

2-2-2024

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### Recommended Citation

Crouch, Connor D., Hofstetter, Richard W., Grady, Amanda M., Edwards, Nylah N. S., Waring, Kristen M. "Oystershell scale (Hemiptera: Diaspididae) population growth, spread, and phenology on aspen in Arizona, USA" *Environmental Entomology*, 2024, pp. 1-12 <https://doi.org/10.1093/ee/nvae006>

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## Plant-Insect Interactions

# Oystershell scale (Hemiptera: Diaspididae) population growth, spread, and phenology on aspen in Arizona, USA

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Subject Editor: Christopher Ranger

Received on 26 October 2023; revised on 26 December 2023; accepted on 19 January 2024

Oystershell scale (OSS; *Lepidosaphes ulmi* L.) is an invasive insect that threatens sustainability of aspen (*Populus tremuloides* Michx.) in the southwestern United States. OSS invasions have created challenges for land managers tasked with maintaining healthy aspen ecosystems for the ecological, economic, and aesthetic benefits they provide. Active management is required to suppress OSS populations and mitigate damage to aspen ecosystems, but before management strategies can be implemented, critical knowledge gaps about OSS biology and ecology must be filled. This study sought to fill these gaps by addressing 3 questions: (i) What is the short-term rate of aspen mortality in OSS-infested stands in northern Arizona, USA? (ii) What are the short-term rates of OSS population growth on trees and OSS spread among trees in aspen stands? (iii) What is the phenology of OSS on aspen and does climate influence phenology? We observed high levels of aspen mortality (annual mortality rate = 10.4%) and found that OSS spread rapidly within stands (annual spread rate = 10–12.3%). We found first, second, and young third instars throughout the year and observed 2 waves of first instars (i.e., crawlers), one throughout the summer and a second in mid-winter. The first wave appeared to be driven by warming seasonal temperatures, but the cause of the second wave is unknown and might represent a second generation. We provide recommendations for future OSS research, including suggestions for more precise quantification of OSS phenology, and discuss how our results can inform management of OSS and invaded aspen ecosystems.

**Key words:** climate, invasive insect, life cycle, mortality, pest management

## Introduction

Oystershell scale (OSS; *Lepidosaphes ulmi* L.) is an invasive insect that threatens sustainability of aspen (*Populus tremuloides* Michx. [Malpighiales: Salicaceae]) populations in the southwestern United States (Crouch et al. 2021, Crouch 2023). Although the native range and introduction history of OSS are uncertain, the species was likely transported to North America by European settlers on infested plant material (Griswold 1925, Beardsley and Gonzalez 1975). OSS was first reported as a pest of apple trees (*Malus* spp.) in the 1700s and is now present throughout much of North America, especially in urban and ornamental settings (Griswold 1925, Miller and Davidson 2005b). Despite the species' polyphagous nature, its pervasiveness in North America, and its long invasion history, OSS has rarely been a

major pest in wildland forest settings (but see Sterrett 1915, DeGroot 1967, Houston 2001). OSS was first reported on aspen in wildland forest settings in Arizona, USA by Fairweather (1992) and Zegler et al. (2012). In both cases OSS abundance was low, and impacts were minimal. However, in 2016 OSS was observed causing dieback and mortality of aspen in wildland forest settings (Grady 2017), and severe outbreaks have since been observed in aspen ecosystems throughout Arizona and in other western states, including Nevada and Utah (Crouch et al. 2021, Crouch 2023).

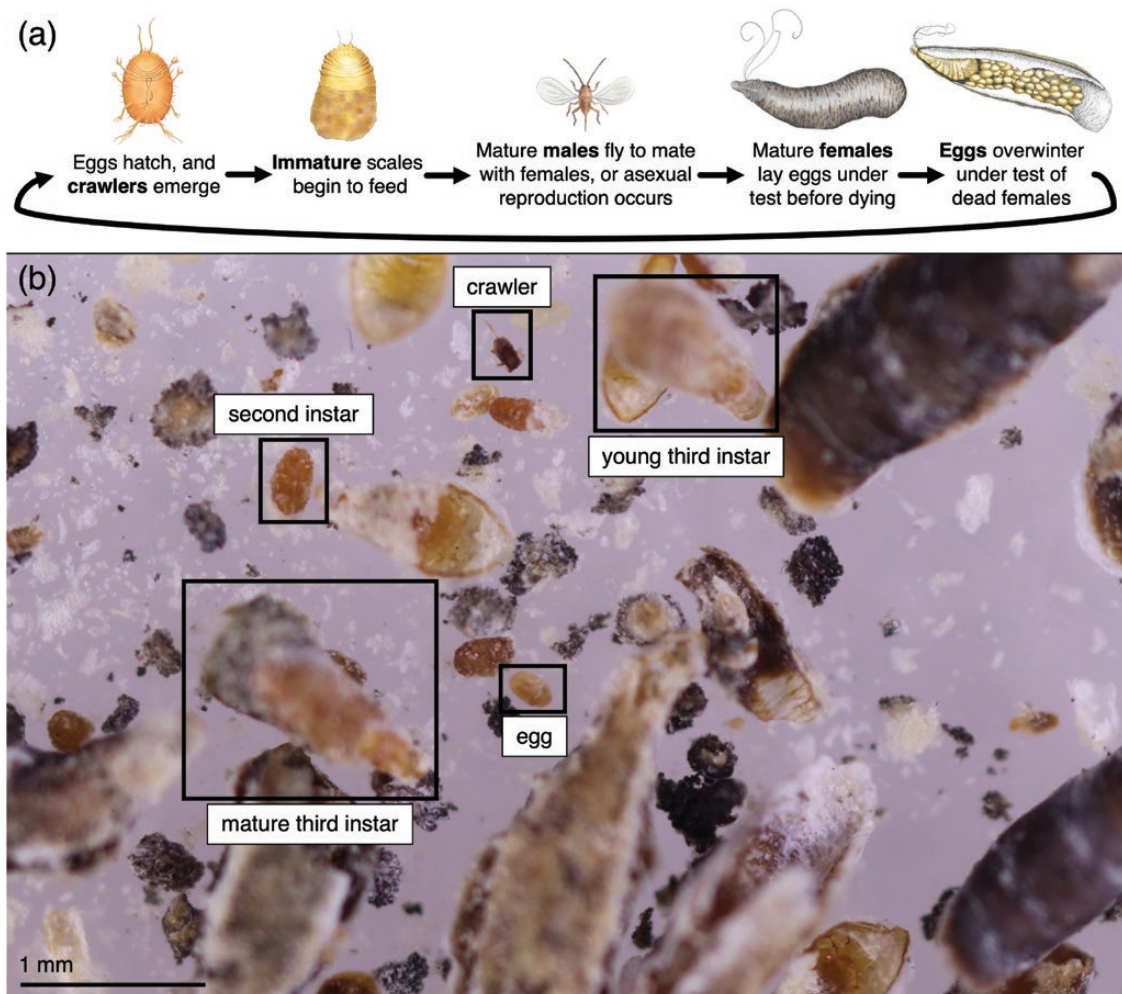
OSS is an armored scale that inserts its stylet through the bark of woody plants to feed on the fluid of nonvascular cells (Griswold 1925, Beardsley and Gonzalez 1975). OSS is polyphagous, feeding on approximately 100 host genera globally, and is most common

on woody, deciduous plants with relatively thin bark (Miller and Davidson 2005b). Twelve plant genera (*Acer*, *Alnus*, *Ceanothus*, *Cornus*, *Frangula*, *Fraxinus*, *Juglans*, *Lupinus*, *Populus*, *Ribes*, *Salix*, and *Symphoricarpos*) have been documented as hosts in the western United States (Crouch et al. 2021). As an obligate parasite, OSS completes its entire life cycle on woody tissues of living hosts (Samarasinghe 1965).

