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Physiological Ecology

Thermal Tolerance of Gloomy Scale (Hemiptera: Diaspididae) in the Eastern United States

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Abstract

An insect species' geographic distribution is probably delimited in part by physiological tolerances of environmental temperatures. Gloomy scale (Melanaspis tenebricosa (Comstock)) is a native insect herbivore in eastern U.S. forests. In eastern U.S. cities, where temperatures are warmer than nearby natural areas, M. tenebricosa is a primary pest of red maple (Acer rubrum L.; Sapindales: Sapindaceae) With warming, M. tenebricosa may spread to new cities or become pestilent in forests. To better understand current and future M. tenebricosa distribution boundaries, we examined M. tenebricosa thermal tolerance under laboratory conditions. We selected five hot and five cold experimental temperatures representative of locations in the known M. tenebricosa distribution. We built models to predict scale mortality based on duration of exposure to warm or cold experimental temperatures. We then used these models to estimate upper and lower lethal durations, i.e., temperature exposure durations that result in 50% mortality. We tested the thermal tolerance for M. tenebricosa populations from northern, mid, and southern locations of the species' known distribution. Scales were more heat and cold tolerant of temperatures representative of the midlatitudes of their distribution where their densities are the greatest. Moreover, the scale population from the northern distribution boundary could tolerate cold temperatures from the northern boundary for twice as long as the population collected near the southern boundary. Our results suggest that as the climate warms the M. tenebricosa distribution may expand poleward, but experience a contraction at its southern boundary.

Key words: *Acer rubrum*, cold tolerance, lethal duration, *Melanaspis tenebricosa*, range limits

Climate, particularly temperature, is a major factor governing the geographic distribution of arthropods (Andrewartha and Birch 1954). Local minimum, mean, and maximum temperatures affect arthropods' fitness and survival and, thus, their sustained presence in any given location. A species' physiological capacity to withstand local temperatures is a robust predictor of current and future distributions (Robinet and Roques 2010, Rezende et al. 2014, Bebber 2015). So, if the temperature is too warm or too cold, a species' geographic distribution could be constrained by thermal tolerances. For example, the southern pine beetle (*Dendroctonus frontalis* Zimmermann; Coleoptera: Curculionidae) suffers significant mortality when exposed to winter temperatures below –12°C (Ungerer et al. 1999). Consequently, the distribution of established populations of *D. frontalis* was restricted to latitudes below 35.4°N until the early 2000s, when warmer winters and a reduced frequency of cold temperature extremes contributed to greater overwinter survivorship (Dodds et al. 2018). Subsequently, *D. frontalis* has since expanded poleward and has established populations above 35.4°N (Lesk et al. 2017). Contrastingly, the distributions of some arthropod species contract under warmer temperatures due to reduced insect

survival (e.g., Thomas et al. 2006, Oliveira et al. 2017). For example, bumble bee species (*Bombus* spp.; Hymenoptera: Apidae) have among the lowest heat tolerances of bee species tested along an urban thermal gradient (Hamblin et al. 2017) and their distributions are contracting due to climate warming (Kerr et al. 2015). Thus, physiological thermal tolerances are an important trait for describing or predicting species' responses to climate and climate change (Youngsteadt et al. 2017, Diamond et al. 2018). However, physiological thermal tolerances are species specific, and most are not known. Determining a species' thermal tolerance will enhance our understanding of how climate delimits the species' distribution (Sunday et al. 2011, Hoffmann et al. 2013), and this will inform predictions of range expansion or contraction for both pests and species of conservation concern (Musolin 2007, Lancaster 2016).

Insects are ectothermic and generally benefit from warmer temperatures through a number of potential mechanisms that may increase their survival or range, including thermal adaptation (Preisser et al. 2008, Janes et al. 2014), increased voltinism (Janes et al. 2014), and increased fecundity (Dale and Frank 2014a), provided that the magnitude and duration of a temperature do not exceed their

thermal tolerances (Musolin 2007, Deutsch et al. 2008, Dukes et al. 2009). When temperature conditions exceed these tolerances, fitness declines and death may occur (Addo-Bediako et al. 2000, Deutsch et al. 2008). There are a number of methods to evaluate the physiological response of insects to temperature and derive estimates of thermal tolerance (Lutterschmidt and Hutchison 1997, Terblanche et al. 2007), including critical thermal duration, which we use in this study. Critical thermal duration is a duration of exposure to a given temperature at which a population can be expected to reach a mortality threshold, e.g., 50% (Hazell et al. 2010a). Because thermal tolerances can be estimated from relatively simple measurements and serve as a good predictor of distributional limits for a number of species, they are a useful metric for quantifying a species' sensitivity to different temperatures (Terblanche et al. 2007, Calosi et al. 2010).

Gloomy scale (*Melanaspis tenebricosa* (Comstock)) is a native insect herbivore in forests of the eastern United States, where its primary hosts are soft maples, including red maple (*Acer rubrum* L.; Sapindales: Sapindaceae) and silver maple (*A. saccharinum* Marshall); (Metcalf 1922). *Melanaspis tenebricosa* occurs at low densities in forests but has been a serious pest of some ornamental landscape tree species, especially soft maples, for over a century (Metcalf 1912, Youngsteadt et al. 2015, Long et al. 2019). Research in urban landscapes, where temperatures are warmer than nearby natural landscapes, has revealed that *M. tenebricosa* has positive responses to warming—as is the case with many other arthropods (Robinet and Roques 2010, Chown and Duffy 2015)—including increased fecundity and survival (Dale and Frank 2014b, 2017). In addition, *M. tenebricosa* is primarily found in the southeastern United States despite the latitudinally widespread distribution of its preferred hosts. Taken together, these observations suggest that *M. tenebricosa* thermal tolerances may limit its distribution, as its peak densities occur in the midlatitudes of its known distribution. This suggests that cold tolerance limits the species distribution at the northern edge of its range and that as the climate warms, the *M. tenebricosa* distribution could expand poleward. Likewise, decreased *M. tenebricosa* densities at lower latitudes suggest the species' distribution may also be heat limited, which can similarly be predicted by its heat tolerance. Thus, the *M. tenebricosa* distribution could expand poleward or forest population densities could increase with warming (Youngsteadt et al. 2015, Long et al. 2019). However, for many native insect herbivores, including *M. tenebricosa*, neither their actual current distribution nor the factors that delimit this distribution (e.g., thermal tolerances) are well documented.

