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EXPERIMENTING WITH MULTISTRESSORS: INTRASPECIFIC VARIATION IN BEHAVIORS, TOLERANCES, AND DISTRIBUTIONS

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EXPERIMENTING WITH MULTISTRESSORS: INTRASPECIFIC VARIATION IN BEHAVIORS, TOLERANCES, AND DISTRIBUTIONS

A DISSERTATION APPROVED FOR THE
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ABSTRACT

Understanding organisms' behavioral, spatial, and physiological responses to stress is critically important for successfully predicting and managing population declines and extinctions. Currently, climate change is accelerating and exacerbating stressors such as heat and desiccation into ecosystems across the globe. A key ecological priority is to determine how organisms respond when these stressors interact with abiotic and biotic stressors already present within ecosystems. Traditionally, intraspecific variation in multistressor responses has been neglected in experimental work and population models. In my dissertation, I adopted experimental and meta-analytic approaches to determine how multiple stressors combine to shape intraspecific behaviors, tolerances, and distributions.

In Chapter 1, I focus on the nesting behavior of the social insect herbivore *Hyphantria cunea* in different host tree taxa, with special attention to their vertical distribution because this is often neglected in ecological assessment and management strategies. In the field, I measured larval nest placement and abundance in tree crowns along with microclimatic variables to determine if the probability of nesting lower in the crown differed from the probability of nesting higher in the crown. I found that there was a low probability of nesting higher in a tree crown, and that this pattern could be attributed to host plant taxonomy. I then ran a laboratory feeding experiment to determine if insect dietary stress was the main driver of this nesting pattern, or if alternative ecological factors were the cause. In the feeding experiment, larval mass and mortality on a diet of upper crown leaves was compared to a diet of lower crown leaves for two common host taxa, and one host taxon is characteristically more toxic than the other. For both plant taxa, lower crown leaves led to greater *H. cunea* larval mass and survival, indicating that dietary stress plays a key role in this insect's vertical distribution. I conclude that population

modeling and management can improve by accounting for vertical distribution patterns and how biotic stress shapes these patterns.

In Chapter 2, I explored how combining dietary and climate stress affected *H. cunea* mass and mortality, and if stress tolerances were locally adapted. I ran a multifactorial common garden experiment with insects extracted from low, moderate, and high rainfall locations in Oklahoma, USA, to test the following hypotheses: 1) there is a stress tolerance trade-off according to Shelford's law of tolerance, which reduces mass and survival when stress is combined 2) there is no stress tolerance trade-off; cross-talk or cross-tolerance should increase mass and survival when stress is combined and 3) local adaptation modulates relationships between stress tolerances. Larval mass and mortality were compared across treatments featuring various levels of dryness, heat, and plant toxicity. Modality of response to multiple stressors differed based on source population. *H. cunea* from low and moderate rainfall locations tolerated plant chemical stress well as the landscape becomes drier and warmer, while populations from high rainfall locations tolerated plant chemical stress well as conditions become warmer only if they are not simultaneously water stressed. It is essential to account for multistressor effects and intraspecific variation in these effects to accurately predict which populations will respond to environmental changes positively, neutrally, or negatively.

In Chapter 3, I continued to assess for local adaptation in *H. cunea* stress responses, but this time I focused on behavioral interactions instead of physiological tolerances. I used a common garden experiment with insects extracted from low, moderate, and high rainfall locations in Oklahoma, USA, to test the following: 1) According to the Stress Gradient Hypothesis, stress increases the probability of cooperative behavior regardless of the genetic relatedness of individuals, and 2) According to Hamilton's rule of kin selection, genetic relatedness increases the probability of cooperative behavior. Water availability and plant diet were manipulated to introduce stress in this study, and behaviors were compared between siblings that were familiar with each other, siblings that were not familiar with each other, and non-siblings. The behaviors investigated included larval predator defense and adult eclosion and inbreeding. Stress increased the probability of cooperative behavior, as did kin relatedness. Behaviors were locally adapted such that *H. cunea* from higher rainfall locations were more selfish as adults and used more water to defend themselves as larvae. The regional variation we detected in this study indicates that climate change should influence evolution by decreasing selfishness regardless of kin relatedness.

