

Population Ecology

High Rainfall May Induce Fungal Attack of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Leading to Regional Decline

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Abstract

Hemlock woolly adelgid (HWA; *Adelges tsugae* Annand (Hemiptera: Adelgidae)) is the cause of widespread mortality of Carolina and eastern hemlock (*Tsuga caroliniana* Engelm and *T. canadensis* (L.) Carrière) throughout the eastern United States (U.S.). Since its arrival in the northeastern U.S., HWA has steadily invaded and established throughout eastern hemlock stands. However, in 2018, anecdotal evidence suggested a sharp, widespread HWA decline in the northeastern U.S. following above-average summer and autumn rainfall. To quantify this decline in HWA density and investigate its cause, we surveyed HWA density in hemlock stands from northern Massachusetts to southern Connecticut and analyzed HWA density and summer mortality in Pennsylvania. As native fungal entomopathogens are known to infect HWA in the northeastern U.S. and rainfall facilitates propagation and spread of fungi, we hypothesized high rainfall facilitates fungal infection of aestivating nymphs, leading to a decline in HWA density. We tested this hypothesis by applying a rain-simulation treatment to hemlock branches with existing HWA infestations in western MA. Our results indicate a regional-scale decline and subsequent rebound in HWA density that correlates with 2018 rainfall at each site. Experimental rain treatments resulted in higher proportions of aestivating nymphs with signs of mortality compared to controls. In conjunction with no evidence of increased mortality from extreme winter or summer temperatures, our results demonstrate an indirect relationship between high rainfall and regional HWA decline. This knowledge may lead to better prediction of HWA population dynamics.

Key words: entomopathogen, fungi, hemlock woolly adelgid, mortality, precipitation

The invasive exotic forest pest hemlock woolly adelgid (*Adelges tsugae* Annand (Hemiptera: Adelgidae), hereafter HWA) has devastated Carolina hemlock (*Tsuga caroliniana* Engelm) and eastern hemlock (*T. canadensis* (L.) Carrière) forests along the eastern coast of the United States (Orwig and Foster 1998, Stadler et al. 2005, Albani et al. 2010) since its introduction from Japan prior to 1951 (Souto et al. 1996, Havill et al. 2014). In the U.S., HWA now ranges from northern Alabama to southern Maine (Havill et al. 2014, USDA Forest Service 2021). In the northeastern U.S., HWA invaded southern Connecticut in the early 1980s (McClure 1987) and continued to spread northward to the northern edge of Massachusetts over the next two decades (Preisler et al. 2008). Despite extensive chemical and biological control efforts (Cheah et al. 2004, Preisler et al. 2014), HWA continues to cause mortality of hemlocks in the eastern U.S. In 2018, however, anecdotal reports from forest health

specialists across the northeastern U.S. indicated a regional collapse of HWA populations in the late summer and early autumn of 2018. The timing of this decline suggests a cause of adelgid mortality other than the regularly observed annual winter mortality (Paradis et al. 2008, Trotter and Shields 2009, Cheah 2017, McAvoy et al. 2017).

In North America, HWA reproduces asexually, producing two generations per year. The progrediens generation hatches in early spring and reaches maturity in early summer. The sistens generation hatches in early summer, disperses as first instar “crawlers”, and settles at the base of hemlock needles, largely on new growth. Once settled, sistens nymphs aestivate until early- to mid-autumn when they begin feeding and eventually reproduce, laying eggs that will hatch into the next progrediens generation (McClure 1989). Typically, population dynamics of HWA are governed by density-dependent competition for adequate space on the new-growth twigs of hemlock

(McClure 1991, Sussky and Elkinton 2014, Crandall et al. 2020). However, mortality due to high summer temperatures (Sussky and Elkinton 2015, Mech et al. 2018), cold winter temperatures (Paradis et al. 2008, Trotter and Shields 2009, Cheah 2017, McAvoy et al. 2017), and low hemlock productivity (McClure 1991) can all contribute to fluctuations in HWA density. The role abnormal or extreme temperatures play in causing HWA mortality can be discerned from temperature records, while the effect of low hemlock productivity is often evident in the late spring with a decline in density of the progrediens generation. Therefore, reports of widespread summer mortality in the northeastern U.S. in 2018 were highly unusual and warranted further investigation.