The exact timing of OSS phenology on aspen in Arizona is unknown (Crouch et al. 2021), but the general life cycle pattern is as follows (Fig. 1a). OSS overwinters as eggs beneath the tests of dead females (Beardsley and Gonzalez 1975), and eggs hatch in late spring or early summer, after which the newly emerged first instars (i.e., crawlers) begin actively dispersing along the host's stem or branches (Miller and Davidson 2005b). This dispersal stage lasts only a few days until a crawler finds a suitable feeding site, where it remains through adulthood (Griswold 1925). As crawlers mature, they develop a waxy outer shell, or test, which protects them from predators and adverse climatic conditions (Beardsley and Gonzalez 1975). In bisexual populations, mature males shed their tests and fly to mate with females; however, female-only populations occur and reproduce via parthenogenesis (Beardsley and Gonzalez 1975, Miller and Davidson 2005b). The female lays 50–100 eggs beneath her test, and

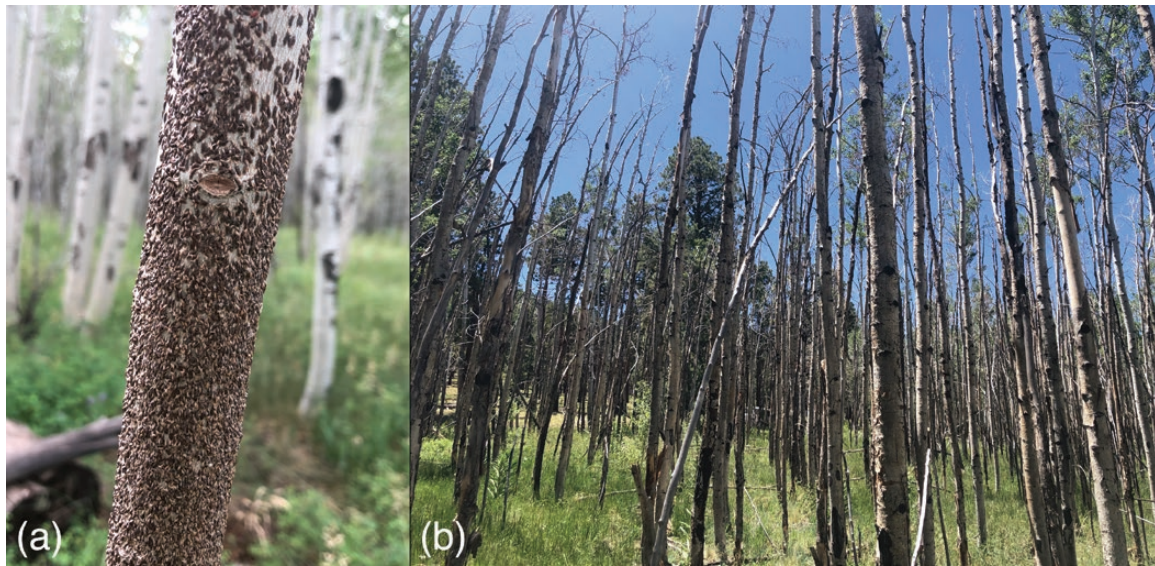
after which she dies, but the eggs remain protected under her test throughout the winter (Griswold 1925, Miller and Davidson 2005b). Questions remain about OSS dispersal mechanisms and distances, but long-distance dispersal is likely driven by human movement of infested plants, whereas short- and intermediate-distance dispersal are likely driven by wind, potentially with animal assistance (Griswold 1925, Beardsley and Gonzalez 1975, Magsig-Castillo et al. 2010). Crawlers do not actively move more than 1 m due to limited energy reserves and susceptibility to adverse climatic conditions (Beardsley and Gonzalez 1975, Magsig-Castillo et al. 2010).

OSS invasions of aspen ecosystems have created challenges for land managers who are tasked with maintaining healthy aspen ecosystems (USDA Forest Service 2014, 2018). Aspen ecosystems are highly valued because of their ecological importance (Campbell and Bartos 2001, Rogers et al. 2020), positive impact on local economies (McCool 2001, Rogers 2017), and aesthetic and cultural values (Dahms and Geils 1997, McCool 2001, Assal 2020). However, OSS threatens these values by contributing to mortality of aspen, especially of intermediate-sized, recruiting stems (Crouch 2023, Fig. 2a). Mortality of aspen recruits threatens long-term sustainability of aspen ecosystems because recruits are the aspen forests of the future (Crouch 2023). OSS invasions also complicate aspen



**Fig. 1.** a) The general pattern of OSS's life cycle. The exact timing of these stages on aspen in Arizona remains unknown, which is a need our study sought to address. Words in bold indicate the life stage shown in the accompanying drawing. b) Photo from 20 January 2022 showing each of OSS's 5 life stages. The white material in the background is woolly flocculants created by OSS (Crouch et al. 2021). OSS illustrations by D.E. DePinte, USDA Forest Service, Forest Health Protection.





**Fig. 2.** Photos showing a) a small aspen stem with a severe OSS infestation and b) aspen mortality in a stand heavily infested by OSS. Both photos were taken at the Nordic Village study site in 2021.

management because OSS is especially common in fenced ungulate exclosures (Crouch 2023), which are 2-m-tall fences built to exclude ungulates and are one of the most effective strategies for promoting aspen recruitment (Shepperd and Fairweather 1994, Bailey et al. 2007, Crouch et al. 2023). OSS invasions may render exclosures ineffective by disproportionately killing recruiting stems, the very size class exclosures are designed to promote (Crouch 2023). Active management is required to suppress OSS populations and mitigate damage to aspen ecosystems; however, before management strategies can be implemented, critical knowledge gaps about OSS biology and ecology must be filled (Crouch et al. 2021). OSS phenology is one such gap because knowing the timing of the crawler stage is imperative for properly timing management interventions (Beardsley and Gonzalez 1975). For example, if silvicultural tactics, such as sanitation thinning, are conducted when crawlers are active, managers may inadvertently spread OSS throughout the stand. In addition, some horticultural oils and insecticides are specifically targeted at the crawler stage, when OSS is the most vulnerable (Miller and Davidson 2005a, Quesada et al. 2018), making knowledge of OSS phenology a prerequisite for treatment implementation. Information on rates of OSS population growth and spread and on how quickly OSS causes aspen mortality is also critical for informing management because such information can help managers determine how quickly a stand may be overrun with OSS and which stands should be prioritized for treatment. However, rates of population growth, spread, and OSS-induced aspen mortality remain unknown (Crouch et al. 2021).

This study sought to fill these knowledge gaps by studying OSS-invaded aspen stands in northern Arizona. We addressed 3 questions about OSS biology and ecology that are critical for informing management of this high-impact invasive insect: (i) What is the short-term rate of aspen mortality in OSS-infested stands? (ii) What are the short-term rates of OSS population growth on trees and OSS spread among trees in wildland aspen stands? (iii) What is the phenology of OSS on aspen and does climate influence phenology?