In Chapter 4, I used a meta-analytic approach to determine if the theme of stress shifting organism interactions positively extended beyond *H. cunea*. This is the first systematic review and specifically the first meta-analysis to address if this pattern is more common in intraspecific or interspecific interactions, and particular kingdoms, ecosystems, habitats, life history stages, response variables, and stressors. The pattern of positive interactions increasing with increasing stress is well supported by studies that feature bacteria, plants, terrestrial ecosystems, interspecific negative interactions, adults, survival instead of growth or reproduction, and drought, fire, and nutrient stress. This review reinforces the novelty of my previous work with *H. cunea* because it shows experimental studies featuring multistressor responses are relatively scarce, and usually intraspecific interactions shift negatively instead of positively.

Chapter 1: Plant identity and fungal symbionts determine tree crown distribution of a social insect herbivore

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Data availability statement
The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Abstract

1. Leaves vary in terms of nutrient content, water potential, and exposure to light and heat depending on their location within the tree crown. Consequently, particular areas of the crown may be more optimal than others for herbivore foraging and shelter.
2. Based on the Plant Growth-Differentiation Balance (GDB) and Vigor hypotheses, we expected higher herbivory in the lower tree crown due to reduced leaf chemical defense. This is opposite of the Plant Stress hypothesis, which emphasizes nitrogen benefits to herbivores expected in upper tree crown leaves.
3. We contrasted host tree structure, microclimate and herbivore nest placement in the field followed by a leaf diet laboratory experiment to test the relative importance of these hypotheses in the vertical distribution of the red-headed fall webworm (*Hyphantria cunea*), a North American native defoliating social moth species.
4. Nests were more abundant in the lowest third of the tree crown, though this pattern was weaker for pecan (*Carya* sp.), which hosted the most larvae per tree, and for redbud (*Cercis canadensis*), which hosted fewer larvae than other hosts. In the lab, we determined instar-specific mortality and found larvae fed upper tree crown leaves had lower mass and survival.
5. Our results indicate tree crown variation in leaves and fungal endophyte distribution may be the main reason fall webworms prefer to nest lower in trees, consistent with the Plant GDB and Vigor hypotheses. Control efforts for this social shelter-building herbivore should be designed to target lower or shaded tree crowns since preference for shade is strong.

Keywords: herbivore, water potential**,** hygrothermal, endophyte, entomopathogenic

Introduction

Ecological assessment and management strategies have traditionally been based on organisms' horizontal distribution and performance. These strategies could be improved by incorporating vertical distribution and performance in relation to vegetation structure, especially in trees. This may include replacing broad-spectrum pesticide spraying with interference at specific tree crown locations, such as targeted sprays, reproductive disruptors, microclimate modifications, or natural enemy attractants (Nicholas et al., 2005; Niether et al., 2018; Gontijo, 2019). In the non-traditional cases when vertical stratification has garnered attention, it has mainly been for herbaceous agricultural crops or vertebrates (Wilson et al., 1983; Muraleedharan et al., 1988; Stiefel et al., 1992).

Invertebrate populations, for which there is ten times less available information on vertical stratification in trees than for birds or mammals, are important tree herbivores (Davies & Asner, 2014). The top 15 invasive non-vertebrate tree pests in North American forests collectively reduce 41.1% of forest biomass annually (Fei et al., 2019). Indigenous insect herbivore outbreaks have been intensifying, competing with this destruction, since abiotic stressors are fluctuating at one of the highest known rates in Earth's history (Pinault et al., 2007; Bentz et al., 2009; Collins, 2009). For example, larvae of the indigenous pale-winged moth (*Iridopsis ephyraria*) kill 40% of mature tree hosts and 90% of saplings during outbreaks, and they are usually vertically distributed in the lower and middle tree crowns of their hosts (Pinault & Quiring, 2008). Improved Integrated Pest Management (IPM) and sampling strategies have been developed based on knowledge of this vertical distribution (Pinault & Quiring, 2008; Pinault et al., 2012). Yet in most cases only the pattern was documented, with little or no attention paid to the main reasons for the vertical distribution.