We hypothesized that high rainfall during the summer and autumn of 2018 may have benefited fungal pathogen growth, leading to increased mortality of HWA. Previous research has documented infection of HWA by native fungal pathogens in New England (Gouli et al. 1997, Reid et al. 2010) and rainfall and rain-splash are known to facilitate the propagation and spread of entomopathogenic fungi (Bruck and Lewis 2002, Reid et al. 2010). Aestivating sistens nymphs may be particularly vulnerable to attack by fungal pathogens because they lack the protection conferred by the waxy “wool” ovisac of later-stage nymphs (Reid et al. 2010). Therefore, potential exists for the emergence of a naturally occurring fungal biocontrol agent that suppresses HWA population density, just as other invasive insect populations are now controlled by entomopathogenic fungi (e.g., Hajek et al. 1990, Reilly et al. 2014).

In order to quantify and document decline in HWA density we re-surveyed 23 hemlock stands along a transect originally set up to track the decline of hemlock after the initial wave of HWA invasion of New England (Orwig et al. 2002, Preisser et al. 2008, Gómez et al. 2015) and examined recent yearly trends in HWA summer mortality and winter density in Pennsylvania. We assessed the effect of rainfall on HWA density by analyzing the relationship between HWA abundance and rainfall. By applying a rain-simulation treatment to hemlock branches with existing HWA infestations in western MA, we tested whether increased rainfall during the progrediens and early sistens generations of HWA may facilitate colonization and proliferation of entomopathogenic fungi, leading to increased infection and mortality of aestivating nymphs of the sistens generation.

Materials and Methods

Weather Data

Contemporary summer precipitation, 30-year normal precipitation, and temperature data were acquired from PRISM (PRISM Climate Group 2021). We compared yearly precipitation data summed across July–November and averaged across survey sites to the 30-year normal (derived from 1980 to 2010 data) and between years. Summer (June–September) and winter (December–March) temperature data from 2018 were compared to the last ten years of temperature means, maximums (for summer temperatures), and minimums (for winter temperatures). Weather data were mapped and extracted using ArcGIS version 10.7.1.

HWA Surveys

To quantify HWA decline and assess the timing of increased mortality, we employed HWA survey data from the Pennsylvania Department of Conservation and Natural Resources (PA DCNR) Bureau of Forestry that documented summer mortality of HWA aestivating sistens across Pennsylvania from 2016 to 2020. An average of 6.4 sites were surveyed for summer mortality each year (ranging

from 3 to 12 sites per year). Summer sistens mortality was recorded from 10 to 20 eastern hemlock branches (each 30–60-cm long) that were heavily infested with HWA. Summer mortality was assessed between November and March (i.e., after aestivation had broken) and was calculated as the number of sistens that did not break aestivation (i.e., did not wool up to create ovisacs) divided by the total number of sistens nymphs, multiplied by 100. We also examined PA DCNR HWA density survey data that included estimates of annual overwintering sistens density from 2013 to 2021. Density surveys scored HWA density on new growth plus approximately 7.5 cm of previous years' growth on each of 10 branches from 3 to 10 eastern hemlocks per site using a scale of 1–4 (1 = no adelgid; 2 = low density: approximately one adelgid settled at the base of 25% of needles; 3 = medium density: approximately one adelgid settled at the base of 50% of needles; 4 = high density: approximately one adelgid settled at the base of every needle).

To further quantify the changes in density of HWA in the northeastern U.S. and document a regional-scale decline in HWA density, we re-surveyed HWA density in 23 HWA-infested stands in 2019 and 2020 that had most-recently been surveyed in 2011 (Orwig et al. 2002, Preisser et al. 2008, Gómez et al. 2015). These stands are located along a north-south transect running along the Connecticut River Valley from coastal Connecticut to the northern border of Massachusetts (Orwig et al. 2002, Preisser et al. 2008, Gómez et al. 2015). For 2019 and 2020 surveys, we used a protocol adapted from that used in previous surveys of the same stands (Preisser et al. 2008, Gómez et al. 2015); at each site, we classified 20 1-m-length sections of hemlock branches on a 0–3 scale for density of HWA ovisacs (0 = 0 ovisacs/m; 1 = 1–10 ovisacs/m; 2 = 11–100 ovisacs/m; 3 = >100 ovisacs/m; Preisser et al. 2008, Gómez et al. 2015). Surveys conducted in 2011 examined two 1-m branches on each tree to rate HWA density of 50 trees per stand using the same HWA density scale (Gómez et al. 2015).

Rain Simulation Experiment

In order to test the effect of rain on HWA mortality, we applied an artificial rain treatment consisting of spraying 1-m-long HWA-infested branches with distilled water three times per week throughout June, July, and early-August 2020. During each water application, branches were sprayed for 15 s using a backpack sprayer (Model MS-401, Echo, Inc.). The rain treatment was applied to one branch in each of 24 branch pairs distributed across 3 sites in Amherst, Pelham, and Sunderland, MA. Branches within each site were paired based on having similar proportions of branchlets with new growth and similar numbers of 2019 sistens ovisacs. Branches were collected in mid-August and using a dissecting microscope, the number of healthy sistens nymphs and dead or dying sistens nymphs with signs of potential fungal infection and disease (e.g., bloating, discoloration, mummification, and fungal growth; Gouli et al. 1997, Reid et al. 2010), or other signs of mortality (i.e., desiccation and lack of a wool ring; personal observations) were counted on the distal 10 branchlets of each branch.