Our goals were to assess the physiological thermal tolerances—upper and lower critical thermal durations—of *M. tenebricosa*. We predicted longer cold tolerance at higher latitudes and longer heat tolerances at lower latitudes of the known *M. tenebricosa* geographic distribution in the eastern United States. To address these goals, we performed laboratory thermal tolerance experiments to expose *M. tenebricosa* to warm and cold temperatures similar to what they might experience at the northern, middle, and southern parts of their range, examining scale mortality at multiple durations per temperature. Given that insects, including scales, can adapt to environmental conditions through genetic or plastic responses (Hanks and Denno 1993, Preisser et al. 2008, Meineke et al. 2014), we predicted that *M. tenebricosa* thermal tolerance would vary among scales collected from different latitudes, such that populations nearer to their northern distribution boundary would be more tolerant of colder temperatures and those nearer the southern boundary would be more tolerant of warmer temperatures. We also measured in situ overwintering survival of *M. tenebricosa* at northern, mid, and southern locations within their geographic distribution collected throughout winter. Because *M. tenebricosa* densities are the greatest in the midlatitudes of their distribution (Metcalf 1922, Just et al. 2019), we predicted that survival would be highest at temperatures from these latitudes. Finally, because scale insect thermal tolerances can vary by season (e.g., Zhang et al. 2010, Wang et al. 2019a), we predicted that cold tolerance would decline in late winter when conditions become milder.

Materials and Methods

Study System

Melanaspis tenebricosa is an armored scale insect native to the eastern United States. It is a primary insect pest of *Acer* spp., particularly *A. rubrum* (Frank 2019). Tree species in the genus *Acer* are among the most common forest and ornamental trees in the eastern United States (Abrams 1998). Within *Acer*, *A. rubrum* are the most commonly planted (Raupp et al. 2006) and are one of the most abundant trees in eastern North American forests, with a continuous geographic distribution that is over 2,500 km long (Burns and Honkala 1990). Importantly, *A. rubrum* is widespread throughout (and beyond) the entire geographic extent of our study, allowing us to focus on thermal tolerance, and not host availability, as the potential factor delimiting the distribution of *M. tenebricosa*. *Melanaspis tenebricosa* feed on the parenchyma cells of their hosts with specialized mouthparts, and heavy infestations reduce tree vigor and may lead to premature death (Frank et al. 2013, Backe and Frank 2019). *Melanaspis tenebricosa* are univoltine and ovoviviparous and nymphs generally begin to emerge in mid to late spring and quickly settle on the bark of the host's trunk or branches; they become sessile after settling and then produce a waxy, protective covering (i.e., a test; Metcalf 1922). Approximately 5 mo after emergence, nymphs become adults and then mate and produce eggs in the autumn. *Melanaspis tenebricosa* overwinter as adult females; thus, their density and distribution are a product of reproduction and survival, not the number of generations completed per year.

Insect Sources

We collected overwintering *M. tenebricosa* adults from infested *A. rubrum* twigs in three eastern U.S. cities: Newark, DE; Raleigh, NC; and Gainesville, FL (Table 1; Supp Fig. A1 [online only]). We collected scales in Raleigh on three dates in winter 2018 (19 and 24 February and 24 March 2018) and five dates in winter 2018–2019 (3 December; 14, 17, and 28 January, and 27 February 2019). We refer to the experiments associated with these dates of collection as *Raleigh 2018* and *Raleigh 2019*, respectively (Table 2). In winter 2018–2019, we collected scales in Newark and Gainesville on two dates (5 December 2018 and 28 January 2019) to match two dates from the *Raleigh 2019* collections. Experiments and analyses associated with these collections from Newark, Gainesville, and Raleigh are referred to as *Three-city 2019*. These collection cities largely encompass the latitudinal extent of the known distribution of *M. tenebricosa* (Metcalf 1922), with Newark, Raleigh, and Gainesville located at the approximate northern, mid, and southern distribution extents, respectively. We collected in Raleigh more often because of greater *M. tenebricosa* densities at this latitude (Just et al. 2019) and practical considerations, such as proximity to the authors. In each city, we identified three sites with infested *A. rubrum* (*n* = 2–4 trees per site). At each site, we collected a total of ten twigs using pole pruners. For each twig, we retained the most distal 15 cm. Cut twigs were placed in plastic zip-top bags and stored in a cooler with ice

Table 1. Study city information

City	Latitude (°)	Winter temperature (°C)			Summer temperature (°C)			Overwinter survival (%)	
		Normals	2018	2019	Normals	2018	2019	2018	2019
Newark	39.68	−3.3	−3.6	−2.7	29.4	28.9	29.3	23.0 ± 7.7	61.0 ± 3.7
Raleigh	35.78	−0.3	0.2	1.6	30.9	31.0	31.3	92.5 ± 0.1	64.2 ± 5.5
Gainesville	29.65	6.8	8.5	9.6	32.5	32.8	32.3	5.5 ± 3.1	42.5 ± 2.9

Latitude (°) is for the city center. Winter temperature (°C) is the mean minimum temperature of December, January, and February. Summer temperature (°C) is the mean maximum temperature of June, July, and August. Temperatures presented are 30-yr normals for each city center (Di Luzio et al. 2008) and means for 2018 and 2019. *Melanaspis tenebricosa* background overwintering survival is the mean proportion of alive to dead scales in 2018 and 2019.

Table 2. Thermal tolerance experiments

City	Collection period	Experimental group	Heat/cold	Temperature (°C)	Start date
Raleigh	Feb.–Apr. 2018	*Raleigh 2018*	Cold	−22.8	21 Feb. 2018
				−12.9	26 Feb. 2018
				−1.0	26 Mar. 2018
			Heat	32.2	26 Mar. 2018
				37.8	21 Feb. 2018
				40.6	26 Feb. 2018
Raleigh	Dec. 2018–Mar. 2019	*Raleigh 2019*	Cold	−4.7	5 Dec. 2018, 30 Jan. 2019, 1 Mar. 2019
				−15.2	16 Jan. 2019
				−12.9	19 Jan. 2019
			Heat	32.8	5 Dec. 2018, 30 Jan. 2019, 1 Mar. 2019
				37.8	30 Jan. 2019
				39	16 Jan. 2019
Newark, Raleigh, Gainesville	Dec. 2018–Mar. 2019	*Three-city 2019*	Cold	−4.7	5 Dec. 2018, 1 Mar. 2019
			Heat	32.8	5 Dec. 2018, 1 Mar. 2019

Scale collection location (City), time period when scales were collected (Collection period), experimental grouping of sales based on collection period (Experimental group), heat or cold thermal tolerance experiment (Heat/cold), treatment temperature (Temperature), and start date of experiment (Start date) for *M. tenebricosa* thermal tolerance experiments. *Raleigh 2019* and Raleigh entries in *Three-city 2019* for experiments starting on 5 Dec. 2018 and 1 Mar. 2019 are referring to the same experiments.

packs for transport to our laboratory. Sites were separated by at least 1 km. Collections were made from publicly owned *A. rubrum* with no history of pest control.