## Materials and Methods

### Study Area

Our study was conducted at 2 sites in northern Arizona, which we refer to as the Nordic Village and Spring Valley study sites. The

Nordic Village study site (35°230 N, 111°460 W) was located 32 km north of Flagstaff, Arizona at an elevation of 2,455 m. This site consisted of 9 study plots in 2 ungulate exclosures, which were 2.8 and 6.8 ha in size and spaced 500 m apart. The Spring Valley study site (35°210 N, 111°580 W) was located 40 km northwest of Flagstaff and 18 km west of the Nordic Village site at an elevation of 2,285 m. This site consisted of 3 plots in 2 ungulate exclosures, which were 0.7 and 3.4 ha in size and spaced 80 m apart. Both study sites are located within the ponderosa pine (*Pinus ponderosa* var. *scopulorum*) forest type, in which small pockets of aspen occur on north-facing slopes or in drainages with increased water availability (Fairweather et al. 2008, Zegler et al. 2012). In Nordic Village, mean basal area of live aspen was 14.2 m<sup>2</sup> ha<sup>-1</sup>, and mean density of live aspen was 3763 trees ha<sup>-1</sup>. In Spring Valley, mean basal area was 0.3 m<sup>2</sup> ha<sup>-1</sup>, and mean density was 2,984 trees ha<sup>-1</sup>. Plots in Spring Valley occurred on Mollisol soils, whereas Nordic Village contained both Mollisols and Alfisols (Poggio et al. 2021). In Nordic Village from 1991 to 2021, mean annual temperature was 6.9 °C, and mean annual precipitation was 58.0 cm, 10.1 cm of which was attributed to snow water equivalent (Wang et al. 2016). In Spring Valley, mean annual temperature was 8.1 °C, and mean annual precipitation was 54.8 cm, 6.7 cm of which was attributed to snow water equivalent (Wang et al. 2016).

These study sites were included in the first peer-reviewed report of OSS in wildland aspen stands (Crouch et al. 2021) and were selected for this study because they were the first sites at which we obtained measurements of OSS presence and severity, allowing for the longest possible chronology of repeated measurements. In addition, the study sites were among the first areas where OSS was observed causing dieback and mortality of aspen in 2016 (Grady 2017), making these high-priority areas for OSS monitoring and management (Fig. 2b).

### OSS Population Growth, OSS Spread, and Aspen Mortality

In each of the 12 study plots, we assessed OSS presence, OSS severity, and aspen tree condition for 3 consecutive years. These data allowed us to quantify annual rates of OSS population growth on trees and OSS spread among trees as well as the rate of aspen

**Table 1.** Summary of sample sizes by study site for OSS phenology data collection, including numbers of study plots, large (>5 cm dbh) and small (<5 cm dbh) aspen stems from which OSS samples were collected, collection dates and vials containing OSS collected, and photos taken of OSS samples

Study site	Sample size					
	Study plots	Large stems	Small stems	Collection dates	Vials collected	Photos analyzed
Nordic Village	9	26	7	25	915	2,608
Spring Valley	3	9	4	19	416	1,172
<b>Total</b>	<b>12</b>	<b>35</b>	<b>11</b>	<b>28</b>	<b>1,331</b>	<b>3,780</b>

mortality. These data were collected in June–August 2020, 2021, and 2022. Each of the 9 monitoring plots consisted of 2 fixed-area, circular plots: an overstory plot (8 m radius) and a nested regeneration plot (4 m radius) sharing the same plot center (Zegler et al. 2012). All trees with dbh  $\geq 10.1$  cm were measured in the 8 m overstory plot, whereas all trees  $<10.1$  cm in dbh were measured in the 4 m regeneration plot. In the regeneration plot, we classified stems into 3 size classes: short regeneration ( $<1.4$  m tall), tall regeneration ( $>1.4$  m tall and  $<5.1$  cm dbh), and saplings (5.1–10.1 cm dbh) (Zegler et al. 2012). For each aspen tree in the monitoring plots, we recorded tree condition (i.e., live or dead), height, dbh, and OSS presence and severity. We assessed OSS severity using the system devised by Crouch et al. (2021), which rates the severity of OSS on each tree from ground level to 6 m. Each tree's stem up to 6 m was divided into thirds, and severity was rated for each 2 m section (or shorter for trees  $<6$  m tall) on both the north and south sides of the tree, resulting in 6 ratings for each tree. Severity was rated from 0 to 3: 0 = no OSS present (light), 1 = only a handful of OSS present (trace), 2 = OSS covers  $<50\%$  of section (light), 3 = OSS covers  $>50\%$  of section (severe). For stems sampled for OSS phenology, we assumed that the scraped off areas had similar OSS severity as the surrounding areas on the stem. To convert the 6 ratings for each tree into a single quantitative variable representing OSS severity, we calculated the mean percentage of each rating (i.e., 1% for 1, 25% for 2, and 75% for 3) and averaged the 6 ratings for each tree. Thus, OSS severity ranged from 0 to 0.75, with 0 indicating a tree without OSS and 0.75 indicating a tree with the highest possible severity rating (i.e., OSS covering  $>50\%$  of all 6 stem sections rated).

To quantify OSS population growth, we summarized mean OSS severity on aspen stems in each of the 3 measurements periods. We used repeated measures analysis of variance (ANOVA) to determine whether there were significant changes in OSS severity over time. Specifically, we used the *nlme* package (Pinheiro et al. 2022) to fit a linear mixed-effects model with OSS severity as the response, measurement year as the fixed effect, and the hierarchical nested structure of our data (i.e., trees [ $n = 366$ ] within plots [ $n = 12$ ] within sites [ $n = 2$ ]) as a random effect. We used the “*anova*” function in R (R Core Team 2022) to conduct repeated measures ANOVA and the *emmeans* (Lenth 2022) and *multcomp* (Hothorn et al. 2008) packages to conduct Tukey-adjusted, post hoc pairwise comparisons. We also used the same ANOVA procedure, without the repeated measures component, to determine if there were differences in OSS severity between the north and south sides of aspen stems. Thus, OSS severity was the response, tree side was the fixed effect, and the hierarchical nested structure of our data was a random effect. To explore the influence of climate on OSS population growth, we obtained precipitation, temperature, and VPD data for both of our study sites from the PRISM database (Daly et al. 2008) and calculated mean values for each of the 3 study years at both sites.

To quantify OSS spread and aspen mortality, we relied on descriptive statistics, specifically means and standard errors, to calculate rates of spread and mortality. We analyzed all data in R version 4.2.1 (R Core Team 2022), using the *dplyr* package (Wickham et al. 2022) for data manipulation and the *ggplot2* package (Wickham 2016) for figure creation.

### OSS Phenology

To document the timing of OSS's life cycle, we collected OSS samples from the 2 study sites at regular intervals from April 2021 to April 2022. During the growing season from April to October 2021, we collected samples approximately every 2 wk (range 10–15 days). In October 2021, we switched to sampling roughly every 3 wk (range 16–24 days) until April 2022 because we expected less life cycle activity to occur outside of the growing season and because of site access challenges due to snow accumulation. We collected OSS samples from 33 trees in 9 plots at the Nordic Village site (2–5 trees/plot) and from 13 aspen trees in 3 plots at the Spring Valley site (4–5 trees/plot) (Table 1). Plots were phased into the study in a piecemeal fashion in spring 2021 as they became accessible with snow melt, and plots were also phased out in a piecemeal fashion in winter 2021–2022 as snow accumulated and sites again became inaccessible. Study trees were selected by locating the first 3 large aspen trees ( $>5$  cm diameter at breast height [dbh]; height = 1.37 m) and the first 3 small aspen trees ( $<5$  cm dbh) nearest plot center. We initially selected 66 trees before the growing season began, but 20 of them proved to be dead. We excluded these dead trees from our study. We selected trees with relatively severe OSS infestations to ensure that enough OSS was present for samples to be collected regularly throughout the year; however, severe infestations were not present in every plot, necessitating selection of trees with lighter infestations. On the large tree closest to plot center, we installed 2 climate data loggers (Onset HOBO Pro v2), 1 each on the north and south sides of the stem. These loggers, which were located 2 m above ground level, recorded temperature and relative humidity every 15 min throughout the duration of the study.