Statistical Analyses

We tested for differences in average HWA density class between years for both the CT-MA and PA surveys using a Wilcoxon signed-rank test since data were not normally distributed (Preisser et al. 2008). Yearly differences in summer mortality of HWA in PA were analyzed using a binomial GLMM with a logit link with year as a fixed effect and forest management region (i.e., north, east, south, west, or central) as a random effect. Overdispersion in this model

was accounted for by including an observation-level random effect (Browne et al. 2005). We analyzed the relationship between July–November 2018 rainfall and 2019 and 2020 HWA density using linear regression. Proportions of sistens nymphs with signs of mortality were analyzed using a binomial GLMM with a logit link. This model included treatment, number of sistens ovisacs from the previous year (2019), and the interaction of treatment and number of 2019 sistens ovisacs as fixed effects, and a random effect for site. To account for overdispersion in this model, an observation-level random effect was also included (Browne et al. 2005). Analyses using GLMM were conducted using the ‘lme4’ package (Bates et al. 2015). Posthoc pairwise comparisons, adjusting for multiple comparisons using Tukey’s method, were conducted using the ‘emmeans’ package (Lenth 2020). All data visualization and analyses were conducted in R version 3.6.2 (R Core Team 2019).

Results

Rainfall and Temperatures

Examination of the difference in total rainfall from July to November between 2018 and the 30-year average for the northeastern U.S. indicated a large region extending from eastern Pennsylvania to southern New Hampshire that experienced precipitation up to 78.5 cm above the 30-year normal during July–November of 2018 (Fig. 1A). Rainfall during July–November 2018 was on average ($\pm$ se) 38.11 ± 1.23 cm above the 30-year normal at CT and MA sites and 28.63 ± 0.22 cm above the 30-year normal at PA sites (Fig. 1B).

During the winters of 2017–2018 and 2018–2019 (i.e., the two winters preceding our 2019 HWA survey), the absolute minimum temperatures at CT and MA survey sites were -22.17 and -24.15°C , respectively (Supp Fig. 1A [online only]). At PA survey sites the absolute minimum temperature was -27.67°C during the winter of 2017–2018 and -28.20°C during the winter of 2018–2019 (Supp Fig. 1B [online only]). Across all CT and MA survey sites, the mean ($\pm$ se) winter temperature (December–March) was $-0.85 \pm 0.23^\circ\text{C}$ and $-1.4 \pm 0.21^\circ\text{C}$ in the winters of 2017–2018 and 2018–2019, respectively, and the maximum number of days below -10°C a site experienced was 44, but on average, sites experienced 17.13 ± 0.74 days and 28.61 ± 2.34 days below -10°C in the winters of 2017–2018 and 2018–2019 respectively. Across PA HWA density survey sites, mean winter temperature (December–March) was $-2.61 \pm 0.02^\circ\text{C}$ and $-1.79 \pm 0.02^\circ\text{C}$ (mean $\pm$ se) in the winters of 2017–2018 and 2018–2019, respectively, and the maximum number of days below -10°C was 61 (in the winter of 2017–2018). On average, sites in PA experienced 40.34 ± 0.17 days and 28.77 ± 0.13 days below -10°C in the winters of 2017–2018 and 2018–2019, respectively.

Summer temperatures across all study locations during 2018 were high, but not outside of the range of temperatures recorded in the last 10 years at these sites. The average summer temperature at HWA survey locations during 2018 was $20.67 \pm 0.45^\circ\text{C}$, whereas the mean ($\pm$ se) summer temperature at these sites from 2010 to 2020, excluding 2018, was $19.95 \pm 0.16^\circ\text{C}$ (19.63 – 20.27°C , 95% confidence interval). The 10-year mean ($\pm$ se) yearly summer maximum temperature across all sites was $36.19 \pm 0.31^\circ\text{C}$ with an absolute maximum temperature of 40.37°C , whereas during the summer of 2018 the maximum temperature across all sites was 37.17°C (Supp Fig. 2 [online only], Supp Table 1 [online only]).