Differential investment in plant defense may be a key determinant in herbivore tree crown distribution and abundance, and this may be more important in herbivores that are social shelter-builders. Shelter-building herbivores comprise ~20% of 126 known Lepidoptera families and 100% of the 1257 species in the spider mite family Tetranychidae, which contribute substantially to biomass loss in agricultural and forested ecosystems (Bolland et al., 1998; Lill & Marquis, 2007; Migeon et al., 2010). Previous work on herbivores that do not build shelters point to the hygrothermal structure of tree crowns playing a large role in the herbivores' vertical distribution. Lower relative humidity and higher temperatures common in the upper tree crown can act directly on arthropods to prolong development and reduce growth and longevity, so lower tree crown levels may be favored (Ram, 1995; Hervieux, 2012; Pinault et al., 2012). Social shelter-building herbivores have the advantage of using the structure to regulate physical factors such as temperature, humidity, and predators, unlike their non-social counterparts (Sullivan & Green, 1950; Knapp & Casey, 1986; Joos et al., 1988; Costa, 1997; Roda et al., 2000; Rehnberg, 2002; Ruf & Fiedler, 2002; Rehnberg, 2006; Lemos et al., 2010; Eouzan et al., 2019). The niche of a social shelter-building herbivore should therefore depend more on its plant host's response to the external environment than its own response to the external environment. Social shelter-builders are ideal for assessing how herbivores respond to variation in plant nutrients or chemical defenses, as confounding effects from the external physical environment acting directly on the insect body should be reduced.

Variation in chemicals among different tree crown levels exists due to exposure to light and the process of transpiration. Leaves growing in high light conditions have smaller surface area and lower water potential (Oishi et al., 2006), which means water potential and C/N ratio decrease farther up a tree (Fig. 1A). Water stress and increased photosynthesis such as that in the upper tree crown results in greater nitrogen per leaf unit area (Coley, 1983; White, 1984; Dudt & Shure, 1994; Reich et al., 1998; Cunningham et al., 1999; Wright & Westoby, 2001; Fortin & Mauffette, 2002; Wright et al., 2003; Oishi et al., 2006; Osier & Jennings, 2007). Nitrogen-based defenses such as alkaloids may be more prominent in the nitrogen-enriched upper crown relative to the lower crown as a result (Scriber & Slansky Jr, 1981; Höft et al., 1998).

Leaf chemical variation also results from a trade-off between plant growth and defense, which Herms and Mattson (1992) proposed in the Growth-Differentiation Balance (GDB) hypothesis. This predicts that the greatest allocation to plant defense chemicals should occur at intermediate resource levels, except when the resource is light. When light increases photosynthesis, secondary metabolite production increases (Stamp, 2004). Foliage in the upper tree crown invest more in defense than growth relative to lower tree crown foliage, including increased production of phenols (Hillis & Swain, 1959). Phenols commonly influence lepidopteran herbivores such as *Lymantria dispar* (Lepidoptera: Erebidae), or gypsy moth (McDonald et al., 1999), and *Hyphantria cunea* (Lepidoptera: Erebidae), or fall webworm (Firidin et al., 2008).

We conducted a field survey followed by a factorial foliage feeding lab experiment to test if vertical distribution of a social shelter-building insect herbivore was due to plant elemental nutrition versus defenses. We derived predictions from contrasting hypotheses: 1) the aforementioned Plant GDB Hypothesis, and the Plant Vigor Hypothesis, in which insect herbivores prefer consumption of rapidly growing, poorly defended plant matter (Fig. 1B; Price (1991)) and 2) the Plant Stress Hypothesis, in which insect herbivores prefer consumption of nutrient and water stressed plant matter due to nitrogen enrichment (Fig. 1B; Mattson (1980); Mattson and Haack (1987)). The Plant Stress Hypothesis minimizes the importance of plant age, herbivory duration, plant defense, and insect tolerances to such defenses (Larsson, 1989; Maschinski & Whitham, 1989). It assumes that nitrogen benefits herbivores, ignoring its possible use in defense compounds.