Thermal Tolerance Experiments

We conducted a total of six thermal tolerance experiments (three heat tolerance, three cold tolerance) in winter 2018 and 10 experiments (five heat tolerance, five cold tolerance) in winter 2018–2019. For each experiment, we exposed *M. tenebricosa* to five experimental durations (0 [control], 6, 18, 30, and 42 h) at the hot or cold temperature of interest (detailed below). Each duration–temperature pairing was a treatment. Sites within a city were replicates. After each collection, twigs were returned to the laboratory and held for 2 d at 9°C to standardize the experimental starting temperature before starting thermal tolerance experiments. Scales were kept under a 10-h day length photoperiod for the duration of our experiments.

Although it may seem counterintuitive to assay overwintering adults for heat tolerance, we believe that there is merit in doing so. First, adult *M. tenebricosa* were first nymphs who developed under warmer conditions. So, when we assayed them for heat tolerance as adults, the experimental temperatures were similar to those they were exposed to during their development. Moreover, thermal history (environmental temperatures at different life stages) has been

shown to affect thermal tolerance and fitness for some insect species (e.g., Bowler and Terblanche 2008, Kellermann et al. 2017). For example, Kellermann et al. (2017) report that thermal conditions during development were more important for determining CT_{max} in adult *Drosophila melanogaster* than thermal acclimation as adults. Second, thermal tolerance can vary seasonally, and acclimation to a cold temperature does not necessarily result in reduced heat tolerance. For example, Terblanche et al. (2005) found that the CT_{max} was greater for beetles acclimated at a cooler temperature than a warmer one. A study of two aphid species found mixed results with regards to acclimation temperature and CT_{max} (Hazell et al. 2010b). Although these studies used smaller temperature differentials, they still highlight a need to understand thermal tolerances with varying parameters. Finally, this is the first assessment of *M. tenebricosa* thermal tolerance, and although these results may not predict the response of the same adults if they were collected in summer, we think there is value in testing individuals collected at different times of the year to provide information that can help guide future work.

Heat Tolerance

For *Raleigh 2018*, we assayed *M. tenebricosa* heat tolerance at the five experimental exposure durations for each of three temperatures

(32.2, 37.8, and 40.6°C). We collected 10 scale-infested twigs from each Raleigh site (for a total of 30 twigs). The first *Raleigh 2018* experiment was conducted at 37.8°C, the record high July temperature for Gainesville, and began on 21 February 2018. The second experiment started on 26 February and was conducted at 40.6°C, the record high temperature in Raleigh. The third experiment was conducted at 32.2°C, the mean high July temperature for Raleigh, and started on 26 March. Record temperature values are from NOAA (https://www.ncdc.noaa.gov/cdo-web/), and mean temperature values are 30-yr normals (1980–2010; Arguez et al. 2010).

To begin each experiment, we placed 24 of the 30 twigs into a climate-controlled chamber (DT2-MP-47L; Tritech Research, Inc. Los Angeles, CA) set at the 9°C holding temperature. Thermal tolerance estimates are considered more reliable when the assay uses temperature ramping instead of abrupt transitions (Terblanche et al. 2011, Diamond et al. 2017), so we increased the temperature from 9°C to the experimental temperature over a period of 6 h. At the end of each experimental duration greater than 0 h (i.e., 6, 18, 30, or 42 h after the chamber reached the experimental temperature), we removed six twigs and held them at 9°C for 7 d. This holding period was included to allow for sufficient time for *M. tenebricosa* that did not survive the experiment to discolor and desiccate (Preisser et al. 2008, Zhang et al. 2010). Control twigs (*n* = 6) remained in the holding chamber at 9°C for the duration of the experiment. To determine scale mortality, we used a dissecting microscope and removed the test from each scale and recorded each individual as alive or recently dead based on discoloration and desiccation (Ishaaya and Swirski 1970, 1971; Zhang et al. 2010) and summed the number of alive and dead scales per treatment per site per experiment.

For *Raleigh 2019* heat tolerance experiments, we repeated the procedures used for *Raleigh 2018* but raised the minimum (from 32.2 to 32.8°C) and lowered the maximum (from 40.6 to 39.0°C) temperatures to better reflect temperatures that scales were likely to encounter (Table 2). On 5 December 2018, and 19 January and 1 March 2019, we assayed the heat tolerance of *M. tenebricosa* at 32.8°C (the mean maximum July temperature in Gainesville). These repeated experiments at 32.8°C were conducted to assess the repeatability of the experiment and to compare seasonal variation in heat tolerance. We also conducted experiments at 39.0°C on 16 January and at 37.8°C on 30 January for *Raleigh 2019* scales. For *Three-city 2019* heat tolerance experiments, we collected ten twigs per site during each collection. Twigs from Newark and Gainesville were shipped overnight with cool packs to Raleigh and then held 1 d at 9°C in a growth chamber for a total of 2 d between collection and the start of an experiment; likewise, Raleigh twigs were held for 2 d at 9°C. For each experiment, we randomly assigned two twigs from each site to a treatment. The *Three-city 2019* experiments were conducted at 32.8°C on 5 December 2018 and 1 March 2019. At the end of each experiment, including the 7-d holding period, we recorded the number of alive and dead scales for up to 50 *M. tenebricosa* individuals per twig and then summed per treatment per site for each experiment. We limited the number of scales we examined to 50 for practical considerations and scales were selected haphazardly for examination.

Cold Tolerance

Our procedures for *Raleigh 2018* cold tolerance experiments were the same as those used in the heat tolerance experiments, except as noted below. We started the first *Raleigh 2018* cold tolerance experiment on 21 February 2018 and it was conducted at –22.8°C, the lowest recorded temperature in Raleigh. The second experiment started on February 26 and was conducted at –12.9°C, which was

chosen for its intermediate value between the temperatures used in the coldest and warmest cold tolerance experiments. The third experiment was conducted at –1.0°C, the mean minimum January temperature in Raleigh, and started on 26 March. For cold tolerance experiments, scales were placed in a climate chamber (Holiday Brand, 142 l Chest Freezer, Lowe's Companies, Inc. North Greensboro, NC) controlled by a CR1000 data logger with type-T thermocouples (Campbell Scientific, Logan, UT). Temperature control and ramping rates were controlled by an author-written program for the data logger. Experiments began at the 9°C holding temperature and decreased linearly until the target assay temperature was reached over the course of 6 h.