HWA Surveys

Mortality of aestivating sistens in PA during the summer of 2018 was $92.6 \pm 3.05\%$ (mean $\pm$ se), which is nearly twice the summer

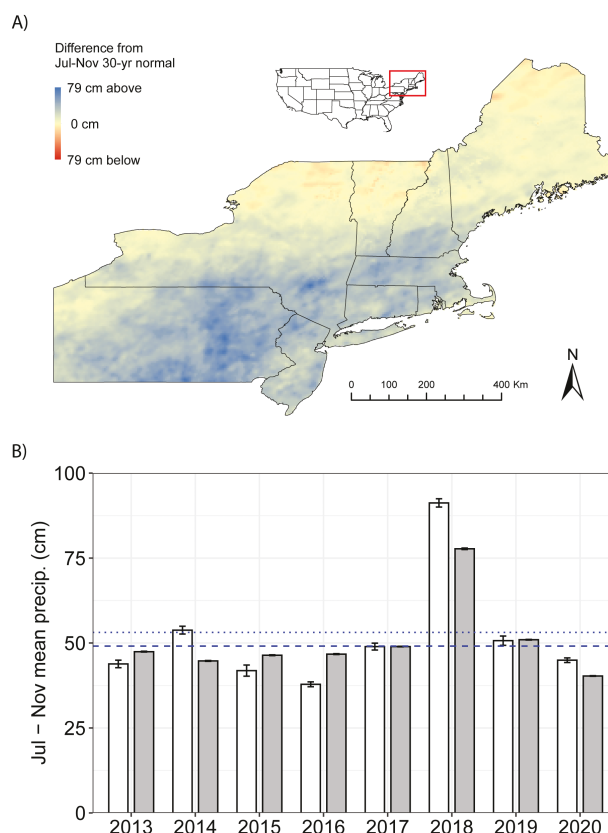


Fig. 1. A) Difference in total rainfall from July to November between 2018 and the 30-year normal for the northeastern U.S. Positive values (blues) indicate above normal rainfall and negative values (oranges/reds) indicate below normal rainfall during July–November of 2018. Areas of near-normal rainfall during July–November of 2018 are represented with yellow. B) Annual July–November precipitation averaged across MA and CT survey sites (open bars) and all PA survey sites (gray bars). The 30-year normal, derived from 1980 to 2010 precipitation, is represented by a dotted line for the MA and CT sites and a dashed line for the PA sites.

mortality observed in 2019 and more than three times the summer mortality observed in all other survey years (2016, 2017, 2020; Fig. 2A). Analyses of surveys of summer mortality in PA indicated a significant effect of year on summer mortality ($X^2 = 105.37$, $df = 4$, $P < 0.001$). Linear contrasts indicated that estimates of summer mortality in 2016, 2017, 2019, and 2020 were all significantly lower than in 2018 (2016 vs. 2018: $Z = -5.89$, $P < 0.001$; 2017 vs. 2018: $Z = -6.37$, $P < 0.001$; 2018 vs. 2019: $Z = 5.13$, $P < 0.001$; 2018 vs. 2020: $Z = 8.77$, $P < 0.001$; Fig. 2A). HWA density in PA during the winter of 2018–2019 was significantly lower than HWA during the winters of 2013–2014 ($P = 0.002$), 2017–2018 ($P = 0.028$), and 2020–2021 ($P < 0.001$), significantly higher than 2015–2016 ($P < 0.001$), but not significantly different than the winters of 2014–2015 ($P = 0.761$), 2016–2017 ($P = 0.843$), or 2019–2020 ($P = 0.1017$; Fig. 2B).

Average HWA density class at our CT-MA survey sites was significantly different between all years: 2011 vs 2019 (Wilcoxon = 248.5, $P < 0.001$), 2011 vs 2020 (Wilcoxon = 54, $P < 0.001$), and 2019 vs. 2020 (Wilcoxon = 20.5, $P < 0.001$). The average site HWA density dropped in 2019 compared to 2011 levels but rebounded in 2020 to levels higher than in 2011 (Fig. 3). Rainfall during July–November of 2018 was marginally negatively correlated with average site-level HWA density class in 2019 ($y = -0.04x + 3.80$, $F_{1,21} = 4.04$, $P = 0.058$, $R^2 = 0.16$; Fig. 4A) and negatively correlated with 2020