In the field, we expected the upper tree crown to be favored over the lower tree crown if nitrogen nutritional benefits were more important to herbivore distribution and abundance than other ecological factors. We expected the lower tree crown to be preferred if the insects were sensitive to an alternative ecological factor, such as water or heat stress, natural enemies, or plant defense chemistry. In the lab, we expected the insects to perform better on a diet of leaves from the upper tree crown if nitrogen nutritional benefits were of central importance to herbivore success. If plant defenses were more important to herbivore success than nitrogen nutritional benefits, then the insects were expected to perform better on a diet of leaves from the lower tree crown and exhibit signs of distress when fed from the upper tree crown.

Methods

Study Organisms

We used the fall webworm (*Hyphantria cunea*; Lepidoptera: Erebidae) to test the relative importance of the 1) Plant GDB and Vigor hypotheses and 2) Plant Stress hypothesis in driving vertical distribution. Hundreds of individuals, all full siblings, nest together in the outer crown of a tree host, creating webs on branch tips that discourage predators and provide temperature and humidity control (Sullivan & Green, 1950; Rehnberg, 2002, 2006). In North America, fall webworms are an abundant nuisance moth species, ranging from southern Canada (latitude 50-55 °N) to the southern United States and parts of Mexico (latitude 19 °N). They spread to Europe and Asia during the 1940’s, becoming invasive in those areas (Morris, 1963; Ge et al., 2019).

Fall webworm larvae come in at least two color morphs: red-headed and black-headed. The red-headed fall webworm is not as well studied as their black-headed counterpart (Vega et al., 2008; Loewy et al., 2013; Murphy & Loewy, 2015; Vidal et al., 2020). Our study featured the red-headed fall webworm for this reason and because it is more abundant than the black-headed color morph in Oklahoma, USA, which is where this study took place (Rebek, 2009). There is ongoing debate regarding the taxonomic status of these color morphs, but they are currently considered the same species based on analyses of mitochondrial haplotypes and interbreeding (Loewy et al., 2013). Black-headed fall webworms are more common in the eastern United States and Canada and are rarely found west of Oklahoma. Red-headed fall webworms are more common in southern and western North America (Loewy et al., 2013; Yang et al., 2017).

In Oklahoma, the number and timing of generations per season differs for the two color morphs, though they have an equivalent number of instars per generation. The first-generation red-headed fall webworm larvae feed for a period of approximately six weeks from mid-June through the first week or two of August, and the second-generation larvae feed for a period of approximately six weeks from September through mid-October (Rebek 2009; Adams unpubl. data). In contrast, black-headed larvae usually have one generation per year that is active at the same time as the second generation of red-headed larvae, but they may have an extra generation following wet and cool weather in May and early June (Rebek, 2009, Marshall, 2017, *personal communication*, OSU Entomology Collection, Adams unpubl. data). Black-headed fall webworms have five instars, molting four times to accommodate an expanding head capsule width (Boucias & Nordin, 1977). We confirmed this was also the case for Oklahoma red-headed fall webworms. In the lab, we observed that larvae consume their exoskeletons during the transition from the first to second instar and the second to third instar. They did not consume their molted exoskeletons during the final two transitions.

