stylets to feed. Like most Diaspididae, *F. externa* reproduces sexually, and adults are sexually dimorphic (Beardsley and Gonzalez 1975). Females are pupillarial and remain permanently at the feeding site, while males develop and eclose with functional wings (Stoetzel 1976). Northern populations are primarily univoltine, while southern populations are bivoltine (Stimmel 1980, McClure 1989, Abell and Van Driesche 2012). The primary overwintering stages are believed to be adult females bundled with their eggs in "tests," the exuviae of second instars (McClure 1989, Petrice et al. 2022).

The cold tolerance or overwintering potential of *F. externa* is poorly understood. Existing research focuses on brief cold exposures. Preisser et al. (2008a) reported that exposure to −15 °C for 36 h reduced survival of *F. externa* individuals from Maryland, but not Connecticut or Massachusetts, even after insects were reared in a common garden for a generation. Venette et al. (unpublished data) found that 90% of *F. externa* would die after acute (in this case, less than about 3 s) exposure to −26 to −38 °C, depending on the month the insects were collected. However, the effects of cold temperatures vary with the severity and duration of exposure, so it is important to consider both acute and long-term cold exposure when modeling effects of winter conditions on insect survival and distributions (Morey et al. 2012, Delisle et al. 2013, Vétek et al. 2020).

Insect cold tolerance is a plastic trait that can vary temporally and spatially (Lee 2010). Many insects acclimatize, becoming more cold-tolerant in preparation for winter, then de-acclimatize as winter transitions into spring (Crosthwaite et al. 2011, Cira et al. 2016, Elkinton et al. 2017, Hefty et al. 2017, Chandler et al. 2020, Vétek et al. 2020). Many insects also adapt to different climates, gaining increased cold tolerance in colder regions (Jing and Kang 2003, Ayrinhac et al. 2004, Zhang et al. 2010, Zhong et al. 2010, Cira et al. 2016, Just and Frank 2020, Vétek et al. 2020, Hafker et al. 2024). Furthermore, because phytophagous insects' production of cryoprotectants is dependent on energy reserves from their food source (Storey and Storey 1991), host plant may influence the cold hardiness of some insects (Liu et al. 2007, Zhong et al. 2009, Morey et al. 2016).

This study quantifies changes in survival of *F. externa* through time in response to prolonged exposure to low temperatures. We collected *F. externa* from sites in Michigan and North Carolina across multiple hosts, months, and years, exposed them to temperatures reflecting winter conditions in its geographical range that are not immediately lethal (−10 and −20 °C), and quantified the time required for 50% or 90% of *F. externa* to die (ie lethal times, $LTime_{50}$ or $LTime_{90}$). We expected *F. externa* to acclimatize, indicated by increasing lethal time estimates from fall to winter, followed by decreasing lethal time estimates from winter to spring. We conjectured that lethal time estimates would be similar between our 2 sites given similarities in winter temperatures. We also hypothesized that *F. externa* from different hosts, Fraser fir and eastern hemlock, would exhibit different cold hardiness. We used the results from these studies to forecast where prolonged exposures to −20 °C might constrain the potential future spread of *F. externa* in North America, which is particularly prudent given that many hosts lie beyond its current distribution.

Materials and Methods

F. externa Sampling and Handling

Infested host material was collected from Covert, Michigan, United States (latitude, longitude: 42.2855, −86.3385), ~300 m from Lake Michigan's eastern coast and at an elevation of ~297 m, and from Waynesville, North Carolina, United States (35.4898, −82.9714; North Carolina Department of Agriculture and Consumer Services' Mountain Research Station) at an elevation of ~840 m. We gathered air temperatures from the weather station KMISTJOS46 in St John, Michigan (42.1021, −86.4887, 25 km northeast of Covert) and from the Mountain Research Station weather station. Covert experienced a minimum temperature of -15.7 °C in the meteorological winter (December to February) of 2022/2023 and −21.5 °C in 2023/2024, with an average temperature across both winters of 1.4 °C. The Mountain Research Station experienced a minimum temperature of −19.0 °C in the winter of 2022/2023 and −16.0 °C in 2023/2024; across the 2 winters, the average temperature was 5.1 °C.

We sampled *F. externa* 7 times over 2 winters: January 2023 to February 2023 and November 2023 to March 2024. Michigan samples consisted of infested eastern hemlock. North Carolina samples consisted of infested eastern hemlock and

Fraser fir. Details for each collection are given in Table 1. Multiple trees were sampled arbitrarily during each collection. Collectors gathered branch tips with the current year's growth, allowing us to target *F. externa* in its first year of life. Foliage from different hosts was sealed in separate plastic bags. Samples were priority shipped on ice to a biosecurity level 2 laboratory in St Paul, Minnesota, in transit ≤4 d. All shipping and handling followed the permit conditions from the US Department of Agriculture, Animal and Plant Health Inspection Service (permits P526P-21-00620 and 526-23-242-11729). Upon receipt, samples in bags were placed in a dark 3 °C incubator until the trials began.

Lower Lethal Time Trials

To test how long *F. externa* could survive sustained cold temperatures, we placed *F. externa* in dark incubators at 3 temperatures (3, −10, and −20 °C) and held them for 32 to 64 d while we monitored survivorship. We chose 3 °C to be the control treatment because it reflects the average temperature across both collection sites in recent winters. *Fiorinia externa* is well established at these sites, so we do not expect this temperature to cause rapid mortality. We chose −10 °C as an intermediate temperature, regularly experienced during winter within the known US range of *F. externa*. We chose −20 °C because it is a common winter temperature in Canada and the northern United States, yet not cold enough to cause instant mortality (Venette et al. unpublished data). For every combination of collection location, date, host, and temperature, we tested 34 to 57 *F. externa* at 4 to 7 exposure times, for a total of 1,438 *F. externa*. Trials typically began about a week after collection; 3 trials began about 3 wk after collection due to logistical constraints or equipment malfunction (Table 1).

To prepare for trials, we clipped the current year of growth, determined by color and position, from *F. externa* infested branches. A cut branch frequently had multiple tips. Branch tips were typically ∼5 cm long and were occasionally cut into segments (∼2.5 cm long) to increase the number of tips available for each temperature × time combination. Some of the eastern hemlock branch tips from the 26 November 2023 sample from North Carolina included the previous year's growth due to a limited supply of material. We assigned 4 to 10 tips (usually 10) to each combination of temperature and exposure time, which included 0 d, using a semi-stratified randomization approach. Branch tips from the same branch were separated into different treatments such that each treatment group comprised foliage tips from different branches (or from the maximum variety of branches). Each set of tips was sealed in labeled plastic bags and placed into its respective incubator. Thereafter, survival was tested approximately weekly. This schedule was altered for feasibility reasons and extended toward the end of the trial if few *F. externa* had died. Exact exposure times are listed in Table 1.