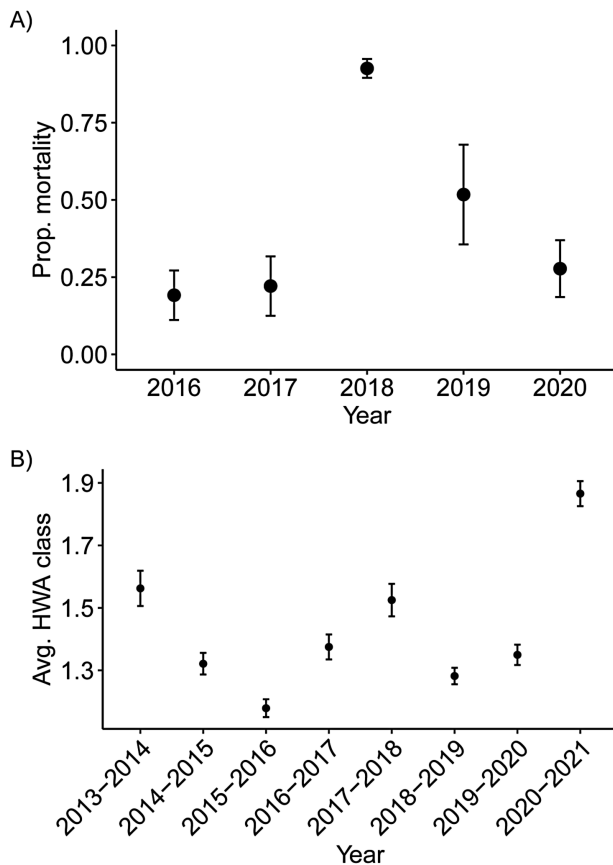


Fig. 2. A) Mean proportion ($\pm$ se) of mortality of hemlock woolly adelgid (HWA) aestivating sistens and B) mean ($\pm$ se) HWA density class values of overwintering sistens at research sites in Pennsylvania by year. Density classes were defined as: 1 = no adelgid; 2 = low: approximately one adelgid settled at the base of 25% of needles; 3 = medium: approximately one adelgid settled at the base of 50% of needles; 4 = high: approximately one adelgid settled at the base of every needle.

HWA density ($y = -0.08x + 9.25$, $F_{1,21} = 7.33$, $P = 0.013$, $R^2 = 0.22$; Fig. 4B). However, in PA, 2018 rainfall was not correlated with HWA summer mortality ($y = 0.02x + 84.78$, $F_{1,10} = 0.002$, $P = 0.969$, $R^2 < 0.001$) nor 2018 HWA density ($y = 0.02x - 0.36$, $F_{1,361} = 42.25$, $P < 0.001$, $R^2 = 0.102$) or 2019 HWA density ($y = 0.01x + 0.65$, $F_{1,491} = 19.07$, $P < 0.001$, $R^2 = 0.04$).

Rain Simulation Experiment

Analyses of the effect of experimental rain simulation on the proportion of sistens nymphs with signs of mortality indicate a significant effect of the interaction of treatment and 2019 sistens ovisac density ($Z = 2.43$, $P = 0.015$). Branches that received a rain simulation treatment had a significantly higher proportion of 2020 sistens nymphs with signs of mortality including fungal infection and/or disease when HWA density was high (i.e., above a 2019 ovisac density of 29.6 ovisacs per 20-cm branch) at the start of the experiment (Fig. 5). The main effects of treatment ($Z = -1.14$, $P = 0.254$) and ovisac density ($Z = 0.189$, $P = 0.850$) on nymph condition were not significant.

Discussion

Observational data and experimental results both indicate that abnormally high rainfall in the northeastern U.S. may have led to a

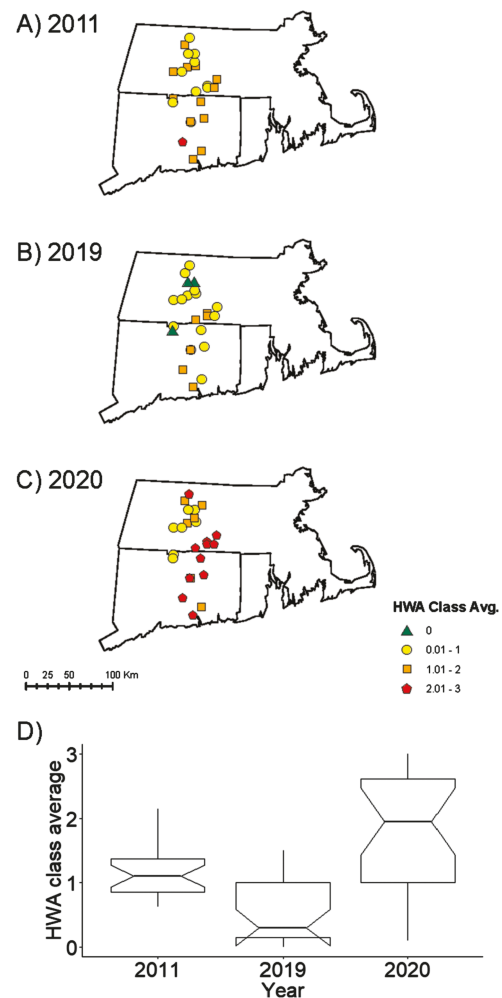


Fig. 3. Map of Connecticut and Massachusetts hemlock woolly adelgid (HWA) monitoring site locations and average HWA density class in A) 2011, B) 2019 C) 2020, and D) the average HWA density class 0 to 3 (0 = no HWA; 1 = 1–10 HWA/m branch; 2 = 11–100 HWA/m branch; 3 $\geq$ 100 HWA/m branch; Preisser et al. 2008, Gómez et al. 2015) observed at each location in 2011, 2019, and 2020.