Raleigh 2019 cold tolerance experiments followed similar procedures to those conducted in *Raleigh 2018* except we raised the minimum temperature from –22.8 to –15.2°C to capture scale responses to cold temperatures that they were more likely to encounter (Table 2) and lowered the maximum temperatures from –1.0 to –4.7°C, the mean minimum temperature in Newark, because –1.0°C did not seem likely to be a lethal temperature based on our *Raleigh 2018* results (Table 3). We tested cold tolerance at –4.7°C on 5 December 2018, and 19 January and 1 March 2019, to evaluate our predictions about seasonal changes in cold tolerance. We also conducted experiments on *Raleigh 2019* scales at –15.2°C on January 16 and at –12.9°C on January 30. The *Three-city 2019* experiments were conducted at –4.7°C and began on 5 December 2018 and 1 March 2019.

Overwinter Survival

We examined in situ *M. tenebricosa* overwinter survival on multiple dates throughout winter for *Raleigh 2018* and *Raleigh 2019* scales, and at different latitudes for *Three-city 2019* scales. We defined in situ overwinter survival as the ratio of living scales to those that had recently died. For these estimates, we examined the scales from our control twigs from the previously described experiments. We also compared in situ overwinter survival between the three cities in 2018 (*Three-city 2018*), here we collected scales, as described above, in Newark and Gainesville once at the end of winter (26 March 2018). We summed alive and dead scales per site by collection date and then calculated overwinter survival per date (*Raleigh 2018* and *Raleigh 2019*) or per city (*Three-city 2018* and *Three-city 2019* [mean survival of the two collection dates]).

Table 3. Melanaspis tenebricosa thermal tolerance for Raleigh 2018 scales

Heat/cold	Temperature (°C)	LD_{50}	SE	LCL	UCL
Heat	40.6	7.36	1.14	5.66	9.57
	37.8	12.41	1.13	9.85	15.63
	32.2	27.69	1.09	23.34	32.85
Cold	–22.8	4.24	1.11	3.44	5.23
	–12.9	20.85	1.07	18.34	23.71
	–1.0	755.39	2.57	118.41	4,819.13

Upper and lower lethal duration 50% (ULD_{50} and LLD_{50}) for *M. tenebricosa* collected from *A. rubrum* in Raleigh, NC, by experimental temperature. SE and upper and lower 95% confidence interval limits (LCL, UCL) are also reported for the ULD_{50} and LLD_{50} estimates. Thermal tolerance per temperature was estimated from a GLM with duration (hours) as the predictor and probability of mortality as the response.

Statistical Analyses

We performed all statistical analyses in R 3.5.1 (R Core Team 2016). For each thermal tolerance experiment, we used generalized linear models (GLM; binomial distribution with a logit link) from the *glm* function from package *MASS* (Venables and Ripley 2013) to estimate the probability of *M. tenebricosa* mortality. For *Raleigh 2018* and *Raleigh 2019* experiments, our models included treatment (i.e., duration at a given temperature) as the predictor and scale mortality status (i.e., dead, alive) as the response. For *Raleigh 2019*, we used mean responses in our models for experiments that were conducted multiple times at the same temperature (i.e., 32.8 or –4.7°C; Table 2). When analyzing *Three-city 2019* experiments, Raleigh data are from two of the five *Raleigh 2019* experiments, 5 December 2018 and 1 March 2019, dates when we also performed experiments with scales from the other two cities. We fit a separate GLM for each city and included treatment and date of collection as predictors and scale mortality as the response.

From these GLMs, we estimated thermal tolerance—upper and lower lethal thermal durations (i.e., ULD_{50}, LLD_{50})—by identifying the duration (hours) at a given experimental temperature at which mortality was expected to be 50% (e.g., ULD_{50} at 32.2°C). We calculated ULD_{50} and LLD_{50} using the *invest* function from package *investr* (Greenwell and Schubert Kabban 2014). We also estimated the 95% CI of these durations. Because we recorded scale mortality in our control (0 h) treatments, we adjusted scale mortality in the other treatments using the Henderson–Tilton mortality correction (Henderson and Tilton 1955). Corrections were calculated per site by twig collection date before modeling *M. tenebricosa* mortality. For *Raleigh 2018* and *Raleigh 2019*, we made pairwise comparisons of ULD_{50} or LLD_{50} between the experimental temperatures. For *Raleigh 2019*, we also tested for seasonal differences in lethal durations for scales assayed at 32.8 or –4.7°C. For *Three-city 2019* results, we tested each city pair for differences in ULD_{50} and LLD_{50}. We used the Wheeler et al. (2006) ratio test for comparing lethal durations, implemented in the *ratio_test* function from the *ecotox* package (Hlina 2019).

We tested for seasonal differences in overwintering survival for *Raleigh 2018* and *Raleigh 2019* and between study cities for *Three-city 2018* and *Three-city 2019* scales. We compared groups with a Kruskal–Wallis test with a Benjamini–Hochberg post hoc *P*-value adjustment (α = 0.05) for multiple comparisons from function *kruskal* in package *agricolae* (de Mendiburu 2015).

Results

In general, for both heat and cold tolerance experiments, we found that the lethal duration was correlated with treatment temperature. For example, the warmest temperature in the heat tolerance experiments and the coldest temperature in the cold tolerance experiments resulted in the shortest lethal durations for those assays, respectively (Tables 3–5). For all GLM models, we found that treatment was a significant predictor of scale mortality. For *Three-city 2019* analyses, we found that the date of scale collection was also a significant predictor of scale mortality.

Heat Tolerance

We assayed a total of 1,373 *M. tenebricosa* in the *Raleigh 2018* heat tolerance experiments (Supp Table 1 [online only]). Upper lethal durations ranged from 7.36 ± 1.14 (SE) to 27.69 ± 1.09 h at 40.6 and 32.2°C, respectively (Table 3; Fig. 1). The longest estimated lethal duration was for scales assayed at the Raleigh mean maximum July

Table 4. Melanaspis tenebricosa thermal tolerance for Raleigh 2019 scales

Heat/cold	Temperature (°C)	LD_{50}	SE	LCL	UCL
Heat	39.0	5.93	1.12	4.79	7.35
	37.8	12.68	1.05	11.46	14.03
	32.8	12.48	1.03	11.82	13.18
Cold	–15.2	4.93	1.22	3.32	7.32
	–12.9	10.3	1.10	8.56	12.4
	–4.7	15.38	1.03	14.62	16.18

Upper and lower lethal duration 50% (ULD_{50} and LLD_{50}) for *M. tenebricosa* collected from *A. rubrum* in Raleigh, NC, by experimental temperature. SE and upper and lower 95% confidence interval limits (LCL, UCL) are also reported for the ULD_{50} and LLD_{50} estimates. Thermal tolerance per temperature was estimated from a GLM with duration (hours) as the predictor and probability of mortality as the response.