At each observation time, a set of branch tips was removed from each of the 3 incubators. They were then left at room temperature for ∼24 h before testing to allow adenosine triphosphate (ATP) to degrade in dead individuals (Kamiji and Kadoi 2017); this was necessary for survival testing (described below), and this period was not considered part of the exposure time. Subsequently, we removed about 10 *F. externa* tests (ie female and eggs in the exuvium of the second instar) per temperature treatment and host from needles using a #0 insect mounting pin. Only tests with adult females were picked because they are the primary overwintering stage. We picked the most terminal test per branch tip. If there were fewer than 10 tips, we picked multiple tests per tip moving to the test(s) next-closest to the terminal end while keeping the number picked per tip as even as possible. If there was more than one test on a needle, we only picked one. We did not select for healthy-looking females but avoided tests that had clearly succumbed to mortality factors other than cold (ie were parasitized, empty, or overgrown with fungus). We also picked 5 live controls from the foliage at 3 °C of either or both hosts, selecting healthy looking females (ie unparasitized, amber colored, well-affixed to needle, with at least one hydrated egg, and lacking fungal growth). We picked 5 dead controls from foliage kept for >36 h in a −80 °C freezer. We could not reliably determine when *F. externa* females were dead from cold exposure based on appearance alone.

Biochemical Viability Testing

To determine if *F. externa* were alive or dead, we utilized an existing colorimetric assay, known hereafter as a biochemical viability test (BVT), that yields formazan (colored blue-purple) from the reduction of a tetrazolium salt through a series of enzymatic reactions in the presence of ATP, described in detail by Phillips et al. (2013). ATP is the rate limiting reagent in the process, so a deeper blue-purple color reflects a greater concentration of ATP. ATP is a universal energy source for all organisms at a (sub-) cellular level; its presence is indicative of life. BVT stain was prepared per the manufacturer's directions (AgResearch, Christchurch, New Zealand). To conduct the assay, we added 2 µl of BVT stain into a 10-µl conical well of a plastic microtiter plate (AgResearch, Christchurch, New Zealand) then transferred an *F. externa* test into the well with a #0

Table 1. Details of lower lethal time trials with *Fiorinia externa* at 3, −10, or −20 °C

Source	Collection date	Trial start date	Exposure times (days)	Sample size
MI	18 January 2023	24 January 2023	0, 8, 17, 21, 42	96[a]
	17 February 2023	9 March 2023	0, 7, 14, 21, 28, 51	140[a]
	6 November 2023	15 November 2023	0, 13, 20, 25, 32	110[a]
NC	26 November 2023	30 November 2023	0, 7, 20, 34	109[a], 105[b]
	21 January 2024	25 January 2024	0, 7, 14, 21, 36, 51	169[a], 167[b]
	22 February 2024	27 February 2024	0, 8, 15, 22, 35, 57	136[a], 145[b]
	19 March 2024	10 April 2024	0, 7, 14, 26, 43, 63	95[a], 166[b]

MI = Covert, Michigan; NC = Mountain Research Station, North Carolina.
[a]host: eastern hemlock.
[b]host: Fraser fir.

insect mounting pin. Under a dissecting microscope, we used 2 pins to remove the female from the exuvium of the second instar, remove the exuvium from the well, and macerate the female in the BVT stain. We immediately covered the microtiter plate with its lid to limit stain evaporation. After exactly 10 min, we placed the microtiter plate under a secondary dissecting microscope, removed the lid, and photographed the well. The microscope was equipped with a mounted smartphone (winter of 2022/2023) or digital camera (winter of 2023/2024). Camera settings are described in Supplementary Table S1. The microtiter plate was on a white background on the stage and illuminated by an LED ring light (ASIN B0CZLM-9JWM, Mactrem, Shenzhen, Guangdong, China) suspended 3.3 cm above the stage. The LED light ring emitted cool white light at maximum intensity. Overhead lights were off when images were taken. A technician blind to the treatment gave a visual description of the stain color 30 s or 1 min before photographing. *Fiorinia externa* were assayed in randomized order with live and dead controls interjected at random. Pins used for insect maceration were rinsed with deionized water and dried with Kimwipes between each use.

We analyzed digital images of BVT reactions with ImageJ (ver 1.52; Schneider et al. 2012). Each image was cropped into a circle around the BVT stain. The image was converted to a Hue-Saturation-Brightness stack, and the relative frequency of pixels in each of 257 hues was determined. We compared histograms of the relative frequencies of hues of live and dead controls. We selected a string of hues that were associated with live controls and thus indicative of viability. These hues were generally at least 4 times more prevalent in live controls than dead controls. Different image capture methods affected the specific string of hues selected, which are listed in Supplementary Table S1. Then, we summed the relative frequency of the chosen hues in each image to create a "live index." We identified the live index value halfway between the dead control with the highest live index and live control with the lowest live index, and *F. externa* with live index values greater than this cutoff value were considered alive. The cutoff was calculated for each day and per batch of BVT stain when more than one was used in a day, as the intensity of BVT staining varied depending on the age of the stain. Due to blurry or lost images, the visual description was used to impute viability for 19 *F. externa* (all controls for the 6 November 2023 collection and 2 controls for the 18 January 2023 collection).

Data Analysis

All analyses were carried out in *R* v4.3.2 (Posit Team 2025). All data manipulation and visualization were performed with the "tidyverse" suite of packages (Wickham et al. 2019). We used a combination of survival analysis and beta regression to estimate the time required to achieve 50% and 90% mortality (the lethal times, $LTime_{50}$ and $LTime_{90}$).

We first used the Kaplan–Meier estimator with the *R* package "survival" to describe the effect of time on survival (Therneau and Grambsch 2000). The Kaplan–Meier method is a non-parametric statistical technique used to analyze time-to-event data that supports censored data. Censoring occurs when the event of interest (ie death) is not observed at a specific time during the study but within a range of observations. Individuals that tested unviable could have died at any point during their exposure period, so the time to death was coded as left censored (-infinity: observed mortality). Individuals that tested viable would die at some future time, so the time to death was coded as right censored (observed survival: infinity). We extracted Kaplan–Meier nodes and applied the Abbott (1925) correction to these data (ie divided all points by the predicted proportion alive at time of 0 to correct for initial mortality, such that all subsequent mortality occurred during cold exposure). This procedure sometimes created values equal to 1, which were transformed to 0.999 prior to beta regression.

Using the "betareg" package, we analyzed these corrected Kaplan–Meier data (nodes) using beta regressions (Cribari-Neto and Zeileis 2010). Beta regression is designed to analyze dependent variables that are proportional (constrained between 0 and 1) and beta distributed. We employed the logit link function to relate expected survival (μ) to the independent variables (x_1, x_2, … x_n) following the equation $\mu = 1 / \left(1 + e^{-(b_0 + b_1 x_1 + b_2 x_2 + \cdots + b_n x_n)}\right)$. Beta regression also estimates a dispersion parameter ϕ. For a given μ, a higher ϕ results in a lower dispersion of the response variable around μ: $\text{var}(\mu) = \mu(1-\mu)/(1+\Phi)$ (Ferrari and Cribari-Neto 2004). In models, the combination of location, collection date, and host were treated as one categorical factor, hereafter called batch. Some batches lacked all 3 temperature treatments: incubator malfunctions led to a lack of reliable data on *F. externa* at −20 °C from eastern hemlock collected on 18 January 2023 from Michigan and the failure of trials with *F. externa* collected on 19 March 2024 from North Carolina. The latter trials were redone using leftover foliage, except for the 3 °C treatment for eastern hemlock, because we ran out of material. As a result, our data are not full rank, so we ran one set of models to analyze the effect of temperature on survival, and another set to analyze the effect of batch on survival.