Larval fall webworms feed on the leaves of a range of deciduous trees, including fruit and nut trees (Morris, 1963; Ge et al., 2019). Black-headed and red-headed fall webworms are found on completely different tree species in Oklahoma, and the diet of red-headed fall webworms is more constrained (Rebek, 2009; Adams unpubl. data). Potent carbon and hydrogen-based phenolics and terpenoids are the main chemical plant defenses of red-headed larvae host trees and are not present in black-headed larvae host foliage (Hedin et al., 1980; Borazjani et al., 1985; Choi et al., 1996; Kim & Wetzstein, 2005; Solar et al., 2006; Villarreal-Lozoya et al., 2007; Zhang et al., 2009; Cosmulescu & Trandafir, 2011). Red-headed fall webworms are found on a wider range of tree species in Oklahoma urban areas, but outside of the urban areas they are found on redbud (*Cercis canadensis*), black walnut (*Juglans nigra*), persimmon (*Diospyros virginiana*), and pecan or hickory (*Carya,* especially *C. illinoiensis*) trees (Rebek 2009; Adams unpubl. data). We confidently identified the redbud, black walnut, and persimmon species for our investigation, but pecan is known to frequently hybridize with other *Carya*. These pecan-hickory hybrids are commonly referred to as either “pecan” or “hican” trees and it is impossible to distinguish them without microscopic examination of parenchyma bands in their wood (McDaniel, 1954). The leaf shape and chemical profiles of these *Carya* do not significantly differ, so this did not affect our investigation (Borazjani et al., 1985). We refer to tree common names because of the taxonomic ambiguity of the *Carya* sp.

Field Survey

A total of 162 host trees representing the four taxa used by red-headed fall webworms were surveyed in uncultivated landscapes dominated by post oaks (*Quercus stellata*) and blackjack oaks (*Quercus marilandica*) in the Cross Timbers ecoregion of Oklahoma, with 54 trees each in western (35° N, 98° W), central (35° N, 97° W), and eastern (35 ° N, 95 ° W) Oklahoma. There were three sites per longitude, and 18 trees were surveyed per site. Host trees in a landscape were assigned numbers and selected for measurement using a random number generator. The measured trees were a minimum of 350 m apart to reduce spatial autocorrelation and account for moth male dispersal distance (Yamanaka et al., 2001). After mating once, females will deposit eggs on only one individual tree host, so this ensured the larvae on the different trees in our dataset did not share parents (Sourakov & Paris, 2014). Geographic coordinates of fall webworm host trees were recorded with a handheld GPS unit. We examined multiple sites along a longitudinal gradient to determine if Oklahoma's precipitation gradient might influence patterns in fall webworm distribution. On average, western Oklahoma receives ≤ 64 cm rain annually, central Oklahoma receives 64-102 cm, and eastern Oklahoma receives 102-143 cm (mesonet.org). Our field data set features the following:

1) Environmental data – edaphic factors measured with a Sonkir probe (soil moisture, pH, temperature) and the following Oklahoma Mesonet monitoring station data: mean daily air temperature (all temperature records are in Celsius, and all sites were located within 30 miles of the nearest monitoring station), rainfall (centimeters), percent humidity.

2) Plant data: host genus, leaf temperature adjacent to nest, total height (centimeters), diameter at breast height (DBH – a measure of stem width used for calculating relative plant age, units were centimeters), and light exposure (recorded as the percent of whole tree shaded over an entire afternoon).

3) Insect data: number of webs, number of larvae per web, total larvae per tree, larval maturation (measured in instars), web height off the ground, total webs per crown level, and web temperature and humidity measured with a Mestek Non-Contact Laser Digital Thermometer and Hygrometer.

Field Statistical Analyses

The probability of a nest occurring in the upper, middle, or lower tree crown was estimated with a Bayesian multinomial model with flat priors (i.e., no assumptions about probability of occurrence at each crown level). Results from a JAGS model implemented in R (R Core Development Team, www.r-project.org) using the *rjags* package (Plummer, 2012) with 3 Markov chains, a burn in of 1,000, and posterior distributions built from 10,000 iterations were plotted using package *bayesplot* in R.

Next, we used multivariate analysis of variance (MANOVA) to test how environmental characteristics drove fall webworm demographics. Our field data included the following categorical independent variables: rainfall region (east, central, west OK), tree crown level (lower, middle, upper), tree taxa (pecan, redbud, persimmon, walnut), soil moisture (dry scored 1-3, moist scored 4-7, wet scored 8-10 with the Sonkir probe), soil temperature (low less than 30 C, medium 30-33 C, high greater than 33 C), tree height (short trees less than 20 feet tall, medium trees 20 to 49 feet tall, and large trees 50 or more feet tall), nest distance from the ground (close less than 250 cm, moderate 250-500 cm, far more than 500 cm), and light exposure (low more than 50% shade, medium 30%-50% shade, high less than 30% shade). There was little to no variation in the other temperature, humidity, and pH measurements we collected, so these were excluded from the statistical analyses. Relative plant age was also included, which we determined by following the International Society of Arboriculture (IBA) protocol for estimating tree age using our measurements of tree DBH and IBA species growth factors (White, 1998). Once absolute age was known, we used IBA definitions of “young,” "middle-aged," and "old" for each tree species to assign a tree to one of these three relative age categories so they could be compared in the analysis.