Table 5. Melanaspis tenebricosa thermal tolerance for Raleigh 2019 scales for repeated experimental temperatures

Date	Temperature (°C)	LD_{50}	SE	LCL	UCL
5 Dec. 2018	32.8	10.47	0.56	9.38	11.56
30 Jan. 2019		15.48	0.66	14.18	16.77
1 Mar. 2019		11.71	0.58	10.56	12.86
5 Dec. 2018	–4.7	12.74	0.6	11.57	13.91
30 Jan. 2019		16.19	0.74	14.74	17.64
1 Mar. 2019		17.62	0.72	16.21	19.03

Upper and lower lethal duration 50% (ULD_{50} and LLD_{50}) for *M. tenebricosa* collected from *A. rubrum* in Raleigh, NC, by experimental start date. SE and upper and lower 95% confidence interval limits (LCL, UCL) are also reported for the ULD_{50} and LLD_{50} estimates. Thermal tolerance per temperature was estimated from a GLM with duration (hours) as the predictor and probability of mortality as the response.

temperature (32.2°C). We found pairwise differences between the ULD_{50} for each of the temperatures assayed (*P* < 0.001; Supp Table 2 [online only]). Heat tolerance was qualitatively similar for experiments performed in 2018 and 2019 on Raleigh-collected scales (Figs. 1 and 2). For the *Raleigh 2019* heat tolerance experiments, we assayed a total of 3611 *M. tenebricosa* (Supp Table 3 [online only]). Lethal durations ranged from 5.93 ± 1.12 to 12.48 ± 1.03 h at 39.0 and 32.8°C, respectively (Table 4; Fig. 2). The ULD_{50} differed between each pair of experimental temperatures, except for the heat tolerance experiments at 32.8 and 37.8°C (Supp Table 4 [online only]). For the *Raleigh 2019* heat tolerance experiments repeated at 32.8°C for three dates throughout winter, we assayed 1,044 *M. tenebricosa* (Supp Table 5 [online only]). Lethal duration lengths were idiosyncratic throughout the winter (Table 5; Fig. 3). The ULD_{50} at 32.8°C differed between dates, except for 5 December 2018 and 1 March 2019 (Supp Table 6 [online only]). We examined a total of 3,436 *M. tenebricosa* from the *Three-city 2019* experiments (Supp Table 7 [online only]). The mean heat tolerance (ULD_{50} at 32.8°C) of Raleigh scales, 11.04 h, was greater than the duration for Newark, 9.61 h, but did not differ from Gainesville, 10.20 h (Tables 6; Supp Table S8 [online only]; Fig. 4).

Cold Tolerance

We assayed a total of 4,732 *M. tenebricosa* in the *Raleigh 2018* cold tolerance experiments (Supp Table 1 [online only]). Lower lethal durations ranged from 4.2 ± 1.1 to 755.4 ± 2.57 h at –22.8 and –1.0°C,
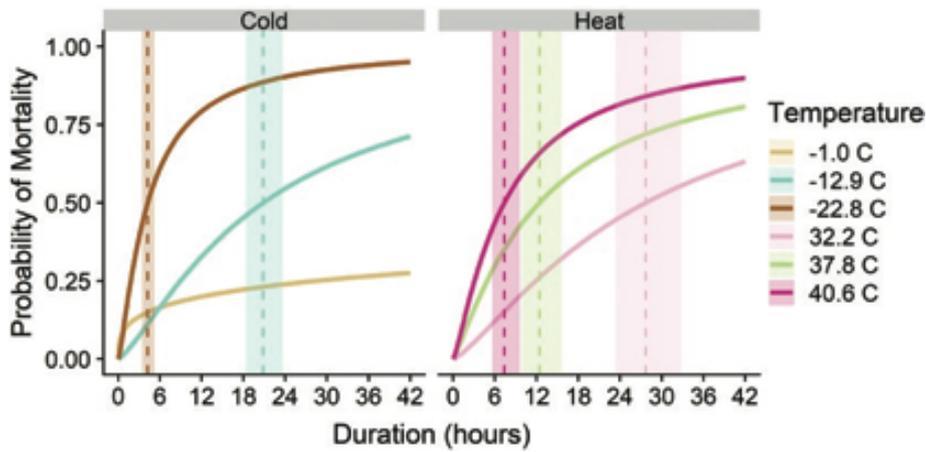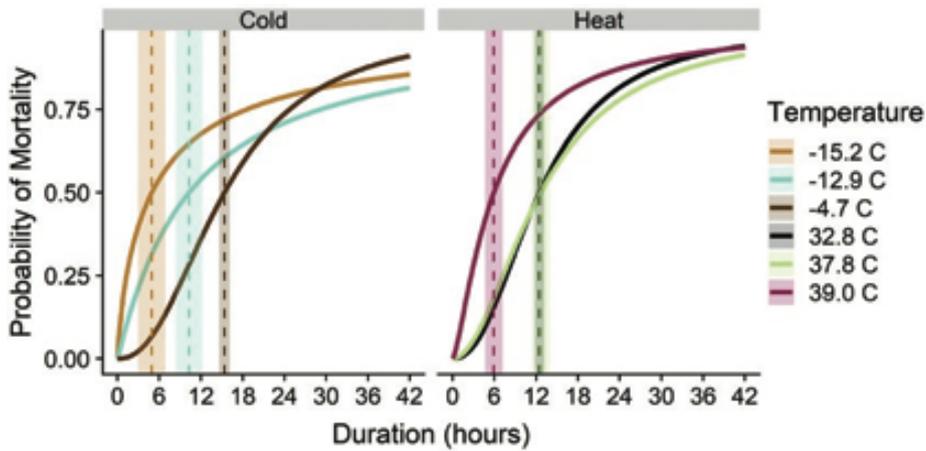
Fig. 1. Raleigh 2018 probability of Melanaspis tenebricosa mortality and thermal tolerance by experimental treatment (duration [hours] by temperature). Lines are GLM estimates of M. tenebricosa mortality by experimental temperature. Vertical lines denote ULD_{50} (right; Heat) and LLD_{50} (left; Cold), i.e., thermal tolerance, for each treatment (LLD_{50} for −1.0°C ≈ 755 h [not shown]) and are estimated from the GLM model fits. Shaded bars are the 95% confidence intervals for LD_{50} by temperature.