To analyze the effect of temperature on the survival of *F. externa* over time, we subset the data by batch, for 11 subsets, and analyzed each subset separately with a model testing for the main effects of time and temperature and the interaction between time and temperature (see Results). To analyze the effect of batch on survival of *F. externa* over time, we subset the data by temperature and analyzed each subset separately with a model testing for the main effects of time and batch and the interaction between time and batch (see Results). From the latter set of models, we elucidated the effects of location, collection date, and host by focusing on certain batch comparisons. For both sets of models, we used the "multcomp" *R* package to apply the Bonferroni correction and view pairwise comparisons between groups (Hothorn et al. 2008) and then used the "cldList" function in the "rcompanion" *R* package to generate compact letter displays (Mangiafico 2025).

Using the latter set of models and the "predict" function provided with base *R*, we estimated the live proportion of *F. externa* over time for each batch and temperature, as well as the 0.025 and 0.975 quantiles of these estimates to generate 95% confidence intervals (CIs) (Fig. 1). This step provided the $LTime_{50}$ and $LTime_{90}$ and their 95% CIs (Fig. 2), though since the lethal times are inverse predictions (predicting y at a given x), we had to characterize survival on a very fine time scale to pinpoint the desired values.

Mapping

Survival data for *F. externa* at −20 °C were most useful for risk mapping because preliminary analyses suggested that few

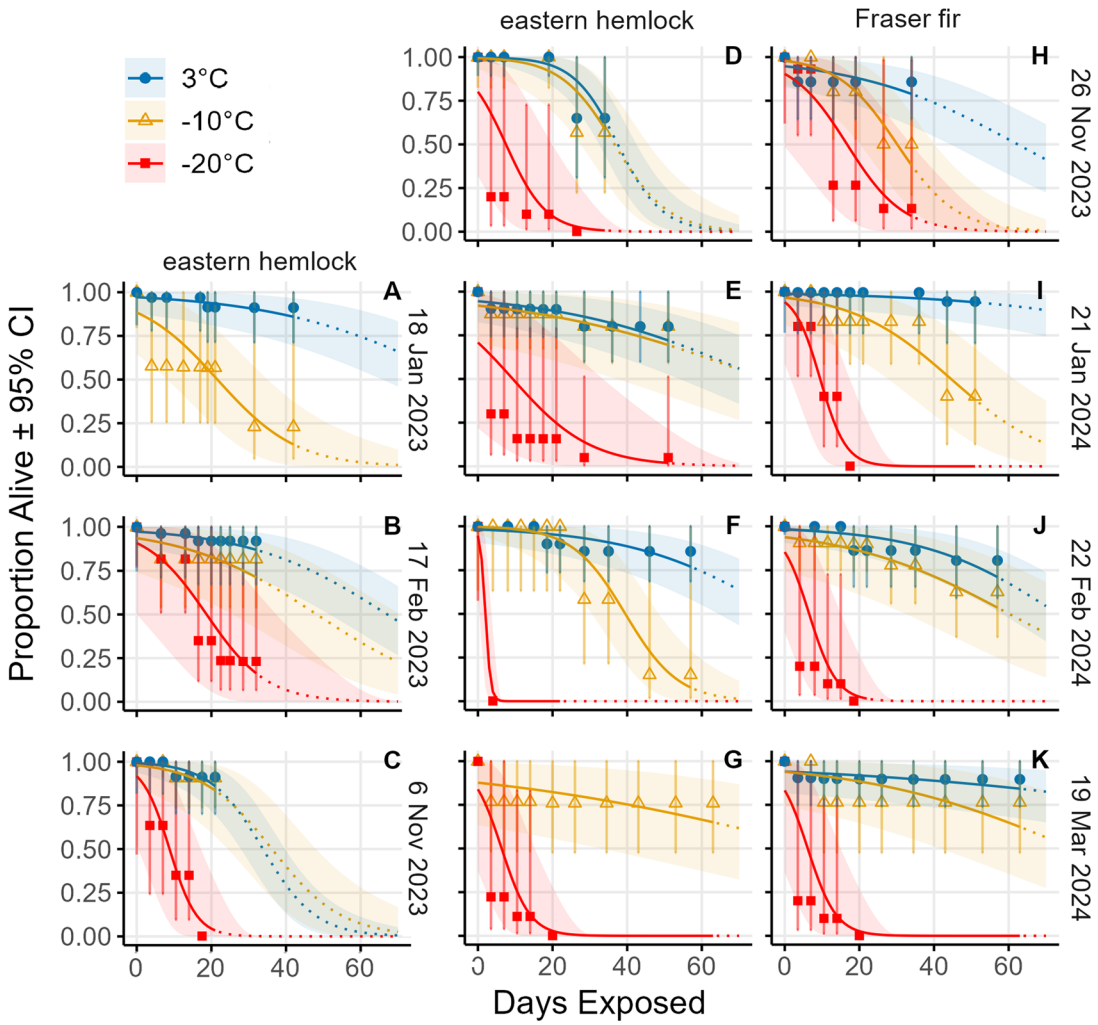

Fig. 1. Mean survival ± 95% confidence intervals of *Fiorinia externa* over time at 3 temperatures. Specimens were collected from eastern hemlock (A–G) or Fraser fir (H–K) from Covert, Michigan (A–C) or Mountain Research Station, North Carolina (D–K) ($n = 34$ to 59 per collection per temperature). Lines indicate fitted estimates ± 95% CI from beta regression models. Dotted lines are projections beyond the limit of the data. Model coefficients are reported in Table 2.

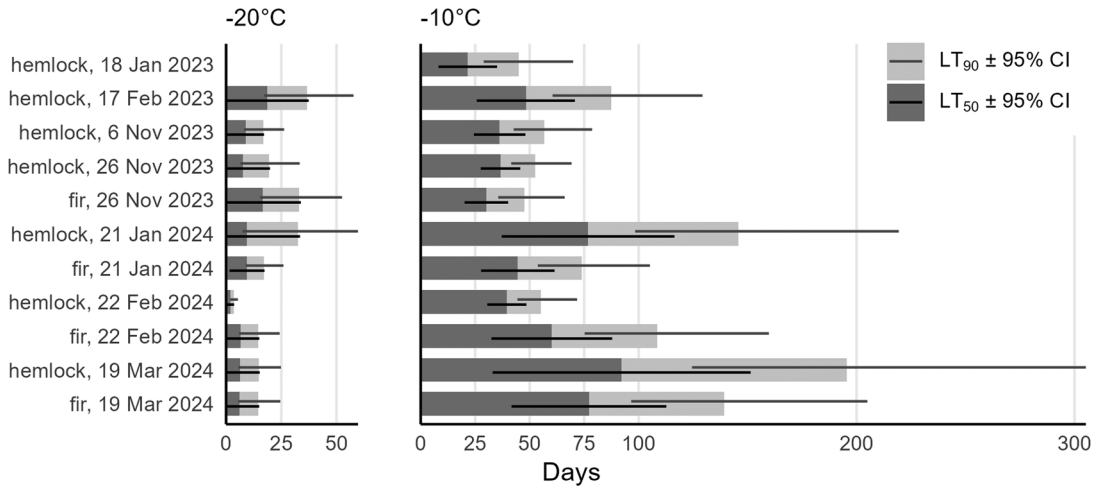

Fig. 2. Lethal time for 50% ($LTime_{50}$) and 90% ($LTime_{90}$) of *Fiorinia externa* at −20 and −10 °C ± 95% confidence intervals. Specimens were collected on 7 dates and 2 hosts (hemlock = eastern hemlock, *Tsuga canadensis*; fir = Fraser fir, *Abies fraseri*) from 2 locations (18 January 2023, 17 February 2023, and 6 November 2023 from Covert, Michigan; the rest from Mountain Research Station, North Carolina) ($n = 34$ to 59 per collection per temperature).