Dependent variables for MANOVA included the trees' herbivore abundance, specifically number of webs per tree and crown level and total larvae per web, tree and crown level. It also included fall webworm growth, specifically larval maturation. We log-transformed dependent variables in order to meet assumptions of normally distributed residuals and homogeneity of variances. When treatments significantly affected overall insect demographic response estimates in MANOVA, we conducted individual ANOVA on each response variable to isolate the treatment effects on different components of insect demographic responses. When treatment interactions were statistically significant, we used a Tukey-Kramer adjustment for multiple comparisons to decompose differences among the means. All multivariate and ANOVA statistics were conducted with IBM SPSS Statistics software.

Lab leaf feeding experiment

In 2017, descendants of red-headed fall webworm moths captured in central Oklahoma (35.2226° N, 97.4395° W) during the 2016 season were hatched and raised through pupation on one of four leaf diets: upper crown persimmon, lower crown persimmon, upper crown pecan, and lower crown pecan. These two tree hosts were chosen for comparison because a) these are the two most common hosts of fall webworms in central Oklahoma and b) fall webworms occupied equal numbers of these two host trees across the state, and c) in the field, the relative position of larval nests in the tree crown was higher in pecan trees than persimmon trees. Our lab environment and equipment matched those described in a rearing protocol for red-headed fall webworms established by Loewy et al. (2013), except average annual humidity is 2% higher and elevation is 1,252 m lower in Norman, Oklahoma relative to Denver, Colorado. We reared a total of 150 caterpillars per diet, for a total of 600 caterpillars. 20 siblings were divided into groups of five and assigned to one of the four treatments. There was a total of 30 different sibling groups per treatment. Twice per week we weighed caterpillars to track growth. We also tracked timing between instars and pupation, and we counted and diagnosed mortality.

Although we focused on crown leaf differences due to chemicals, Carpenter and Smith (1981) found that North American hardwood tree leaves growing in direct sun are physically thicker than shaded leaves. They confirmed red-headed fall webworm host tree leaves in direct sun are 5-10% thicker than shaded leaves. According to Lobregat et al. (2018), leaf thickness needs to be increased by 50% or more before it will significantly reduce plant consumption of chewing Lepidoptera. Even though it was unlikely increased toughness from leaf structural carbohydrates in the upper tree crown leaves contributed much to our results, we still accounted for it by tracking the mass of foliage consumed.

The larval caretaker followed certified guidelines for Lepidoptera disease prevention (Smith, 2016). This included disinfecting tools and hands between larval measurements, and checking for fungi to verify that any present in the experiment were naturally occurring in the leaves, not spread by flawed sanitation. We confirmed the presence of fungi within persimmon leaves by staining thin sections of the inner leaf with lactophenol cotton blue and examining at 200X (Bacon, 1994; Marques et al., 2013; Raja et al., 2016). Fungal presence was consistent between lab and field leaves from unshaded upper tree crowns and did not cause apparent harm to the plants. It was not detected often in leaves from lower tree crowns, and it was not detected in pecan leaves.

Lab Statistical Analyses

We used multivariate analysis of variance (MANOVA) to test how leaf characteristics drove fall webworm demographics. Our approach matched that used for the field data, but lab data included the following categorical independent variables: host identity as either pecan or persimmon, sibling group identity, and upper or lower crown leaf origin. Dependent lab variables included larval mass, mass of consumed foliage, and time between instars.