Fig. 2. Raleigh 2019 probability of Melanaspis tenebricosa mortality and thermal tolerance by experimental treatment (duration [hours] by temperature). Lines are GLM estimates of M. tenebricosa mortality by experimental temperature. Vertical lines denote ULD_{50} (right; Heat) and LLD_{50} (left; Cold), i.e., thermal tolerance, for each treatment and are estimated from the GLM model fits. Shaded bars are the 95% confidence intervals for LD_{50} by temperature.
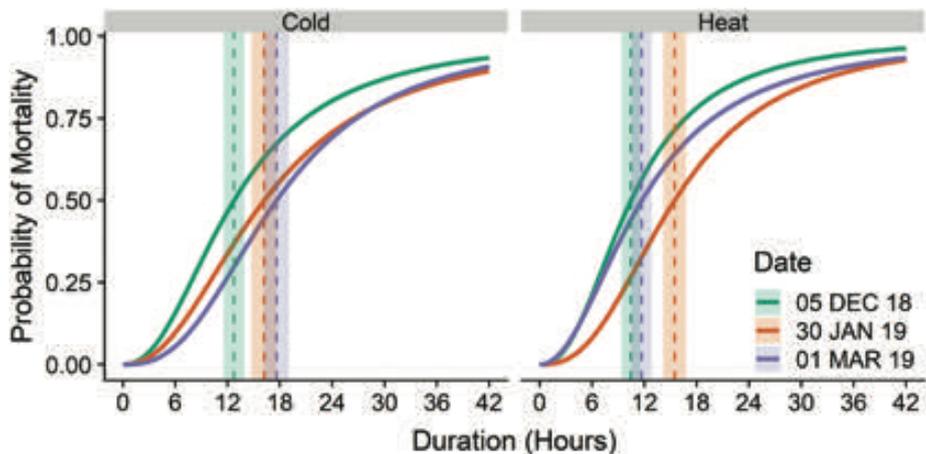

Fig. 3. Raleigh 2019 probability of Melanaspis tenebricosa mortality and thermal tolerance by experimental treatment (duration [hours] by temperature) by date. Lines are GLM estimates of M. tenebricosa mortality by date. Vertical lines denote ULD_{50} (right; 32.8°C [Heat]) and LLD_{50} (left; −4.7°C [Cold]), i.e., thermal tolerance, for each treatment by date and are estimated from the GLM model fits. Shaded bars are the 95% confidence intervals for LD_{50} by temperature by date.

Table 6. Three-city 2019 Melanaspis tenebricosa thermal tolerance

City	Temperature (°C)	Collection date	LD_{50}	SE	LCL	UCL
Newark	−4.7	5 Dec. 18	14.549	0.6	13.373	15.725
		1 Mar. 19	24.018	1.187	21.691	26.344
	32.8	5 Dec. 18	6.492	0.322	5.861	7.123
		1 Mar. 19	12.737	0.563	11.633	13.842
Raleigh	−4.7	5 Dec. 18	12.863	0.566	11.755	13.972
		1 Mar. 19	17.579	0.758	16.093	19.065
	32.8	5 Dec. 18	10.294	0.555	9.206	11.382
		1 Mar. 19	11.795	0.561	10.695	12.894
Gainesville	−4.7	5 Dec. 18	10.152	1.282	7.64	12.664
		1 Mar. 19	9.279	1.131	7.063	11.494
	32.8	5 Dec. 18	12.965	1.256	10.504	15.426
		1 Mar. 19	7.429	1.089	5.294	9.563

Upper and lower lethal duration 50% (ULD_{50} and LLD_{50}) for *M. tenebricosa* by experimental temperature. SE and upper and lower 95% confidence interval limits (LCL, UCL) are reported for the LD_{50} estimates. Thermal tolerance (hours) per city and experimental temperature (LD_{50}) was estimated from a GLM with duration and date of collection as predictors and probability of mortality as the response.
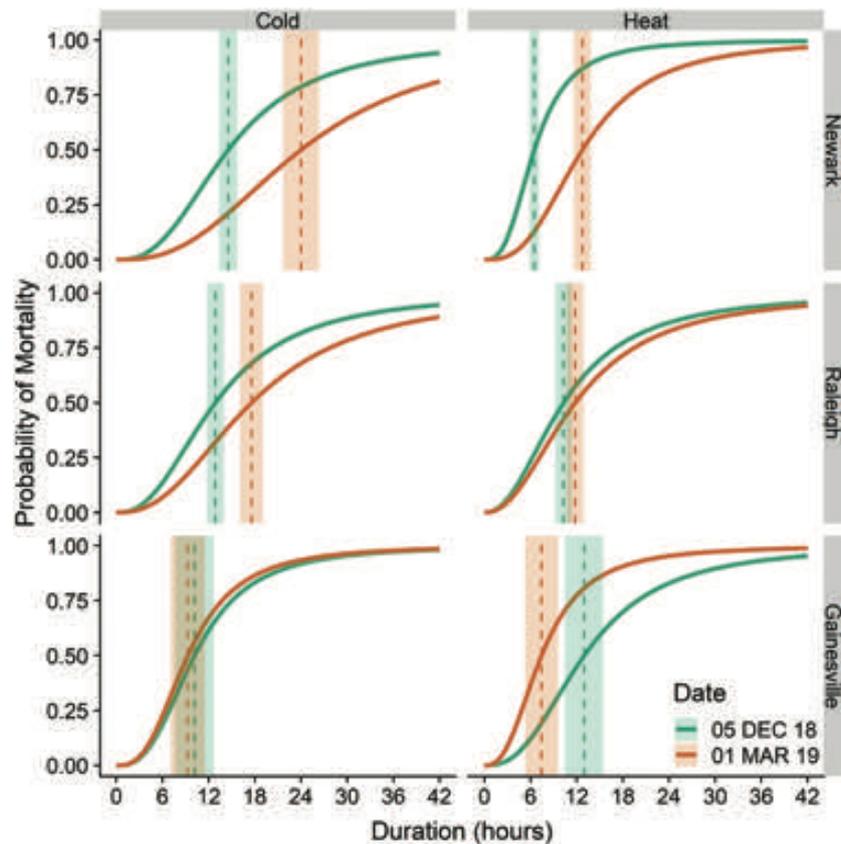

Fig. 4. Three-city 2019 probability of Melanaspis tenebricosa mortality and thermal tolerance by experimental treatment (duration [hours] by temperature) by city. Lines are GLM estimates of M. tenebricosa mortality by date. Vertical lines denote ULD_{50} (right; 32.8˚C [Heat]) and LLD_{50} (Left; −4.7˚C [Cold]), i.e., thermal tolerance, for each treatment and are estimated from the GLM model fits. Shaded bars are the 95% confidence intervals for LD_{50} by temperature by date.