decline in HWA density. At our study sites, above-average rainfall coincided with increased summer and autumn mortality and a decline in HWA density that we were able to model with a rain-simulation experiment. The effect of increased rain on HWA may have led to an increase in growth of entomopathogenic fungi that killed a significant percentage of the HWA population. Low winter temperatures were not a large source of mortality at our study sites, and there is little evidence for other potential causes of regional HWA decline. Although we did not survey for fungal-induced HWA mortality during the 2018 period of high rainfall, a lack of evidence for alternative sources of mortality in conjunction with negative correlation between rainfall and HWA density in CT and MA, as well as experimental evidence of moisture-induced mortality, indicated that above-average rainfall was indirectly associated with the 2018 decline in HWA abundance in the northeastern U.S.

Although our results support high rainfall as the most probable driver for the regional decline in HWA density we observed, there are potential alternative explanations for the region-wide HWA density decline in 2018. For example, a decline in hemlock productivity might have led to increased HWA mortality during the summer and autumn, or high HWA density in 2017 might have

led to density-dependent mortality in the following year (McClure 1991). McClure (1991) recorded a bi-annual boom-bust pattern of

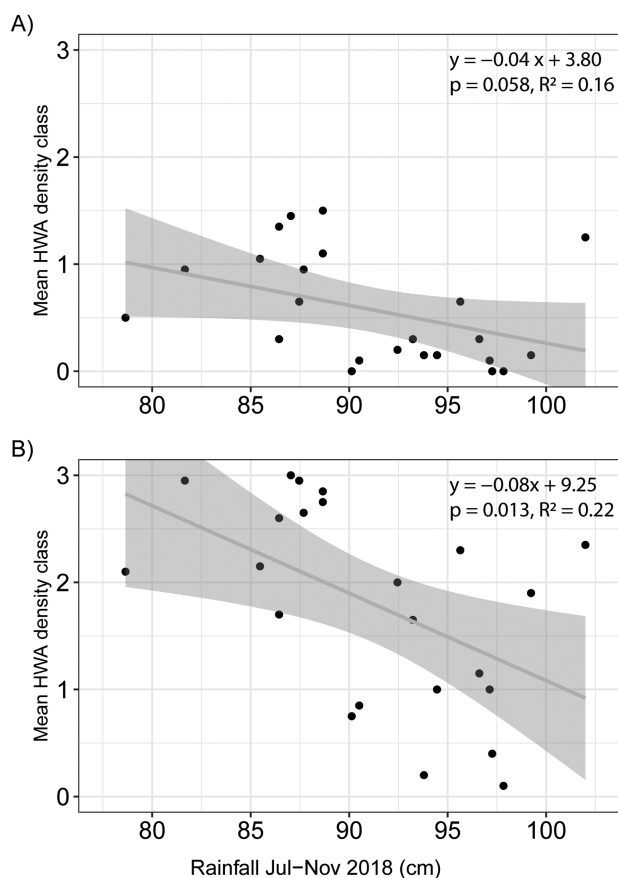


Fig. 4. Average site-level hemlock woolly adelgid density class in A) 2019 and B) 2020 at Connecticut and Massachusetts long-term transect sites plotted by sum of rainfall from July to November 2018.