places outside the Arctic had mean temperatures ≤–10 °C for long enough (~56 or 102 d) to cause 50% or 90% mortality during the winters of 2011/2012 to 2020/2021. Therefore, we pooled all Kaplan–Meier data for *F. externa* from hemlock and Fraser fir at –20 °C and re-ran a beta regression using a log-link function (405 *F. externa*, 61 Kaplan–Meier nodes). We excluded observations from the 22 February 2024 collection of eastern hemlock in North Carolina because survival was poorer than on any other collection date. We created maps using the software SAFARIS (Takeuchi et al. 2023, Center for Integrated Pest Management 2025) and 10 yr of Daymet version 4 weather data (Thornton et al. 2022). Specifically, we queried for the number of days (consecutively and non-consecutively) with a mean temperature ≤–20 °C for 10 winters (2011/2012 to 2020/2021). In ArcGIS Pro, we queried the maps to visualize where the number of days exceeded estimates for $LTime_{50}$ or $LTime_{90}$ (both rounded up to the next whole number). We then tallied and visualized the number of winters out of 10 that met the conditions across Canada and the continental United States.

Results

Effects of Prolonged Exposure to 3 Temperatures on F. externa Survival

As temperature decreased from 3 to –10 to –20 °C, survival time decreased in every batch (Fig. 1). At 3 °C, the control, *F. externa* experienced >75% survival while being observed, with models typically estimating <40% decline by 8 wk (Fig. 1), such that $LTime_{50}$ and $LTime_{90}$ could not be reliably estimated.

At –10 °C, *F. externa* experienced intermediate decline in survival over time (Fig. 1). The slopes of the survival curves at this temperature generally fell between that of the control and –20 °C but closer to the control (Table 2). Survival of *F. externa* at –10 °C declined at a statistically similar rate to the control in every batch ($|Z|$ = 0.196 to 2.357, $P \geq 0.091$), except the batch of eastern hemlock collected on 22 February 2024 from North Carolina, for which survival of *F. externa* at –10 °C was initially higher (ie greater intercept; $Z = 2.758$, $P = 0.033$) and declined more quickly than the control (ie steeper slope; $Z = 4.318$, $P < 0.001$; Table 2). At –10 °C, the $LTime_{50}$ ranged from 21.6 to 92.2 d, and the $LTime_{90}$ ranged from 45.0 to 195.4 d, depending on the batch (Fig. 2).

At –20 °C, *F. externa* experienced substantial declines in survival over time (Fig. 1). The $LTime_{50}$ ranged from 2.0 to 18.7 d and the $LTime_{90}$ ranged from 3.6 to 36.6 d, depending on the batch (Fig. 2). Survival declined more quickly at –20 °C than at 3 °C ($Z = 3.071$ to 5.834, $P < 0.013$), except for the batch of eastern hemlock collected on 26 November 2023 from North Carolina ($Z = 1.535$, $P = 0.461$; Table 2). Survival also declined more quickly at –20 °C than at –10 °C ($Z = 3.128$ to 5.102, $P < 0.01$), except for insects from the batches of Fraser fir ($Z = 1.357$, $P = 0.486$) and eastern hemlock ($Z = 1.462$, $P = 0.543$) collected on 26 November 2023 from North Carolina (Table 2).

Effects of Collection Location, Host, and Date on F. externa Survival

To assess the effect of location, we compared survival of *F. externa* on eastern hemlock collected on 6 November 2023 from Michigan to *F. externa* on eastern hemlock collected on 26 November 2023 from North Carolina and found that survival curve intercepts and slopes were statistically similar within each temperature treatment ($|Z|$ = 0.557 to 1.243, $P \geq 0.214$; Table 3).

To assess the effect of host, we compared survival curves of *F. externa* from the same temperature treatment, collection date, and location, but different hosts, and found that host rarely affected survival. Of 11 such combinations, all from North Carolina, an effect of host was detected 3 times (Table 3). An effect was detected for *F. externa* collected on (i) 26 November 2023 at 3 °C, when survival on eastern hemlock was initially less ($Z = -4.529$, $P < 0.001$) and declined more quickly than on Fraser fir ($Z = 3.894$, $P < 0.001$), (ii) 22 February 2024 at –10 °C, when survival on eastern hemlock was initially greater ($Z = 4.480$, $P < 0.001$) and declined more quickly than on Fraser fir ($Z = 4.844$, $P < 0.001$), and (iii) 22 February 2024 at –20 °C, when survival on eastern hemlock declined more quickly than on Fraser fir ($Z = 3.112$, $P = 0.025$; Table 3).

To assess the effect of collection date, we considered comparisons between survival curves of *F. externa* from the same temperature treatment, location, and host, but different collection dates, and found that collection date rarely affected survival. Across the 4 collections of Fraser fir from North Carolina (November 2023 and January, February, and March 2024), we did not detect differences in initial survival or decline in survival over time within each temperature treatment ($|Z|$ = 0.006 to 5.097, $P \geq 0.071$; Table 3). In contrast, for the 7 collections of eastern hemlock (3 from Michigan: January, February, and November 2023; 4 from North Carolina: November 2023 and January, February, and March 2024), intercepts and/or slopes of *F. externa* survival curves varied between collection dates within each temperature treatment. These differences (Table 3) are primarily within the 3 and –10 °C treatments and do not follow a temporal pattern. Notably, at –20 °C, survival of *F. externa* on eastern hemlock collected on 22 February 2024 from North Carolina declined significantly more quickly than all other collection dates ($Z = 3.099$ to 3.612, $P = 0.009$ to 0.048; Table 3). All individuals in this batch died by 7 d, the first survival assessment (Fig. 1), making the batch an outlier with a steep survival curve slope. Otherwise, all survival curves of *F. externa* at –20 °C, on either host, had similar intercepts and slopes ($|Z|$ = 0.017 to 3.612, $P \geq 0.055$).

Risk Maps

We pooled data from eastern hemlock and Fraser fir across collection dates, except the 22 February 2024 collection from North Carolina, and re-ran statistical models on these data to inform risk maps. We found that survival, μ, of *F. externa* on Fraser fir at –20 °C through time was best described by a log-link function: $\mu = \exp(b_0 + b_1 x)$, where b_0 = –0.070, b_1 = –0.091, and x is days. From this relationship, we found that the $LTime_{50}$ is 6.8 d and the $LTime_{90}$ is 24.5 d (95% CIs: 0.2 to 27.0, 5.2 to 51.9 d, respectively; Fig. 3).