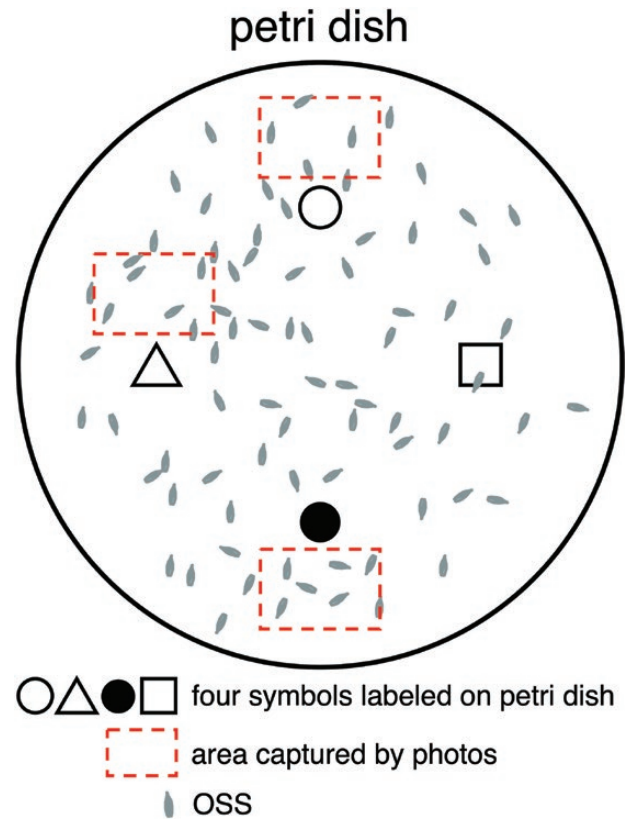
To collect OSS from the study trees for phenology analysis, we scraped approximately 1 ml in volume of OSS into vials, which were 12 cm tall and 1.2 cm wide, for each of the 46 study trees during each sampling visit (Table 1). We based our sample collections on volume, rather than area, because we wanted to ensure we collected adequate OSS for photo analysis. Less than 1 ml was scraped into the vial for some trees, such as smaller trees with lighter infestations or trees later in the study period when their OSS populations had depleted. One sample was obtained from small trees around the entire circumference of the stem when possible, whereas 2 samples were taken from large trees, 1 each on the north and south sides of the stem. An important limitation of our sampling approach is that we collected a portion of all OSS individuals on a given tree, not just the individuals that emerged during the study period. Using this



method, we collected individuals, including dead ones, that were established on study trees prior to the measurement period in addition to those that were newly established during the study period. During sampling, we tried to collect from patches of OSS that were clearly newer and likely alive, rather than from patches of OSS that had likely established in previous years and were clearly dead at the time of sampling. To help us distinguish between previously and newly established OSS, we cleared a section of OSS on each stem to provide a reference area in which only OSS from the study year would recolonize. On small trees, this cleared section was 10 cm in height and wrapped around the circumference of the stem. On large trees, we cleared a 10 cm × 10 cm section on both the north and south sides of the stem. These sections were located at breast height (1.37 m) for large trees or at the center of the stem, which was 1.37 m in height or less, for small trees. Once these cleared sections began to be colonized by new OSS, we scraped the entire section of OSS into a vial to serve as a reference sample. These reference samples helped us determine which OSS individuals we collected in the nonreference samples were established during the study period versus which ones were present on trees before our study began. In total, 39 reference samples were collected from June to December 2021. After collection, OSS samples were kept in cold storage for laboratory analysis.

We took photos of the OSS in each sample and analyzed the photos to determine which life stages were present. To take photos of the samples, each sample vial was emptied onto a petri dish, and the OSS were spread into a single layer on the dish. The petri dish was labeled using 4 different symbols, and up to 3 photos were taken either directly above or below 3 randomly selected symbols, whichever direction had more OSS (Fig. 3). This allowed us to obtain a stratified random sample from each sample vial. Some vials contained insufficient OSS to take 3 photos, such as for smaller trees with lighter infestations or for trees later in the study period that had depleted OSS populations. In these cases, only 1 or 2 photos were taken. We took photos using a Canon 6D/Macro 100 mm lens, with each photo capturing an area of 4 mm × 6 mm. Once the photos were obtained, we analyzed each photo by counting the number of individuals present in each life stage, including eggs, first instars (i.e., crawlers), second instars, young third instars, and mature third instars (Miller and Davidson 2005a, 2005b, Fig. 1b).

We calculated the proportion of each of these life stages present in each photo and used descriptive statistics to summarize changes in the proportion of life stages present in each of the 13 mo we collected samples. We averaged the temperature and relative humidity data obtained from each plot at a daily resolution, allowing us to compare OSS phenology and climate patterns. Because we wanted to explore the relationship between OSS phenology and climate in more depth, we calculated correlation coefficients between proportion of crawlers observed each month, local mean temperature and humidity obtained from climate data loggers installed in each plot, and additional downscaled climate data obtained at the site level from the PRISM database (Daly et al. 2008) at a monthly resolution. These downscaled climate variables included mean precipitation, minimum and maximum vapor pressure deficit (VPD), and minimum and maximum temperatures. We then used the *nlme* package (Pinheiro et al. 2022) to fit 7 linear mixed-effects models with OSS crawler density as the response, each of the 7 climate variables as predictors, and the sampling month as a random effect. We selected crawlers as the life stage of interest for this correlation analysis because, unlike later life stages, crawlers are more prone to desiccation (Miller and Davidson 2005a) and less likely to persist on trees after dying than other life stages. Therefore, crawlers should provide the clearest phenological signal of all the life stages.



**Fig. 3.** Diagram of petri dish and sampling method used to take photos of OSS samples for phenological analysis. Vials of OSS were emptied onto the petri dish, and the OSS were spread into a single layer. The petri dish was labeled using 4 different symbols, and up to 3 photos were taken either directly above or below 3 of the randomly selected symbols, whichever direction had more OSS.

## Results

### Aspen Mortality

In 2020, there were 366 live aspen trees across the 12 study plots. Before the 2021 survey, 48 of those aspen died, and between the 2021 and 2022 surveys, an additional 28 aspen died, resulting in an annual mortality rate of 10.4% (SE = 1.6%) (Table 2). Of the 76 aspen that died, 24 were short regeneration, 21 were tall regeneration, 15 were saplings, and 16 were overstory trees. OSS was present on all but 5 of the 76 trees that died, and the 5 that lacked OSS were all short regeneration stems. Looking at all 76 trees that died, mean OSS severity in the year before death was 0.32 (SE = 0.05). Short regeneration stems that died had the lowest mean OSS severity (mean = 0.09, SE = 0.06), followed by saplings (mean = 0.33, SE = 0.12), tall regeneration (mean = 0.45, SE = 0.11), and overstory trees (mean = 0.50, SE = 0.12). For comparison, mean OSS severity for trees that remained alive throughout the study was 0.20 (SE = 0.01). Short regeneration that remained alive had the lowest mean OSS severity (mean = 0.03, SE = 0.02), followed by saplings (mean = 0.19, SE = 0.03), overstory trees (mean = 0.22, SE = 0.02), and tall regeneration (mean = 0.29, SE = 0.06).

### OSS Population Growth

Mean OSS severity for the aspen that were alive in 2020 was 0.21 (SE = 0.01). In 2021, severity increased to 0.27 (SE = 0.01), which included trees that died between sampling in 2020 and 2021

**Table 2.** Summary of aspen mortality across 4 stem size classes in each sampling year

Size class	# Trees alive (# trees that died) in each sampling year			Annual mortality rate (%)
	2020	2021	2022	
Overstory trees	201	196 (5)	185 (11)	4.0
Saplings	72	62 (10)	57 (5)	10.4
Tall regeneration	40	23 (14)	20 (7)	26.3
Short regeneration	53	34 (19)	24 (5)	22.6
<b>Total</b>	<b>366</b>	<b>315 (48)</b>	<b>286 (28)</b>	<b>10.4</b>

Aspen stem size classes: short regeneration (<1.37 m tall), tall regeneration (>1.37 m tall and <5.1 cm dbh), saplings (5.1–10.1 cm dbh), overstory trees (>10.1 cm dbh).