HWA density and hemlock response that was corroborated in subsequent studies (Paradis et al. 2008, Elkinton et al. 2012). However, there is no evidence that this natural boom-bust cycle would synchronize among hemlocks on a regional scale. Although it is possible that the timing of high rainfall coincided with the bust stage of the HWA cycle, the negative correlation of site-level rainfall with HWA density at CT-MA sites is opposite of the pattern of HWA density driven by hemlock productivity; a positive rainfall-HWA density correlation would be expected if rainfall increased hemlock productivity and as a result, HWA density. The negative relationship between 2018 rainfall and HWA density at CT-MA sites in 2019 and 2020 indicates that the effect of rainfall on HWA may persist for two years following a high rainfall. Although we saw a positive correlation between 2018 rainfall and HWA density in PA, this relationship is likely driven by the high number of sites with no HWA; when sites without HWA are removed from analyses, we see the same negative relationship between 2018 rainfall and HWA density in 2018 and 2019 that we observed at CT-MA sites. High rainfall may also have directly affected adelgid survival by knocking HWA nymphs off branches, especially during the crawler stage, as heavy rain is known to cause mortality of balsam woolly adelgid (*Adelges picea*), a congener of HWA (Balch 1934). However, our ability to increase summer mortality with a relatively gentle mist of artificial rain indicates that rainfall likely did not directly mediate HWA mortality, but instead indirectly influenced the patterns of HWA density we observed across the landscape through a mechanism such as increasing fungal propagation. Increased signs of HWA mortality on high-density branches with a modest 15-s rain treatment three times per week, demonstrate the potential for substantial 2018 summer and autumn increase in rainfall (up to 78 cm above normal) to cause an increase in HWA mortality due to fungal infection.

Increased predation is another alternative explanation for the 2018 HWA decline. While there are no parasitoids of Adelgidae or specialist natural enemies of HWA in North America (Montgomery and Lyon 1996), multiple predator species have been released as part of HWA biocontrol programs. However, evidence suggests that current populations that have resulted from introductions of these

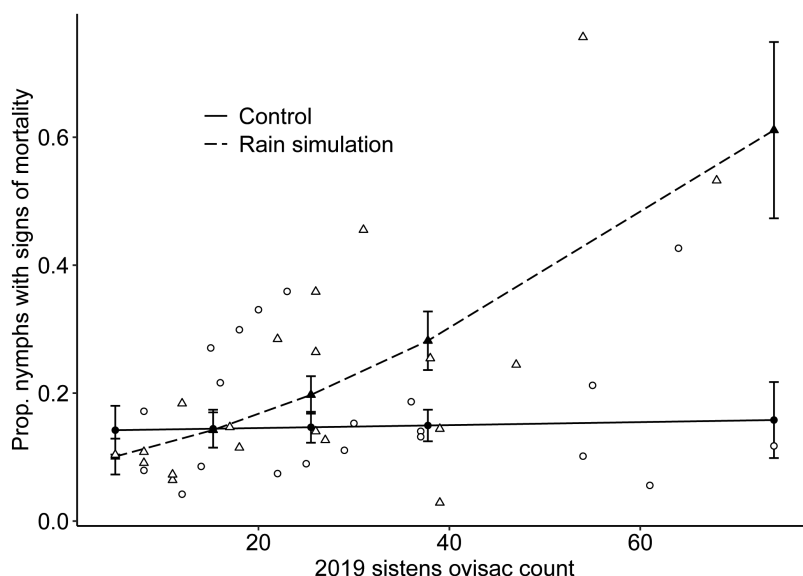


Fig. 5. The proportion of 2020 hemlock woolly adelgid sistens nymphs with signs of mortality as predicted by the interaction of rain simulation treatment and the number of 2019 sistens ovisacs present at the start of the experiment. Open shapes represent raw data and closed shapes represent least square means from a generalized linear mixed model. Triangles represent data from branches with simulated rainfall and circles represent controls.

predators are insufficient to significantly reduce HWA populations, especially across broad geographical regions at the same time, and successful biocontrol will likely require introduction of a suite of biocontrol agents including summer-active predators (Montgomery and Lyon 1996, Preisser et al. 2014, Crandall et al. 2020, Jubb et al. 2020). Research into establishment and potential effects of predators on HWA in New England is still ongoing, but any effect of predators on HWA summer mortality would not likely correlate with rainfall or exhibit only in years of high summer/autumn precipitation.