Based on this model, we displayed the annual likelihood of *F. externa* experiencing temperatures across North America that would cause substantial (≥50 or ≥90%) mortality from prolonged cold exposure, that is, where the average temperature remains ≤–20 °C for at least 7 or 25 d, respectively (Fig. 4). We make the assumption that winter temperatures >–20 °C do not substantially affect mortality of *F. externa*. The ability of *F. externa* to recover from cold injury when it is not in inclement temperatures is unknown; therefore, we created models reflecting 2 scenarios.

Table 2. Coefficients (± SE) of beta regression models describing the change in survival of *Fiorinia externa* over time and significance tests describing the effect of temperature on survival for 11 collection date × host combinations (hemlock = eastern hemlock, *Tsuga canadensis*; fir = Fraser fir, *Abies fraseri*), which were each modeled separately

Treatment (°C)	Intercept (b_0)	Slope (b_1)	*n* (individuals)	*n* (Kaplan–Meier nodes)
MI, 18 January 2023, hemlock (Φ = 9.853 ± 3.493, df = 12)				
3	3.091 ± 0.587a	−0.035 ± 0.023a	48	8
−10	2.010 ± 0.430a	−0.093 ± 0.021a	48	9
MI, 17 February 2023, hemlock (Φ = 18.865 ± 5.242, df = 12)				
3	3.541 ± 0.614a	−0.052 ± 0.027a	46	9
−10	2.878 ± 0.514a	−0.060 ± 0.022a	47	9
−20	3.036 ± 0.481a	−0.167 ± 0.023 b	47	9
MI, 6 November 2023, hemlock (Φ = 13.113 ± 4.768, df = 12)				
3	4.349 ± 0.720a	−0.126 ± 0.047a	36	7
−10	4.026 ± 0.801a	−0.112 ± 0.051a	36	6
−20	3.054 ± 0.589a	−0.349 ± 0.059 b	38	6
NC, 26 November 2023, fir (Φ = 9.306 ± 3.182, df = 12)				
3	2.573 ± 0.557a	−0.041 ± 0.028a	35	6
−10	3.724 ± 0.698a	−0.124 ± 0.029ab	36	6
−20	2.791 ± 0.543a	−0.179 ± 0.031 b	34	7
NC, 26 November 2023, hemlock (Φ = 2.631 ± 0.994, df = 12)				
3	3.441 ± 0.755a	−0.078 ± 0.035a	36	6
−10	3.329 ± 0.703a	−0.083 ± 0.025a	35	6
−20	1.303 ± 0.699a	−0.169 ± 0.051a	38	6
NC, 21 January 2024, fir (Φ = 14.552 ± 4.493, df = 12)				
3	3.995 ± 0.534a	−0.027 ± 0.017a	55	10
−10	3.490 ± 0.469a	−0.078 ± 0.014a	56	10
−20	3.737 ± 0.618a	−0.384 ± 0.059 b	56	6
NC, 21 January 2024, hemlock (Φ = 6.648 ± 1.763, df = 12)				
3	2.389 ± 0.475a	−0.031 ± 0.016a	56	11
−10	2.253 ± 0.471a	−0.029 ± 0.017a	57	10
−20	1.187 ± 0.407a	−0.134 ± 0.022 b	56	9
NC, 22 February 2024, fir (Φ = 8.972 ± 2.664, df = 12)				
3	3.530 ± 0.593a	−0.048 ± 0.016a	56	9
−10	2.664 ± 0.444a	−0.044 ± 0.013a	55	11
−20	2.091 ± 0.586a	−0.351 ± 0.061 b	34	6
NC, 22 February 2024, hemlock (Φ = 10.506 ± 3.836, df = 12)				
3	3.616 ± 0.607a	−0.044 ± 0.016a	48	9
−10	5.542 ± 0.606 b	−0.140 ± 0.018 b	50	11
−20	3.805 ± 1.030ab	−1.887 ± 0.377c	38	2
NC, 19 March 2024, fir (Φ = 6.599 ± 1.886, df = 12)				
3	2.289 ± 0.466a	−0.014 ± 0.013a	57	11
−10	2.537 ± 0.514a	−0.033 ± 0.013a	56	10
−20	1.842 ± 0.591a	−0.321 ± 0.060 b	53	6
NC, 19 March 2024, hemlock (Φ = 5.125 ± 1.768, df = 12)				
−10	1.734 ± 0.460a	−0.019 ± 0.013a	46	11
−20	1.797 ± 0.640a	−0.294 ± 0.064 b	49	6

Within each collection date × host combination, Φ is used to estimate the variance about the expected survival rate; coefficients with different letters are significantly different based on the outcome of 2-sided *Z* tests.
MI = Covert, Michigan; NC = Mountain Research Station, North Carolina.
The expected proportion of *F. externa* alive, $\mu = 1/\left(1+e^{-(b_0+b_1 x)}\right)$, after *x* days.
ϕ is used to calculate the variance: $\mathrm{var}(\mu) = \mu(1-\mu)/(1+\Phi)$.

First, if *F. externa* can recover from cold injury when the average temperature is >−20 °C, then the days with an average temperature ≤−20 °C must be consecutive (Fig. 4A and B). In this scenario, from the winters of 2011/2012 to 2020/2021, most of the contiguous United States did not experience sufficient consecutive days below −20 °C to reach the LTime_{50}. Northern Minnesota, northern Wisconsin, North Dakota, and northeastern Montana reached the LTime_{50} in less than half of the winters (Fig. 4A). More southern areas of Minnesota and Montana, northern Iowa, northern Maine, and northern New York reached the LTime_{50} in just one of 10 winters (Fig. 4A). None of the contiguous United States nor much of Canada experienced enough consecutive dates with a mean temperature below −20 °C to reach the LTime_{90} (Fig. 4B).

Second, if *F. externa* cannot recover from cold injury, then the days with an average temperature ≤−20 °C needed to cause 50% and 90% mortality would not need to be consecutive, and thus higher rates of winter mortality would be expected to occur (Fig. 4C and D). In this scenario, in the winters from 2011/2012 to 2020/2021, the LTime_{50} was reached in most winters in northern Minnesota, North Dakota, and the northeastern corner of Montana, and in less than half of the winters in southern Minnesota, the rest of Montana, South Dakota,

Table 3. Coefficients (± SE) of beta regression models describing the change in survival of *Fiorinia externa* over time and significance tests describing the effect of the collection date × host combination (hemlock = eastern hemlock, *Tsuga canadensis*; fir = Fraser fir, *Abies fraseri*) on survival at 3 temperatures, which were each modeled separately