(Fig. 4a). In 2022, severity decreased to 0.19 (SE = 0.01), again including trees that died between sampling in 2021 and 2022. Sample sizes decreased over time (366 trees in 2020, 349 in 2021, 313 in 2022) due to study trees dying. Repeated measures ANOVA indicated that there were significant ( $F = 73.8$ ;  $df = 2$ ;  $P < 0.001$ ) differences in OSS severity across the 3 measurement periods. OSS severity in 2021 was significantly greater than in 2020 and 2022, and severity in 2020 was significantly greater than in 2022 (Fig. 4b). When comparing differences in OSS severity between tree sides, north sides had significantly ( $F = 365.7$ ;  $df = 1$ ;  $P < 0.001$ ) greater severity (mean = 0.25, SE = 0.01) than south sides (mean = 0.18, SE = 0.01) based on ANOVA.

### OSS Spread

During the first survey in 2020, 25 of 366 live aspen were not infested with OSS across the 12 study plots. Only 2 of these uninfested stems were located in the Nordic Village site, while the remaining 23 were located in the Spring Valley site (Table 3). Uninfested stems represented 51.1% (SE = 7.5%) of all live stems in Spring Valley and only 0.6% (SE = 0.4%) of live stems in Nordic Village in 2020. One of the 2 uninfested stems in Nordic Village, an overstory tree, became infested in 2021, while the other stem, which was short regeneration, died before the 2021 survey. In Spring Valley, 4 of the 23 uninfested stems became infested in 2021, and no new stems were infested in 2022. Thus, across both study sites, OSS spread to 20.0% (SE = 8.0%) of previously uninfested stems during the 2-yr study period (Table 3). OSS also spread to aspen suckers that regenerated after the 2020 survey. In 2021, 39 new suckers established in the study sites, and in 2022, 6 new suckers established. Of these 45 new stems, 12 became infested by OSS: 8 in 2021 and 4 in 2022. When considering these newly established stems, the rate of OSS spread to uninfested stems was 24.3% (SE = 5.1%) over the 2-yr study period (Table 3).

### OSS Phenology

During our first collections in April 2021, most individuals were eggs or mature third instars (Fig. 5). Throughout the summer, density of eggs decreased, whereas density of mature third instars increased, before density of both life stages stopped fluctuating in the fall and remained relatively static thereafter. A few crawlers were present in April, and their presence increased through July when crawlers made up 1.5% (standard error [SE] = 0.2%) of samples (Fig. 6a). Presence of second instars and young third instars also increased during the summer (Fig. 6a) as temperature and relative humidity increased (Fig. 6b and c). The first wave of crawlers tapered out in the fall, with crawler densities approaching zero in October and November 2021. However, a second wave of crawlers emerged mid-winter in

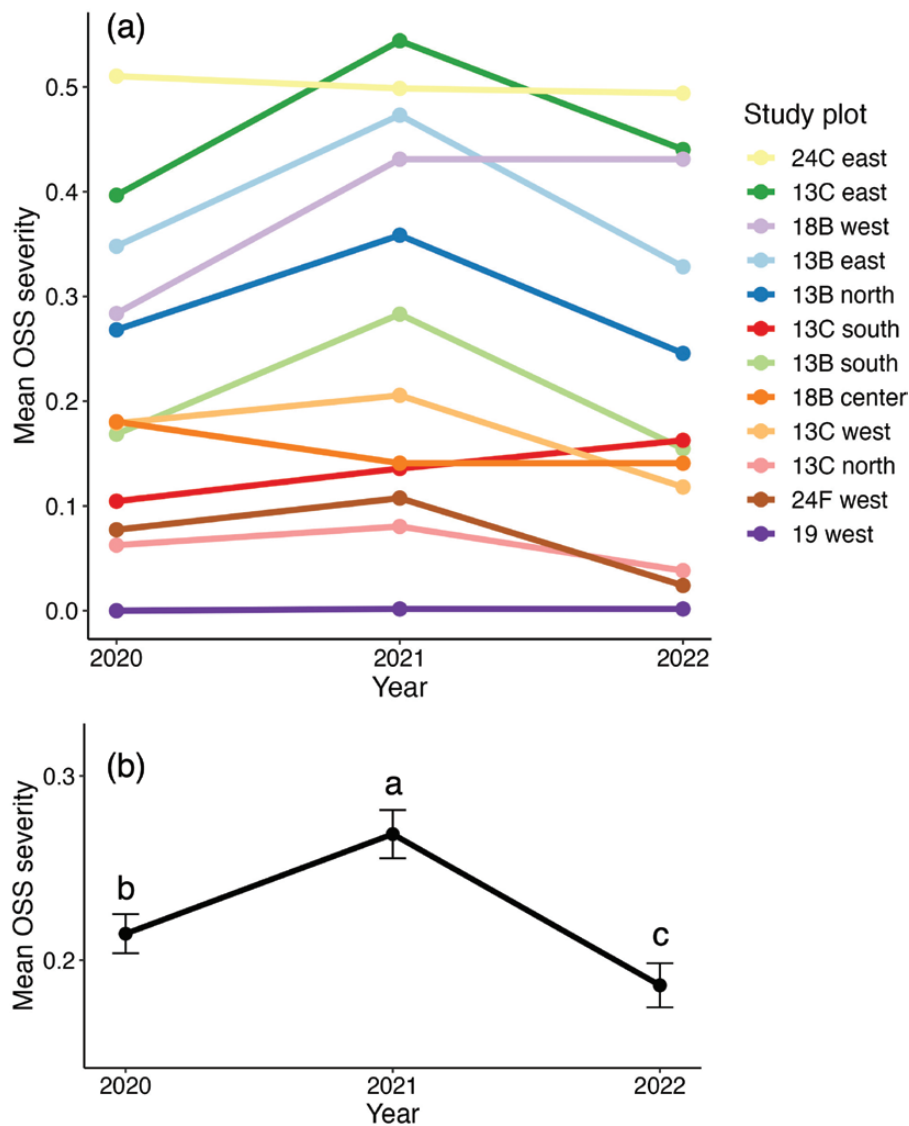
both study sites, reaching a peak in January 2022 that approached the summer 2021 peak in density. Second instar and young third instar densities also decreased in fall and early winter. Second instar densities remained low throughout winter with increases in December, January, and March. Young third instars reached a clear second peak in March 2022.

Climate variables, particularly those capturing temperature, had strong relationships with proportion of crawlers observed in our study sites (Table 4). This included local climate data collected from plot-level climate data loggers as well as downscaled climate data obtained at the site level from PRISM (Daley et al. 2008). Mean, minimum, and maximum monthly temperatures all had significant ( $P \leq 0.021$ ) positive relationships with crawler density, as did maximum VPD ( $P = 0.021$ ). Mean relative humidity, precipitation, and minimum VPD had insignificant relationships ( $P \geq 0.111$ ) with crawler density.

OSS phenology varied little between the 2 study sites. Both sites experienced peak crawler emergence in July, with a second crawler emergence in mid-winter, although the winter peak occurred in December for Spring Valley and in January for Nordic Village. Comparing samples taken on the north versus the south side of trees, there was generally more crawler activity observed on the north side (mean = 0.8%, SE < 0.01%) than on the south side (mean = 0.6%, SE < 0.01%). The same was true for second (north mean = 1.7% [SE = 0.01%], south mean = 0.7% [SE < 0.01%]) and young third instars (north mean = 4.6% [SE = 0.02%], south mean = 3.1% [SE = 0.02%]), whereas differences in tree side patterns for eggs and mature third instars were negligible (data not shown).