To analyze larval mortality for the lab diet experiment, we created Kaplan-Meier survival plots using R. These were separated by larval instars. Log-rank tests determined if survival times between treatments significantly differed. Cox proportional hazards regression models were used to test if other independent variables affected larval survival.

Results

Field Results

Fall webworms had a 30% and 40% higher probability of nesting in the lower tree crown than the middle and upper levels, respectively (Fig. 2). Maturation and number of larvae per nest did not differ among tree crown levels, nor did mean annual rainfall affect webworm nest location in tree crowns or the number of larvae per nest (Supp. Table S1). Eastern and central Oklahoma host trees contained similar numbers of webs, but western Oklahoma host trees contained 40% fewer webs than trees in the other two regions (Fig. 3A). Eastern and western larvae appeared to mature at a similar rate, while larvae from central Oklahoma matured more quickly except on walnut trees (Fig. 3B). By the final instar, central Oklahoma larvae often appeared double the size of larvae from other regions except for those raised on walnut trees. Eastern Oklahoma larvae did not appear to develop a fifth instar and consequently abandoned webs to pupate sooner than larvae in western and central Oklahoma.

Host identity affected all variables. Larvae developed twice as quickly on the three other hosts relative to redbud in eastern and western Oklahoma. In central Oklahoma, redbud larval development was about half an instar behind walnut, 1-1.5 instars behind pecan, and 1.5 instars behind persimmon on average three weeks after hatching (Fig. 3B). In redbud trees larvae had a 53% and 57% higher probability of nesting in the middle tree crown than the upper and lower tree crown, respectively (Fig. 4A). Larvae in pecan trees were twice as likely to nest in the upper crown relative to the middle crown and had a 10% lower probability of nesting in the upper tree crown relative to the lower tree crown (Fig. 4B). In persimmon and walnut trees, fall webworms were approximately three times more likely to nest in the lower tree crown (Fig. 4C&D). On average, redbud nests contained 53%, 50%, and 36% fewer larvae than pecan, persimmon, and walnut nests, respectively. Persimmon nests contained 7% fewer larvae on average than pecan nests while walnut nests contained 27% and 22% fewer larvae than pecan and persimmon, respectively (Fig. 5A). Redbud hosts contained 41%, 31%, and 20% fewer webs on average than pecan, persimmon, and walnut, respectively. Walnut hosts contained 26% and 14% fewer webs on average than pecan and persimmon, respectively, while persimmon hosts contained 14% fewer webs on average than pecan (Fig. 5B).

Although fall webworms were found on more pecan and persimmon trees than walnut trees in central Oklahoma, across the state the most common tree hosts ranked in this order: 1) 52% of hosts were walnut (*Juglans*) 2) persimmon (*Diospyros*) tied with pecan (*Carya*), each comprising 19% of hosts and 3) 10% of hosts were redbud (*Cercis*). Fall webworms were found on more redbud in the eastern part of the state relative to the two regions to the west, though the pattern of most common hosts was still 1) walnut 2) pecan and persimmon and 3) redbud in western and eastern Oklahoma. Redbud trees were occupied the least in each region (Supp. Table S2).

We accounted for potential fall webworm maternal oviposition preference by measuring tree and nest heights and relative plant age in our fall webworm field survey. Host tree height varied from 5 feet to 85 feet, and 31% of the hosts were ~12 feet tall and 25% of the hosts were around ~30 feet tall. Height varied dramatically among the remaining 44%, with small (19 feet tall or less), medium (20 to 49 feet tall), and large (50 to 80 feet tall) trees occupied in relatively equal proportions. Tree height of fall webworm hosts did not differ between rainfall regions, and neither did age. There were no fall webworm demographic differences detected based on tree size or age or height of nest above the ground (Supp. Table S1).

Light exposure influenced larval abundance while soil moisture influenced larval age. Larvae on hosts with high soil moisture matured more rapidly than larvae on hosts with lower soil moisture by one instar on average, irrespective of soil temperature (Fig. 6). Other fall webworm demographic responses did not change based on soil moisture and temperature (Supp. Table S1). There were 25% more webs per tree for hosts with low light exposure relative to medium and high light exposure (Fig. 7A). There were 5% more larvae per web for hosts with high light exposure (Fig. 7B). There was no difference in the other demographic variables based on light exposure (Suppl. Table S1).