Location	Collection date	Host	Intercept (b_0)	Slope (b_1)
3 °C (Φ = 23.034 ± 3.829, df = 65)				
MI	18 January 2023	hemlock	3.525 ± 0.484a	−0.041 ± 0.019 b
	17 February 2023	hemlock	3.633 ± 0.581a	−0.054 ± 0.025 b
	6 November 2023	hemlock	4.793 ± 0.639ab	−0.142 ± 0.042ab
NC	26 November 2023	fir	2.889 ± 0.433a	−0.046 ± 0.021 b
		hemlock	6.293 ± 0.628 b	−0.168 ± 0.023a
	21 January 2024	fir	4.355 ± 0.482ab	−0.032 ± 0.016 b
		hemlock	2.850 ± 0.339a	−0.037 ± 0.011 b
	22 February 2024	fir	4.060 ± 0.486ab	−0.055 ± 0.012 b
		hemlock	4.058 ± 0.496ab	−0.050 ± 0.013 b
	19 March 2024	fir	2.766 ± 0.338a	−0.017 ± 0.009 b
−10 °C (Φ = 10.629 ± 1.592, df = 76)				
MI	18 January 2023	hemlock	2.027 ± 0.410a	−0.094 ± 0.020ab
	17 February 2023	hemlock	2.698 ± 0.589ab	−0.056 ± 0.026abc
	6 November 2023	hemlock	3.870 ± 0.770abc	−0.107 ± 0.052abc
NC	26 November 2023	fir	3.804 ± 0.649abc	−0.126 ± 0.028ab
		hemlock	5.090 ± 0.637bc	−0.139 ± 0.026a
	21 January 2024	fir	3.335 ± 0.481ab	−0.075 ± 0.015abc
		hemlock	2.442 ± 0.411a	−0.032 ± 0.015bc
	22 February 2024	fir	2.737 ± 0.409ab	−0.046 ± 0.012bc
		hemlock	5.557 ± 0.500c	−0.141 ± 0.016a
	19 March 2024	fir	2.740 ± 0.448ab	−0.035 ± 0.012bc
		hemlock	1.962 ± 0.360a	−0.021 ± 0.010c
−20 °C (Φ = 3.435 ± 0.626, df = 42)				
MI	17 February 2023	hemlock	2.302 ± 0.719a	−0.123 ± 0.035a
	6 November 2023	hemlock	2.407 ± 0.742a	−0.274 ± 0.071a
NC	26 November 2023	fir	2.237 ± 0.629a	−0.134 ± 0.034a
		hemlock	1.395 ± 0.642a	−0.186 ± 0.046a
	21 January 2024	fir	2.759 ± 0.749a	−0.291 ± 0.071ab
		hemlock	0.890 ± 0.490a	−0.095 ± 0.023a
	22 February 2024	fir	1.770 ± 0.736a	−0.271 ± 0.067a
		hemlock	2.898 ± 1.039a	−1.436 ± 0.371 b
	19 March 2024	fir	1.607 ± 0.689a	−0.261 ± 0.063a
		hemlock	1.649 ± 0.690a	−0.259 ± 0.063a

Within each temperature, coefficients with different letters are significantly different based on the outcome of 2-sided *Z* tests.
MI = Covert, Michigan; NC = Mountain Research Station, North Carolina.
The expected proportion of *F. externa* alive, μ =1/(1+e-(b0+b1x)), after x days.
ϕ is used to calculate the variance: var(μ) = μ(1 - μ)/(1 + Φ).

Wisconsin, the Upper Peninsula of Michigan, and the northern edge of New England (Fig. 4C). The $LTime_{90}$ was reached in less than half of the winters in northern Minnesota and northern North Dakota and in one of 10 winters in northern Wisconsin (Fig. 4D).

Discussion

Our results demonstrate the capacity of *F. externa* to survive prolonged exposure to winter temperatures that are not immediately lethal. For *F. externa* collected from eastern hemlock and Fraser fir, the estimated time to achieve 50% or 90% mortality at −10 °C exceeded the length of winter in most of North America (Fig. 2), suggesting that winter temperatures ≥−10 °C typically cause little mortality of this insect. In contrast, at −20 °C, 50% mortality occurred after 6.8 d and 90% mortality after 24.5 d (Fig. 3). One individual on eastern hemlock, an outlier, survived over 51 d (our maximum) at −20 °C. Conversely, for one collection (eastern hemlock from North Carolina in February 2024), *F. externa* died rapidly (>90% mortality in 7 d) at −20 °C (Fig. 1). We excluded these latter data from our composite characterization of survival at −20 °C, so our final model may slightly underestimate mortality rates.

Our results suggest that prolonged cold exposure alone will not prevent the spread of *F. externa* in North America. In recent winters, some (ie >50% but less than 90%) mortality from prolonged exposure to temperatures <−20 °C may have occurred near the northern limit to the range of eastern hemlock (Fig. 4). Such mortality levels might slow population growth and the onset of plant symptoms in the Upper Midwest, southern Ontario, and northern New England but would be unlikely to stop the spread of *F. externa*. Furthermore, prolonged cold exposure alone would not necessarily prevent *F. externa* from colonizing wild populations of other hosts like mountain hemlock, *Tsuga mertensiana* (Bong.) Carr.; western hemlock, *Tsuga heterophylla* (Raf.) Sarg.; cascade fir, *Abies amabilis* Douglas ex Forbes; Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco; white fir, *Abies concolor* (Gord. & Glend.)

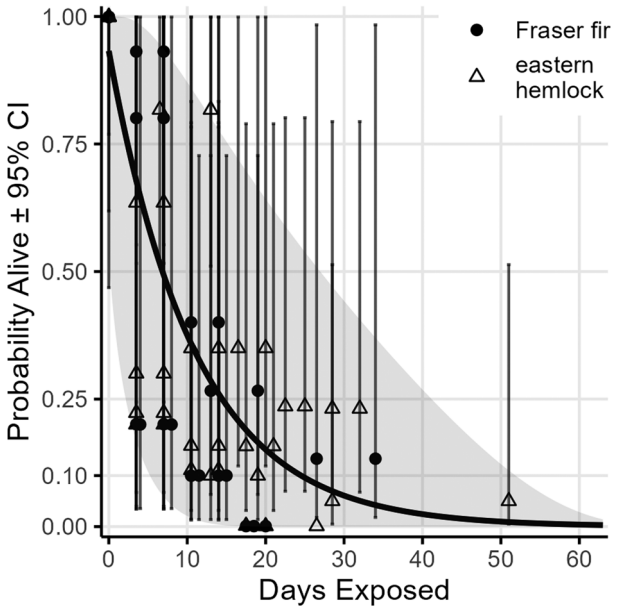

Fig. 3. Survival ± SE of *Fiorinia externa* over time at −20 °C. Fraser fir was collected from North Carolina and hemlock was collected from Michigan and North Carolina ($n = 405$). The thick line indicates fitted estimates ± 95% confidence intervals of a beta regression model: log(y) = −0.070 −0.091(number of days).

Hildebr.; or Colorado blue spruce, *Picea pungens* Engelm (Fig. 5). However, the capacity of these hosts to support damaging densities of *F. externa* without eastern hemlock nearby remains unknown (Venette et al. 2024).

The ability of overwintering *F. externa* females to routinely survive several weeks at −20 °C was surprising because *F. externa* coevolved with native hosts that are less cold hardy than eastern hemlock. Southern Japanese hemlock, *T. sieboldii* Carr., is hardy to USDA Plant Hardiness Zone 6 (with an average annual low of −23.3 to −17.8 °C), and northern Japanese hemlock, *T. diversifolia* (Maxim.) Masters, is hardy to Zone 5 (−28.9 to −23.3 °C; Bannister and Neuner 2001). Eastern hemlock is hardy to Zone 3 (−40 to −34.4 °C). Traditionally, the presumption has been that an herbivore would not evolve stress tolerances that would allow it to spread beyond the geographic range limits of its hosts. Consistent with that notion, we project the likelihood of mortality from prolonged cold exposure to increase dramatically at (or near) the northern range limit for eastern hemlock. However, had cold tolerances for *F. externa* been set by its native hosts, projections of extensive mortality from prolonged cold exposure likely would have extended farther south in North America.