### Discussion

Based on existing studies of OSS phenology on other hosts, we expected OSS to progress through a distinct univoltine life cycle, with a wave of crawlers followed by a wave of second instars and so on. Previous studies of OSS in western North America have documented OSS egg hatch in May through July (Schuh and Mote 1948, Madsen and Arrand 1971, Spackman 1980), and we did not find evidence in the literature of OSS egg hatch lasting more than 2 mo or of multiple generations occurring in western North America. Therefore, our study is the first to document the persistence of first, second, and young third instars throughout the year, albeit at low densities compared to eggs and mature individuals. Our observation of continued crawler emergence during winter was a particularly novel finding (Fig. 6a). We observed an initial wave of crawlers in June through August, which coincided with warming seasonal temperatures and tapered off in the fall as temperatures decreased (Fig. 6b). Indeed, increased crawler density was significantly associated with warmer mean, minimum, and maximum temperatures



**Fig. 4.** Mean OSS severity on aspen trees through time a) in each of the 12 study plots and b) averaged across all study plots, with different letters indicating significant differences ( $P < 0.05$ ) among years based on repeated measures ANOVA. Across all 12 plots, OSS severity in 2021 was significantly greater than in 2020 and 2022, and severity in 2020 was significantly greater than in 2022. OSS severity was assessed using the system devised by Crouch et al. (2021) (see *Methods* for details on the rating system and subsequent severity calculations). OSS severity ranged from 0 to 0.75, with 0 indicating a tree without OSS and 0.75 indicating a tree with the highest possible severity rating (i.e., OSS covering >50% of all 6 stem sections rated).

**Table 3.** Summary of OSS spread, showing the number of uninfested and newly infested (in parentheses) aspen trees during each sampling year, along with the cumulative infestation rate

Study site	# Trees uninfested by OSS (# trees newly infested by OSS)			Cumulative infestation rate (%)
	2020	2021	2022	
Nordic Village <sup>orig</sup>	2	0 (1)	0 (0)	50.0
Spring Valley <sup>orig</sup>	23	12 (4)	12 (0)	17.4
<b>Total<sup>orig</sup></b>	<b>25</b>	<b>12 (5)</b>	<b>12 (0)</b>	<b>20.0</b>
Nordic Village <sup>new</sup>	2	12 (9)	12 (4)	42.9
Spring Valley <sup>new</sup>	23	31 (4)	31 (0)	11.9
<b>Total<sup>new</sup></b>	<b>25</b>	<b>43 (13)</b>	<b>43 (4)</b>	<b>24.6</b>

<sup>orig</sup> indicates count for only the trees that were established at the beginning of our study in 2020.

<sup>new</sup> indicates count for all trees including new aspen that established after 2020.



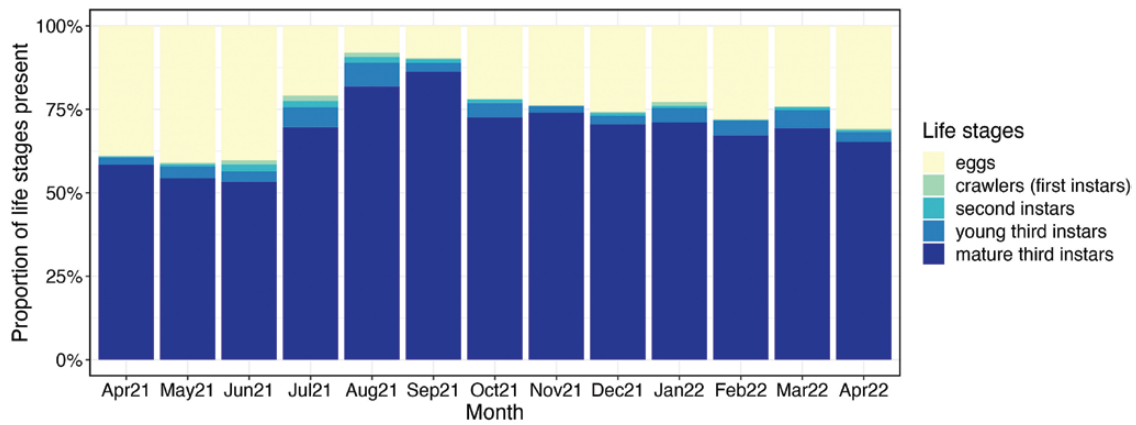


Fig. 5. Proportion of OSS life stages present in samples collected from aspen trees in northern Arizona, summarized by month from April 2021 to April 2022.

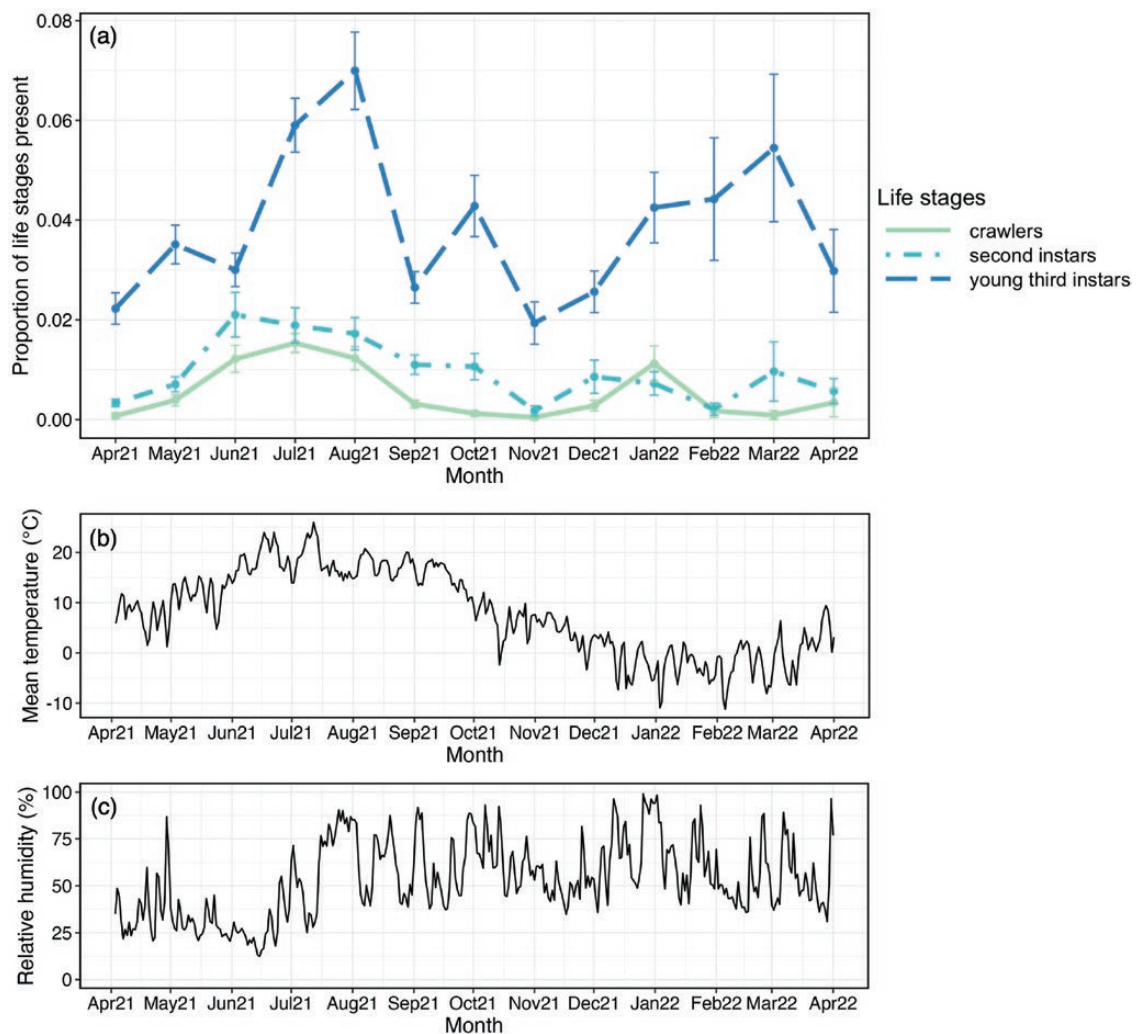


Fig. 6. a) Proportion of OSS crawlers, second instars, and young third instars in samples collected from aspen trees from April 2021 to April 2022 alongside mean daily b) temperature and c) relative humidity during the study period. These climate data were obtained from local data loggers placed in each of the 12 study plots.