respectively (Table 3; Fig. 1), where the longest lower lethal duration, 755 h, was found for scales assayed at the mean minimum Raleigh January temperature. The LLD_{50} differed between each experimental temperature pairing (P < 0.001; Supp Table 2 [online only]). Cold tolerance was qualitatively similar between *Raleigh 2018* and *Raleigh 2019* experiments (Figs. 1 and 2), where lower lethal duration was shorter as experimental temperature decreased. For the *Raleigh 2019* cold tolerance experiments, we assayed a total of 3,757 *M. tenebricosa* (Supp Table 3 [online only]). Lethal durations ranged

from 4.9 ± 1.22 to 15.4 ± 1.02 h at −15.2 and −4.7°C, respectively (Table 5; Fig. 3). The LLD_{50} differed between each pair of experimental temperatures (Supp Table 4 [online only]). For the *Raleigh 2019* cold tolerance experiments repeated at −4.7°C on three dates throughout winter, we assayed 2,517 *M. tenebricosa* (Supp Table 5 [online only]). The length of the LLD_{50} increased throughout the winter, ranging from 12.74 ± 0.60 to 17.62 ± 0.72 h on 5 December 2018 and 1 March 2019, respectively (Table 5; Fig. 3). The ULD_{50} at −4.7°C differed between pairs of these three dates, except for 30

January and 1 March 2019 (Supp Table 6 [online only]). For the *Three-city 2019* cold tolerance experiments, we examined a total of 3,481 *M. tenebricosa* individuals (Supp Table 7 [online only]). The LLD_{50} at –4.7°C became shorter as latitude of the scale collection city decreased, at 19.28, 15.22, and 9.72 h for Newark, Raleigh, and Gainesville, respectively (Table 6, Fig. 4). The LLD_{50} differed between each city pair (*P* < 0.001; Supp Table 8 [online only]).

Overwintering Survival

Overwintering survival did not differ seasonally for *Raleigh 2018* scales (χ^2 = 1.24, df = 2, *P* = 0.538; Supp Fig. S2a [online only]): mean survival was 91%. Overwinter survival for *Raleigh 2019* scales did not differ seasonally (χ^2 = 6.81, df = 4, *P* = 0.146; Supp Fig. S2b [online only]): mean survival was 62%. In 2018, overall in situ overwintering survival varied among the three cities (χ^2 = 10.26, df = 2, *P* = 0.006; Supp Fig. S3a [online only]), with the greatest mean survival found in Raleigh (92%). Gainesville (34%) and Newark (23%) scales had lower survival and did not differ from each other (Table 1). For *Three-city 2019* scales, we found that mean overwintering survival varied among the cities (χ^2 = 8.76, df = 2, *P* = 0.013; Supp Fig. S3b [online only]). Raleigh had the greatest mean survival at 64%, although it did not differ from Newark (61%). Gainesville had the lowest mean survival at 43% (Table 1).

Discussion

In this study, we present the first assessment of *M. tenebricosa* physiological thermal tolerance, including tolerances at representative temperatures from throughout their geographic distribution. Given that the density of *M. tenebricosa* peaks in the mid latitudes of its distribution (Just et al. 2018, 2019), we predicted that temperatures representative of the midlatitudes (e.g., Raleigh, NC) would be the least lethal for *M. tenebricosa* and, thus, their thermal tolerance, measured here as upper and lower lethal thermal durations for a given temperature, would be the longest. Looking at our heat tolerance experiments, we found support for our prediction as *Raleigh 2018* scales tolerated the Raleigh-derived temperatures best, providing the longest ULD_{50} at 32.2°C (mean maximum July temperature) of 27.7 h (Table 3). Contrastingly, we predicted that the temperatures found at the southern edges of the *M. tenebricosa* distribution would be more lethal and, thus, thermal durations would be shorter when compared with Raleigh temperatures. We found *Raleigh 2019* scales succumbed faster at the Gainesville mean maximum July temperature (32.8°C), 12.5 h, when compared with Raleigh temperatures (Table 4, Fig. 2). Our predictions were similarly supported from our cold tolerance experiments, we found a LLD_{50} at –1.0°C (mean minimum January temperature in Raleigh) of 755.4 h for the *Raleigh 2018* assayed scales, whereas *Raleigh 2019* scales had an estimated LLD_{50} of 15.4 h at –4.7°C, the mean minimum January temperature from Newark. These results support the role of temperature in bounding the *M. tenebricosa* distribution, as do our previous observations that the greatest *M. tenebricosa* densities occur in the midlatitudes of their distribution. This was also corroborated by the different in situ overwintering survival of *M. tenebricosa* in each city (Table 1). Yet, we acknowledge that we did not assay *M. tenebricosa* collected from Newark or Gainesville at the Raleigh-based temperatures and assays in addition to this first assessment are needed to confirm that these are general patterns of *M. tenebricosa* thermal tolerance.

We also predicted that thermal tolerance would vary based on the latitude of where the scales were collected because scales and other arthropods can become acclimated or adapted to local climates (Preisser et al. 2008, Zhang et al. 2010). The most poleward study city, Newark, had *M. tenebricosa* that survived the longest in the cold tolerance experiments. Whereas, in Gainesville, the city closest to the equator, scales perished the quickest, with a mean LLD_{50} at –4.7°C that was less than half as long as Newark: 9.7 versus 19.3 h (Table 6, Fig. 4). A study of elongate hemlock scale (*Fiorinia externa* Ferris; Hemiptera: Diaspididae) found that northern populations were more cold tolerant than those in the south and suggested that this may be due to adaptation driven by more frequent low-temperature periods in the north (Preisser et al. 2008). Thus, we might expect the poleward movement of the *M. tenebricosa* distribution due to greater overwintering survival of northern populations aided by warmer winters in the eastern United States. This expansion may occur even with possible periods of lower than normal temperatures, if *M. tenebricosa* at the northern range boundary are becoming more cold tolerant, which is suggested by our results. Moreover, we found that cold tolerance for Newark and Raleigh scales differed seasonally, where scales collected in late winter had a longer LLD_{50}. Other scale species, including *Hemiberlesia pitysophila* Takagi (Hemiptera: Diaspididae) and crapemyrtle bark scale (*Acanthococcus* (=*Eriococcus*) *lagerstroemiae* (Kuwana); Hemiptera: Eriococcidae) (Zhang et al. 2010, Wang et al. 2019a), exhibit seasonal differences in cold tolerance. In one study (Wang et al. 2019b), *A. lagerstroemiae* had lower body water content during colder parts of the year, thereby increasing the concentration of cryoprotective compounds, suggesting a possible physiological mechanism for its cold tolerance. We do not know the thermal tolerance mechanisms in our system. For example, to improve cold tolerance and overwintering survival, *M. tenebricosa* may reduce water content to concentrate cryoprotective compounds or it could be that they inactivate ice-nucleating compounds by sequestering them in lipids (Baust and Zachariassen 1983). In any case, improved cold tolerance at the poleward range edge and a warming climate may provide opportunities for range expansion at the northern edge of the *M. tenebricosa* distribution.