Geographic origin had little effect on the capacity of *F. externa* to survive prolonged cold exposure, after adjusting for collection date, contrary to the findings of Just and Frank (2020) for gloomy scale, *Melanaspis tenebricosa* (Comstock),

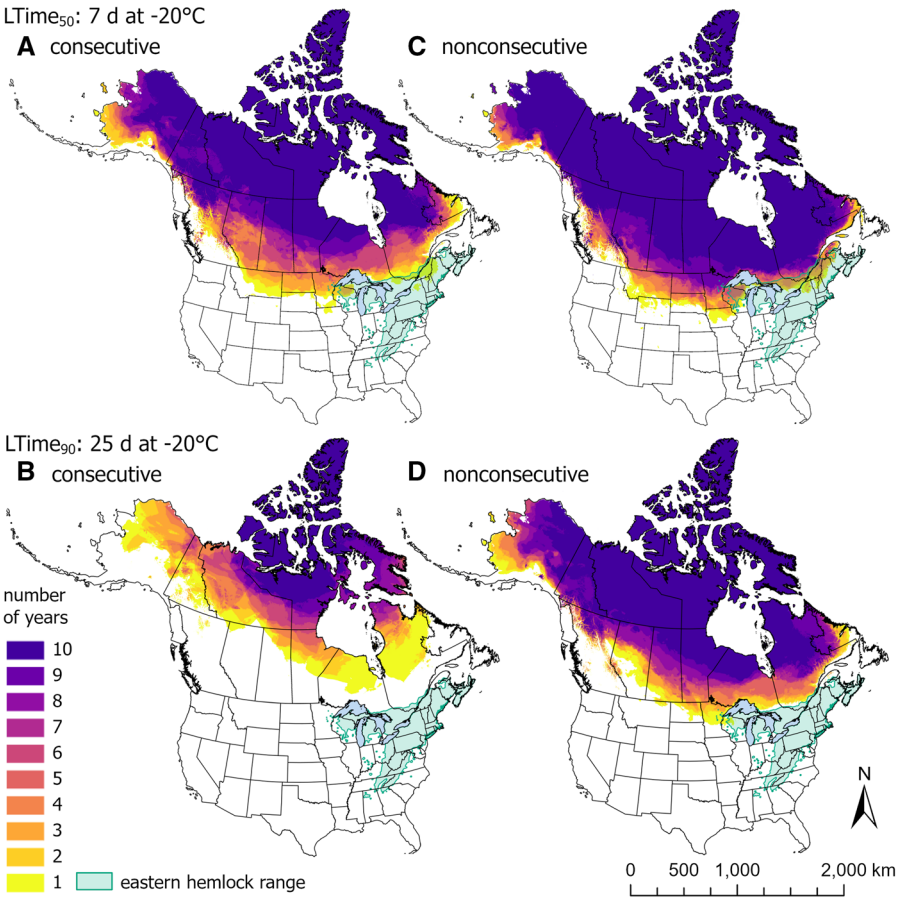

Fig. 4. Maps of *Fiorinia externa* overwintering mortality risk in the USA and Canada, quantified as the number of winters in which the respective lower lethal time at −20 °C was reached out of 10 winters (2011/2012 to 2020/2021) in the scenario that days below −20 °C must be consecutive (A, C) or need not be consecutive (B, D) to be lethal for 50% ($LTime_{50}$; A, B) or 90% of the population ($LTime_{90}$; C, D). Range of eastern hemlock, *Tsuga canadensis* (L.) Carr. is overlaid.

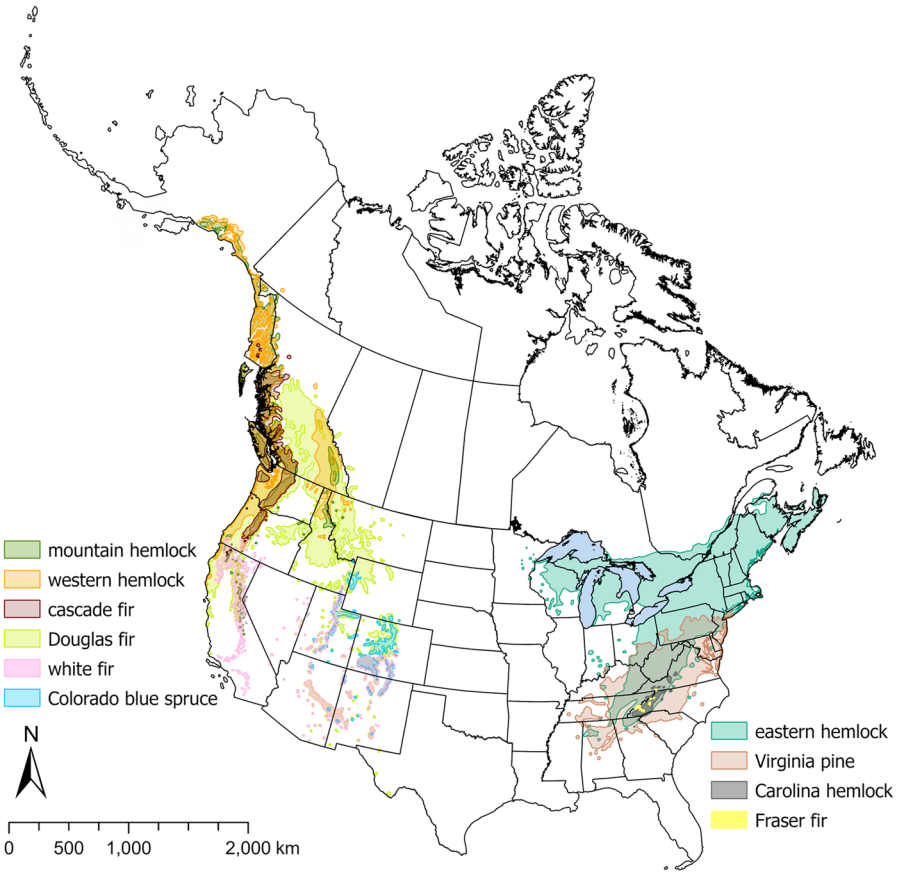

Fig. 5. Distributions of some quality hosts for *Fiorinia externa*. Quality hosts support multiple generations of *F. externa* at densities >40% of levels observed on the primary host, eastern hemlock (based on literature reviewed in Venette et al. 2024): mountain hemlock, *Tsuga mertensiana* (Bong.) Carr.; western hemlock, *Tsuga heterophylla* (Raf.) Sarg.; cascade fir, *Abies amabilis* Douglas ex Forbes; Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco; white fir, *Abies concolor* (Gord. & Glend.) Hildebr.; Colorado blue spruce, *Picea pungens* Engelm.; eastern hemlock, *Tsuga canadensis* (L.) Carr.; Virginia pine, *Pinus virginiana* Mill.; Carolina hemlock, *Tsuga caroliniana* Engelm; and Fraser fir, *Abies fraseri* (Pursh) Poir.

or Preisser et al. (2008a) for *F. externa*. Both studies suggested that individuals from northern populations could survive cold exposure longer than from southern locations. We conjecture that the lack of a location effect in our study may have been because our sites had similarly severe winters in 2022 and 2023 despite their latitudinal difference. Our use of biochemical methods to determine viability complicates comparisons between studies.