and with greater VPD, all of which were obtained as downscaled climate data except for mean temperature, which was obtained locally. This pattern is consistent with the previously documented relationship between OSS egg hatch and accumulation of degree-days

(Kozár 1990), although our study and Kozár's (1990) used different methods. The second wave of crawlers observed in January is unprecedented in the OSS literature and, perplexingly, did not coincide with an increase in temperature. Humidity was relatively high before

**Table 4.** Univariate relationships between proportion of first instars (i.e., crawlers) observed in our study samples and 7 climate variables, all of which were summarized at a monthly resolution

Predictor	Correlation coefficient	Model coefficient	Standard error	P value	Marginal R <sup>2</sup>
Mean temperature (°C) <sup>a</sup>	0.45	0.0004	0.0002	0.021	0.12
Relative humidity (%) <sup>a</sup>	0.07	<0.0001	0.0001	0.879	<0.01
Precipitation (mm) <sup>b</sup>	0.35	<0.0001	< 0.0001	0.111	0.09
Minimum temperature (°C) <sup>b</sup>	0.51	0.0006	0.0002	0.002	0.19
Maximum temperature (°C) <sup>b</sup>	0.47	0.0005	0.0002	0.011	0.15
Minimum VPD (hPa) <sup>b</sup>	0.31	0.0014	0.0011	0.204	0.06
Maximum VPD (hPa) <sup>b</sup>	0.43	0.0005	0.0002	0.021	0.13

<sup>a</sup>Local climate data obtained at the plot-level from data loggers installed on the north and south sides of 1 aspen tree in each study plot.

<sup>b</sup>Downscaled climate data obtained at the site level from PRISM (Daly et al. 2008) at a monthly resolution.

Marginal R<sup>2</sup> is based solely on the linear mixed model's fixed effects, which was the climate variable.

this second crawler wave, with a 10-day period from December 26 to January 4 when mean humidity exceeded 80% (Fig. 6c). However, the summer wave of crawlers emerged during a dry period, and humidity had an insignificant relationship with crawler density, which casts doubt upon the role of humidity in promoting OSS egg hatch.

The prolonged summer crawler wave, which exceeded 3 mo, and the second wave in winter could be explained by diapause and/or the initiation of a second generation. Diapause is a temporary state of dormancy triggered by adverse environmental conditions (Tougeron 2019) and has been observed in certain armored scale species (Beardsley and Gonzalez 1975), including OSS (Fountain et al. 2012). However, diapause in scale insects has only been documented in summer (Beardsley and Gonzalez 1975, Fountain et al. 2012), which would fail to explain the winter wave of OSS crawlers, unless OSS in Arizona experiences winter diapause. Instead, the second wave may be a second generation of OSS, a phenomenon that has been documented in Europe and eastern North America (Schmutterer 1951, Turnipseed and Smith 1953, Garrett 1972, Miller and Davidson 2005b). Because we only studied 1 yr of OSS phenology, we cannot say with certainty that the patterns we observed occur every year. The winter wave of crawlers may have been a 1-yr anomaly, although the fact that we observed this second wave at both studies sites is notable. Further research into patterns and drivers of OSS phenology is needed to determine if the second wave of OSS crawlers occurs every winter in Arizona and possibly in other areas where OSS outbreaks have recently occurred. If the winter wave of crawlers is observed in other years and/or areas, further research is warranted to determine whether the second wave is a second generation or is caused by unprecedented winter diapause and to assess which factors, climatic or otherwise, cause this second wave of crawlers to emerge.

OSS second instar density generally mirrored that of crawlers, albeit at higher densities, whereas patterns of young third instar density did not track neatly with crawler emergence. This could be due to challenges in identifying young third instars, long-term persistence of this life stage on aspen stems even after dying, or drawbacks with the sampling method we used. In contrast to eggs, crawlers, and second instars, distinguishing between young third and mature third instars is less precise because of the visual similarities between these life stages (Fig. 1b). Young third instars might also persist longer on aspen stems than crawlers, which becomes a potential issue when considering our sampling approach. We collected a portion of all OSS individuals on a given tree, which meant our samples included individuals that established on study trees prior to the measurement period and were likely dead at the time of sampling in addition to those that were newly established during the study period.

This could explain the relatively high density of young third instars throughout the year, even when crawlers and second instars were almost completely absent. Three approaches to OSS collection might result in a more precise assessment of the species' phenology. First, sticky traps could be used more precisely detect crawler emergence (Fountain et al. 2012). Second, scraping off small reference areas of OSS (<10 cm × 10 cm) and tracking these cleared reference areas with high-resolution photos, rather than collecting the re-colonizing OSS as we did, might be more effective for tracking OSS development throughout the year. Finally, live samples of aspen branches or stems infested with OSS might be tracked for development of OSS life stages, although killing aspen branches or stems might affect OSS development.

OSS severity increased between 2020 and 2021, but we observed a significant decrease in OSS severity in 2022, the third and final year of our study (Fig. 4b). We have 4 hypotheses regarding why severity declined in 2022. First, OSS severity may have decreased because the most severely infested trees may be the most likely to die (Crouch 2023), resulting in a perceived reduction in stand-level OSS severity on the remaining live trees. However, we explored the effect of removing trees that eventually died from our dataset, and although the size of the decrease in OSS severity was smaller when excluding trees that eventually died, the overarching pattern of a decrease in OSS severity in 2022 remained. Second, the decline in OSS could be due to a density-dependent population collapse. Our study plots had relatively high rates of OSS infestation and severity compared to other OSS-invaded aspen ecosystems (Crouch 2023), perhaps because these plots were among the first to experience OSS outbreaks (Crouch et al. 2021). Thus, the observed decline in OSS severity could be due to a lack of suitable sites for new OSS to colonize. If this were the case, though, plots with the highest OSS severity in 2020–2021 should have had the greatest decrease in severity in 2022, which was not a consistent pattern among study plots (Fig. 4a).

Given the lack of support for these first 2 hypotheses, we suspect that climate or the winter wave of crawler emergence caused OSS severity to decrease in 2022. Climate is the most important driver of OSS abundance at the stand-level, with warmer and drier conditions resulting in more OSS (Crouch 2023). On average, 2022 was wetter at both sites, with higher precipitation and lower minimum and maximum VPD than 2020 and 2021 (Table 5), lending support to our hypothesis that a less favorable climate drove the decline in OSS severity in 2022. Alternatively, the wave of crawlers that emerged in winter 2021–2022 may have experienced high mortality due to cold temperatures. If this was the case, then losing a substantial portion of the next OSS generation might also explain

**Table 5.** Downscaled climate data obtained from the PRISM database (Daly et al. 2008) for both our study sites during each measurement year

Study site and sampling year	Precip (mean)	Temp (min)	Temp (mean)	Temp (max)	VPD (min)	VPD (max)
NV 2020	39.5	0.3	8.1	16.0	2.6	16.4
NV 2021	35.0	0.5	8.3	15.9	2.5	15.8
NV 2022	49.1	0.9	8.1	15.3	1.9	13.7
SV 2020	41.2	0.8	8.7	16.5	2.7	17.3
SV 2021	37.6	1.0	8.9	16.9	2.7	17.3
SV 2022	48.0	1.6	9.0	16.4	2.1	15.3

Study site abbreviations: NV (Nordic Village), SV (Spring Valley). Climate abbreviations: precip (precipitation), temp (temperature), VPD (vapor pressure deficit). Climate units: precip (mm), temp (°C), VPD (hPa). Climate data for each study year represent means from the previous September (e.g., 2019 for sampling year 2020) to August of the present sampling year (e.g., 2020) because August was the latest that we assessed severity in our study plots in any year.

why severity significantly decreased the following summer. To better understand the rate of OSS population growth, more research must be conducted in stands with lower levels of infestation. The same is true for understanding rates of OSS spread because our findings related to spread were limited due to high levels of infestation at the start of the study.