Other studies on scale thermal tolerance have concluded that cold tolerance is more important than heat tolerance for defining species distributions (Zhang et al. 2010, Wang et al. 2019a). Here, we agree that cold tolerance is important with regard to current and future *M. tenebricosa* distributions because, like other pest insects [e.g., *D. frontalis*, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Notodontidae)], poleward movement facilitated by warmer winters seems more likely than southward expansion (Ungerer et al. 1999, Battisti et al. 2005). Yet, differing from those studies, we posit that heat tolerance is also important for *M. tenebricosa* distributions. For example, in this study, the smallest *M. tenebricosa* densities were found in the lowest latitude city, Gainesville. Additional warming is unlikely to benefit scales in Gainesville, where climate and urban warming may combine to generate scale-lethal temperatures and, thus, contribute to range contraction (Tobin et al. 2014, Mech et al. 2018). Still, we acknowledge that *M. tenebricosa* responds positively to warming, provided that the warming is within its thermal tolerance thresholds. For example, within Raleigh, *A. rubrum* in warmer locations hosted 200% more *M. tenebricosa* than locations just 2°C cooler (Dale and Frank 2014b). Herbarium specimens have also revealed that historical *M. tenebricosa* abundances tracked periods of warmer and cooler climates, where warmer periods had greater densities (Youngsteadt et al. 2015). Under increased warming, *M. tenebricosa* may retreat to cooler areas within the south like forests (Long et al. 2019) and become a less-important pest of ornamental trees, but may become more important forest pests. However, some scales may adapt to the heat [e.g., heat adaptation has been suggested for oak lecanium scale; *Parthenolecanium*

quercifex (Fitch); Hemiptera: Coccidae) (Meineke et al. 2014)] and thrive in the additionally warmed cities. Future research should consider heat tolerance for all *M. tenebricosa* life stages with individuals collected during seasons associated with those stages.

Our research on *M. tenebricosa* thermal tolerance supports previous work, suggesting the primacy of temperature as a delimiting factor of insect distributions (e.g., Andrewartha and Birch 1954, Diamond et al. 2017, Hamblin et al. 2017). Host availability does not appear to be a significant delimiter of *M. tenebricosa* distribution as *A. rubrum* and other host tree species occur throughout the eastern United States, but *M. tenebricosa* does not (Metcalf 1922, Burns and Honkala 1990). This distributional mismatch supports temperature rather than host availability as a more important limiting factor. Moreover, scales can be readily transported by humans via horticultural activities (e.g., Dehnen-Schmutz et al. 2007), and *M. tenebricosa* would have probably reached those geographies containing host trees. Yet, they have not become established in most of these areas to our knowledge. For example, we have found *M. tenebricosa* on *A. rubrum* in Queens, NY (unpublished data), but they are not abundant nor a well-established insect pest there. In contrast, if we look to geographies more distant from the *M. tenebricosa* distribution of the eastern United States, e.g., to the insect pests of the Galápagos Islands we find that *M. tenebricosa* is an established pest of *Zanthoxylum fagara* (L.) Sarg. (Sapindales: Rutaceae) on Santa Cruz (Lincango et al. 2010). The monthly mean minimum and maximum temperatures of the Galápagos Islands range from 11 to 23°C (Trueman and D'Ozouville 2010), values that are not expected to be lethal based on our findings from this study. Although we did not study sublethal effects of temperature, *M. tenebricosa* has become established on Santa Cruz. Thus, this example provides further support that *M. tenebricosa* can be transported to distant geographies and become established if temperatures are salubrious or at least sublethal.

We observed variation in thermal tolerance at the repeated temperatures (i.e., 37.8°C, –12.9°C) in *Raleigh 2018* and *Raleigh 2019* assays (Tables 3, 4) as well as in in situ survival across all three cities. We found greater variation in our cold tolerance results than our heat tolerance results, and within species, cold tolerance variability has been documented for other insect taxa (e.g., Ayrinhac et al. 2004, Terblanche et al. 2007, Sinclair et al. 2012). In our study, scales assayed at –12.9°C had a LLD_{50} of 20.85 ± 1.07 or 10.30 ± 1.10 h in 2018 and 2019, respectively. We sampled 5 wk earlier in 2019 than in 2018 and 2018 was a colder winter on average (Table 1), so the scales that were still alive when we collected and assayed in 2018 may have been those that were the most cold tolerant. Shorter lethal durations are more likely to limit species distributions as a temperature duration that meets or exceeds these thresholds is more likely to occur if it is shorter. Extreme temperatures, which are more likely to be lethal at shorter durations, have been increasing in frequency and duration (DeGaetano and Allen 2002, Perkins et al. 2012), though there are cases of climate variability increasing thermal tolerance (Colinet et al. 2011, 2016). Moreover, there are many factors that may have varied between these years that we did not study (e.g., humidity, parasitism, microbial symbionts), which may have contributed to thermal tolerance and background survival. Cold tolerances did vary between years, but our results show similar patterns where the coldest assay temperature had the shortest LLD_{50} and the warmest the longest (Tables 3 and 4).

In summary, our results suggest that both heat and cold tolerance are important delimiters of the *M. tenebricosa* distribution. Our experiments revealed that *M. tenebricosa* from Raleigh survived the longest when assayed with temperatures from the midlatitudes of its distribution, where its densities are currently greatest. Suggesting that the current *M. tenebricosa* distribution and abundance patterns are delimited by environmental temperature and thermal tolerance, though additional assays from scale populations from different latitudes are needed for confirmation. Moreover, the northernmost scale population had the greatest cold tolerance when assayed with temperatures from that latitude. Whereas the southernmost scale population had the least tolerant scales for both heat and cold assays. Thus, we contend that future expansion, if any, of the *M. tenebricosa* distribution would likely be directed poleward due to projected winter warming for the eastern United States (Fitzpatrick and Dunn 2019) and the increased cold tolerance of northern populations. On the other hand, low densities and poorer thermal tolerance in the south may lead to a range contraction at the southern distribution edge with additional warming. Finally, we assayed scales collected from eastern U.S. cities, which are on average 2–3°C warmer than natural areas at the same latitudes. This matches predictions of temperature increase over the next 50–100 yr (Kunkel et al. 2013); thus, cities provide the opportunity to make predictions about how warming may affect forests (Lahr et al. 2018) and their potential thermal suitability for *M. tenebricosa*.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

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