The results of this project did not support other hypotheses. We did not detect any effect of host on the survival of *F. externa*. Although *F. externa* collected on 22 February 2024 survived longer on Fraser fir than on eastern hemlock at –10 and –20 °C, survival curves were similar between hosts in all other cases. Host species have affected the acute cold responses of other insects (eg Liu et al. 2007, Morey et al. 2016), including the pine armored scale, *Hemiberlesia pityophila* (Zhong et al. 2009), but host effects on insect survival under prolonged cold conditions remain understudied.

We did not find a pattern of acclimatization (ie increased cold tolerance indicated by longer survival times) from fall to winter nor de-acclimatization from winter to spring. Although the survival rate at –10 °C varied significantly between collection dates, it did not vary in a seasonal or interannual pattern (Table 2). Survival at –20 °C was generally consistent between collection dates (Figs 1 and 2). Notably, the collection of *F. externa* on eastern hemlock from 22 February 2024 is the only case where survival at –10 °C declined significantly more quickly than at 3 °C (Table 1). All individuals from this collection also died within 7 d at –20 °C (Fig. 1). It is unclear why this batch was less cold tolerant than all others, as survival of *F. externa* at –10 and –20 °C the following month was similar to earlier collections. Other insect species show a clear pattern of seasonal (de-) acclimatization in acute cold exposure responses (eg Crosthwaite et al. 2011, Cira et al. 2016, Hefty et al. 2017, Chandler et al. 2020, Vétek et al. 2020, Hafker et al. 2024), including the hemlock wooly adelgid (Elkinton et al. 2017). Seasonal changes in long-term cold exposure responses are less often studied. Gloomy scale exposed to prolonged cold survived for a shorter or similar duration in December as in March (Just and Frank 2020), a result similar to ours.

BVT provided an efficient and scalable method to determine the viability of *F. externa*, and its ability to assess mortality relatively quickly after cold treatment helped isolate the effect of cold on mortality from other causes such as desiccation. BVT had previously been used to develop cold-storage quarantine treatments for white peach scale, *Pseudaulacaspis pentagona* (Stannard et al. 2019) among other applications. Individuals that we judged to be alive (positive controls) consistently had greater ATP concentrations than individuals that were known to be dead (frozen at –80 °C) but only after 24 h at room temperature to allow ATP in dead individuals to degrade. However,

we observed somewhat healthy-looking *F. externa* females (ie hydrated and well-affixed to the conifer needle) that the BVT deemed nonviable, especially after removal from −20 °C. We also noted individuals that looked unhealthy (ie were easily removed from the needle or had dried eggs) that were deemed viable. Therefore, BVT likely improved the consistency and accuracy of survivorship estimates over visual assessment alone. Whilst most colorimetric changes were unambiguous, some intermediate cases existed. Future BVT testing with comparisons to other measures of viability (eg respirometry or fitness) could provide additional insights about the condition of such individuals (ie convalescent or terminal).

Our use of a combination of Kaplan–Meier analysis and beta regression was novel and could be applied to future research. Cold tolerance data are often censored (ie mortality is not recorded at the exact time it occurred) and this is generally not accounted for. Our data analysis method accounts for censored data while producing more robust predictive models than Kaplan–Meier alone would allow.

Fiorinia externa currently does not occur throughout the geographic range of its primary host, eastern hemlock. It is absent in Minnesota, Wisconsin, northern Maine, and southern Ontario. This pattern suggests that historical temperatures could have slowed the northern movement of *F. externa*. However, the limited dispersal capacity of *F. externa* (McClure 1977) is a likely alternative explanation for its absence in northern eastern hemlock stands. This pest has also established on Fraser fir on Christmas tree farms in the southern Appalachians, becoming a problematic pest (Dale et al. 2020) and a concern for the Christmas-tree industry. Shipping infested, cut host material could lead to detrimental infestations of Fraser fir, other firs (*Abies* spp.) or spruces (*Picea* spp.) growing on Christmas tree farms (Manz et al. 2025). Natural stands of eastern hemlock or other hosts could also be affected. Traditionally cold regions in the United States or Canada cannot rely on their winter weather for biosecurity from this insect. Therefore, inspecting holiday greenery shipments pre- and post-transport could limit opportunities to inadvertently disperse the insect. Preliminary results from this project were used to justify continued Christmas tree inspections in Minnesota (Angie Ambourn, personal communication).

Our models did not account for microclimate effects that would be present in the field. For example, *F. externa* could experience insulation from surrounding foliage or snow. The majority of *F. externa* settle at a height of 0 to 4 m, so some individuals may overwinter below the snow line, and thus stay insulated at 0 °C and have a better chance at survival (McClure 1977). These factors, along with other potential factors like tree health and parasitism, were not quantified and have unknown implications for the accuracy of our model.

Trends for warmer winters are moving USDA Plant Hardiness Zones northward and increasing the area in North America where *F. externa* might overwinter (IPCC 2023). Perhaps ironically, climate change is causing an increase in the frequency and intensity of cold snaps (IPCC 2023). This study does not account for mortality in *F. externa* that might arise from cold snaps, in this case, brief exposure to temperatures ≤ −26 °C that are capable of causing ≥90% mortality (unpublished data). Such temperatures are common in USDA Plant Hardiness Zones 1a to 5a. Mortality of *F. externa* in portions of the upper Midwest, southern Ontario, and northern New England from acute exposure to extreme low temperatures might be more extensive than our maps (Fig. 4) suggest.

This study provides insights into a unique aspect of insect cold tolerance. Lower lethal time is less frequently studied than other cold tolerance metrics like lower lethal temperature or supercooling points. Nevertheless, intensity and duration of cold exposure interact to determine survival rates (eg Morey et al. 2012, Delisle et al. 2013). The lethal times estimated in this study could be a key component of more advanced modelling of *F. externa* establishment risk in the future, as specific knowledge of population responses to cold can improve the predictive success of species distribution models (Venette et al. 2010, Teets et al. 2023).

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Author Contributions

Clarissa Moore (Data curation [lead], Formal analysis [lead], Investigation [equal], Methodology [equal], Project administration [equal], Visualization [equal], Writing—original draft [lead], Writing—review & editing [equal]), Brian H. Aukema (Conceptualization [equal], Formal analysis [supporting], Funding Acquisition [equal], Investigation [supporting], Project administration [equal], Resources [supporting], Supervision [equal], Writing—review & editing [equal]), Julia B. Leone (Data curation [equal], Formal analysis [supporting], Investigation [equal], Methodology [equal], Project administration [equal], Writing—review & editing [equal]), Toby R. Petrice (Investigation [equal], Resources [equal], Writing—review & editing [equal]), Robert M. Jetton (Investigation [equal], Resources [equal], Writing—review & editing [equal]), Angie Ambourn (Conceptualization [equal], Investigation [equal], Funding Acquisition [equal], Writing—review & editing [equal]), and Robert C. Venette (Conceptualization [equal], Formal analysis [supporting], Funding Acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Writing—oringal draft [supporting], Writing—review & editing [lead])

Supplementary Material

Supplementary material is available at *Environmental Entomology* online.

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Conflicts of Interest

The authors declare no conflicts of interest.

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