We observed higher OSS severity and increased life cycle activity on the north side of aspen trees compared to the south side, confirming initial observations that OSS tends to be more abundant on the north side of trees (Crouch et al. 2021). These initial observations explain why the OSS severity rating system assesses the north and south sides of stems separately (Crouch et al. 2021), and our study suggests that this approach is merited due to differences in OSS severity and development between tree sides. To determine if these differences were driven by microclimate, we installed climate data loggers on the north and south sides of stems. However, there were only minor differences in climate between the 2 sides. On average, north sides were 0.2 °C cooler (SE = 0.02) and 0.3% more humid (SE = 0.05) than south sides. Therefore, we suspect that less direct sunlight, rather than a cooler or more humid microclimate, drove increased OSS development and severity on the north versus south sides of trees. Other scale insect species have shown a preference for feeding sites exposed to less sunlight (Gentile and Summers 1958, Sussky and Elkinton 2015, Mech et al. 2018, Mayfield and Jetton 2020). If this is true for OSS, thinning infested aspen stands may decrease aspen susceptibility to OSS because a more open canopy resulting in increased sunlight exposure to stems might make trees less suitable for OSS infestation. However, sudden, direct exposure of aspen stems to sunlight after thinning may cause sunscald (Krasnow et al. 2012), creating potential infection courts for pathogens and leading to mortality. Aspen is also sensitive to mechanical damage from logging equipment and susceptible to subsequent infection by canker-causing fungi and decay diseases (Walters et al. 1982, Jones and Shepperd 1985), so thinning must be implemented with care.

We observed an annual aspen mortality rate of 10.4%, which is relatively high compared to previously documented rates of aspen mortality in Arizona and to rates of tree mortality from other invasive insects and diseases. For example, Fairweather et al. (2008) documented an annual aspen mortality rate of 7.9% from 2000 to 2007 during an acute aspen mortality event caused by interactions among drought, a severe frost event, defoliation by western tent

caterpillar (*Malacosoma californicum*), and other factors. Similarly, Kane (2012) documented an annual aspen mortality rate of 5.2% from 1996 to 2008 in mixed-conifer forests. Our observed mortality rate was higher than both these studies, although our observation period was shorter, and our study area was smaller. For further comparison, the documented annual rate of mortality of eastern hemlock (*Tsuga canadensis*) from hemlock woolly adelgid (*Adelges tsugae*) is ~2–12% (Eschtruth et al. 2006, Ford et al. 2012), of coast live oak (*Quercus agrifolia*) and California black oak (*Quercus kelloggii*) from *Phytophthora ramorum* is ~3–5% (Cobb et al. 2020), and of American elm (*Ulmus americana*) from Dutch elm disease (*Ophiostoma novo-ulmi*) is ~10–20% (Hauer et al. 2020). Again, many of these studies were conducted at larger spatial and longer temporal scales than our study, and our observed 10.4% annual aspen mortality rate is likely on the high end of OSS-induced mortality because OSS presence and severity were relatively high in our study sites (Crouch 2023). Despite these caveats, our study is the first to estimate annual mortality rates of aspen caused by OSS, and our findings can give forest managers a high-end approximation for how quickly aspen stands may deteriorate after OSS invades.

The findings of our study can be used to inform management of OSS and aspen ecosystems invaded by OSS. We found that OSS caused high levels of mortality and appeared to spread rapidly within aspen stands, emphasizing the need for early intervention when invasions are detected. The prolonged crawler emergence that we observed complicates management strategies, such as sanitation thinning, that seek to avoid the crawler stage. Our findings suggest that there may be no time of year when crawlers can be completely avoided, but crawler density is lowest in October through November and February through March. On the other hand, strategies that target the crawler stage, such as application of horticultural oils or insecticides, should be applied in June through August when crawler density is highest. Although horticultural oils and insecticides are generally more suitable strategies for managing OSS on high-value urban trees, systemic insecticides are being considered as a potential tool for managing OSS in natural aspen stands (Crouch et al. 2021, Grady et al. 2022). For example, systemic insecticides applied via basal bark spray could be used to treat infested overstory aspen in areas where cutting infested aspen may be impractical because either there are few large aspen remaining in the area or regeneration is expected to fail to due chronic ungulate browse. Alternatively, systemic insecticides applied via soil drench could be used as a follow-up to treat aspen regeneration after overstory removal or sanitation thinning (Grady et al. 2022). However, the efficacy of management interventions, including silvicultural treatments, prescribed fire, and systemic insecticides, must be assessed before treatment recommendations can be made (Crouch et al. 2021). In the case of systemic insecticides, impacts on nontarget species must also be assessed (Grady et al. 2022). Finally, important questions remain regarding the long-term trajectory of aspen stands infested by OSS, such as whether adequate regeneration and recruitment will occur to replace dying trees, providing motivation for continued monitoring of aspen ecosystems invaded by OSS.

## Acknowledgments

We are immensely grateful to Mark Nabel, Jessi Ouzts, Elwood Rokala, and Michael Sedgeman who not only helped us identify study sites and facilitate research permission but also asked the critical questions regarding OSS management that prompted us to initiate this study. We also thank Al Hendricks, Kyle Price, Julia Totty, Gabe Traver, and Nick Wilhelmi for helping with data collection and



Simon Baker, Jules Barab, Nylah Edwards, Kaitlin Swang, and Gabe Traver for helping with lab work. Finally, we are thankful to Danny DePinte for lending his artistic talents and time to drawing OSS illustrations. We acknowledge that Northern Arizona University sits at the base of the San Francisco Peaks, on homelands sacred to Native Americans throughout the region. We honor their past, present, and future generations, who have lived here for millennia and will forever call this place home. Our research takes place on these homelands and others sacred to Native Americans.

## Funding

This research was funded through McIntire-Stennis appropriations from the USDA National Institute of Food and Agriculture to the Northern Arizona University School of Forestry and the state of Arizona, Northern Arizona University's Presidential Fellowship Program and Interns-to-Scholars Program, ARCS Foundation Phoenix, the Arizona Mushroom Society, and USDA Forest Service, Forest Health Protection, Emerging Pests Program.

## Author contributions

Connor Crouch (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Funding acquisition [supporting], Investigation [lead], Methodology [equal], Project administration [equal], Validation [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Richard Hofstetter (Conceptualization [equal], Investigation [supporting], Methodology [equal], Resources [lead], Writing—review & editing [supporting]), Amanda Grady (Conceptualization [equal], Funding acquisition [supporting], Methodology [equal], Project administration [equal], Writing—review & editing [supporting]), Nylah Edwards (Investigation [supporting], Methodology [supporting], Validation [supporting], Writing—review & editing [supporting]), and Kristen Waring (Conceptualization [equal], Data curation [supporting], Funding acquisition [lead], Methodology [equal], Project administration [equal], Supervision [lead], Visualization [supporting], Writing—review & editing [supporting])

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