High rainfall in 2018 likely led to spread and propagation of fungal entomopathogens that reduced survival of aestivating sistens. Previous studies have identified naturally occurring entomopathogenic fungi that cause mortality of HWA in New England (Gouli et al. 1997, Reid et al. 2010). Furthermore, we have identified known entomopathogenic fungal genera (Reid et al. 2010) from HWA samples collected after the regional decline at our long-term CT-MA research sites by culturing samples of HWA nymphs and extracting DNA from pure cultures for DNA analysis (unpublished data). Previously, other invasive insect species in North America have become largely suppressed by entomopathogenic fungi in years of sufficient rainfall. For example, an inadvertently-introduced fungus, *Entomophaga maimaiga* Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) became the dominant source of mortality for the moth *Lymantria dispar* (L.) (Lepidoptera: Erebidae) (Hajek et al. 1990, 2015), vastly reducing the intensity and/or frequency of *L. dispar* outbreaks, especially in the northern part of its range in North America (Morin and Liebhold 2016). *E. maimaiga* causes *L. dispar* moth mortality that fluctuates with rainfall (Elkinton et al. 1991) and suppresses population levels below outbreak levels except in years of spring drought when moisture levels are too low to support fungal propagation (Reilly et al. 2014). In addition to affecting mortality of HWA from fungal attack, it is possible that the high rainfall of 2018 similarly contributed to the fungal epizootic (caused by two unrelated native fungal pathogens) documented in an outbreak population of spotted lanternfly (*Lycorma delicatula* White (Hemiptera: Fulgoridae)) in PA during Oct of 2018 (Clifton et al. 2019). Due to their efficacy as biocontrol agents, fungal entomopathogens have been introduced as agents of classical biological control of insect and mite pests (Hajek et al. 2021). It is possible that a native or recently-introduced species of fungus could emerge as an important source of mortality for HWA, reducing HWA density when summer rainfall is sufficient to allow for spread and propagation of fungi on aestivating adelgid nymphs, especially as precipitation is projected to increase in the northeastern U.S. with climate change (Kunkel et al. 2013). Multiple fungal species obtained from HWA cadavers in our rainfall simulation experiment have been cultured and isolated and future research will include identifying fungal isolates via DNA sequencing and testing their pathogenicity against HWA.

The 2018 decline in aestivating sistens survival in PA corroborates reports of a drastic regional decline in HWA density that occurred during the summer or autumn of 2018. Although 2018 summer temperatures were on the high end of the range of temperatures recorded at these sites across the past 10 yr, HWA thrives at lower latitudes in the U.S., despite the lack of evidence that HWA populations in the southern U.S. have adapted to higher summer temperatures (Mech et al. 2018). HWA mortality can reach 100% after exposure to high summer temperatures (i.e., 35 and 40°C) for 48 hours in laboratory studies (Mech et al. 2018) and temperature data derived from weather station observations indicate that maximum 2018 temperatures at our survey sites were as high as 37°C. While it is possible that summer temperatures contributed to summer mortality observed in 2018, summer maximum temperatures recorded at weather stations

can be more than 5°C above the actual temperature in the forest understory (Sussky and Elkinton 2015) and maximum daily temperatures are not sustained for 48 hours at a time. We acquired temperature data from datasets produced by PRISM (PRISM Climate Group 2021), which accounts for the effects of factors such as location, elevation, and coastal proximity while interpolating weather station data. However, the PRISM interpolation method does not weight weather data by station landcover type (Daly et al. 2008). Since many weather stations are in open rather than forested locations, the summer temperatures experienced by adelgids at our study sites were likely significantly lower than PRISM weather data indicates and were unlikely to have resulted in the high levels of summer mortality observed in 2018. Typically, landscape-scale HWA survival and spread is heavily influenced by winter minimum temperatures (Paradis et al. 2008, Trotter and Shields 2009). Such cold-induced winter mortality is evident in the 2014–2016 PA HWA density survey data when a polar vortex resulted in extreme low temperatures in February of 2015 (Cheah 2017). However, there is no evidence of temperatures in the winter of 2018 or 2019 sufficiently cold or of long-enough duration to induce above-average HWA mortality. Laboratory studies have found that HWA in our study area can survive short-term exposure to temperatures as cold as –30°C (Parker et al. 1998, Elkinton et al. 2017). Complete (100%) HWA mortality is likely to result from a mean winter temperature of –5°C, 93 d with a minimum temperature below –10°C, or an absolute minimum winter temperature of –40°C (Paradis et al. 2008). Mortality of 91% or more is required to inhibit expansion of HWA populations, which is likely to occur when there are 79 d or more with a minimum temperature below –10°C in a single winter or if the absolute minimum winter temperature is –35°C (Paradis et al. 2008), but none of these cold-tolerance thresholds were crossed during the two winters prior to our 2019 HWA survey (Supp Fig. 1 [online only]). Furthermore, models show that HWA mortality during the early spring is quickly negated by a reduction in density-dependent mortality of crawlers during the progrediens stage and therefore large intra-annual variation in population density is common while interannual density remains stable (Elkinton et al. 2012, McAvoy et al. 2017, Tobin et al. 2017, Crandall et al. 2020).

Our results strongly suggest that the 2018–2019 decline in HWA density observed in CT, MA, and PA occurred as a result of increased mortality that indirectly resulted from abnormally high rainfall during the summer and autumn of 2018 in the northeastern U.S. Application of knowledge of the effects of rainfall on HWA survival may lead to better management and prediction of spread and impact of HWA at a regional scale. Furthermore, isolation of fungi that mediated the HWA in response to high rainfall may yield potential novel biocontrol agents.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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