Feeding Experiment Results

This laboratory feeding experiment excluded predation, parasitism, and transplant shock as causes of mortality. In previous work excluding predation, parasitism, and transplant shock, higher mortality occurred in the second and fourth instars of the black-headed fall webworm

(Boucias & Nordin, 1977). We found mortality related to plant defense occurred in the first and third instars of the red-headed fall webworm. First instar mortality was higher for larvae fed upper crown leaves, and third instar mortality was higher for larvae fed lower crown leaves (Fig. 8A). This suggests insect resistance to toxins and pathogens is lower during these larval stages, although the underlying physiological cause is currently unknown and should be determined in future work.

Larvae fed pecan leaves from the upper tree crown did not survive beyond the first larval instar. They were able to chew and ingest foliage, but they began writhing afterwards and did not successfully digest and excrete it. For both pecan and persimmon, there was not a difference between the mass of upper crown leaves chewed and ingested compared to the lower crown, suggesting leaf toughness was not an obstacle for the webworms ($F_{3, 596} = 0.500$; $p = 0.997$). Diarrhea, especially cases of green diarrhea, is a strong indication of poisoning in lepidopteran larvae and this was produced by larvae fed persimmon upper crown leaves. Leaf fungal infection corresponded with larval diarrhea production and mortality for the persimmon leaf diet. By the final instar, larvae fed lower crown persimmon leaves had 60% higher survival and grew 32% larger than larvae fed lower crown pecan leaves. By the final instar, larvae fed upper crown persimmon leaves had 44% higher survival and grew 38% smaller than larvae fed lower crown pecan leaves. Larvae fed lower crown persimmon leaves had 46% higher survival and 58% higher mass on average by the final instar than those fed upper crown persimmon (Fig. 8B). They also pupated a week earlier on average than larvae fed upper crown persimmon and lower crown pecan (Fig. 8C). These patterns held up regardless of how related the webworms were to each other, and there were no patterns in growth and mortality detected based on descent from certain parents ($F_{29, 570} = 0.999$; $p = 0.475$).

Discussion

We observed a strong preference for the lower tree crown and shade conditions in fall webworms. Our findings are inconsistent with prior studies indicating the upper tree crown is ideal for insect herbivores (Scriber & Slansky Jr, 1981; Coley, 1983; Lincoln & Mooney, 1984; Dudt & Shure, 1994; Hollinger, 1996; Cunningham et al., 1999; Le Roux et al., 1999; Fortin & Mauffette, 2002; Frak et al., 2002; Frak et al., 2006; Łukowski et al., 2015; Łukowski et al., 2017). Our results do not align with Mattson's 1980 Plant Stress Hypothesis regarding leaf nitrogen. We conclude that vertical distribution is driven mainly by foliar defense over other ecological factors, consistent with Price's 1991 Plant Vigor Hypothesis and Herms and Mattson's 1992 Growth-Differentiation Balance Hypothesis.

Although leaves from upper tree crowns may have higher nitrogen content, there have been cases of increased defenses in these leaves that reduce their appeal to herbivores (Lincoln & Mooney, 1984; Sagers, 1992; Höft et al., 1998; Karolewski et al., 2013). Based on the pronounced mortality results obtained from our lab leaf diet experiment, it seems that higher, sunnier levels of the fall webworm host tree crown are generally better defended from herbivory. Although feeding experiment results suggest fall webworms should occupy lower tree crowns of pecan trees since there was high mortality on the upper tree crown diet, this was not the case in the field. There was also higher mass and survival on a lab diet of persimmon leaves even though in the field larval abundance was higher in pecans. We believe this difference is due to light exposure independent of tree crown level. In the lab experiment, we used leaves from upper tree crowns exposed to high levels of sunlight. Upon further inspection of the field data, we found that pecan trees hosting fall webworms were more likely to be at least 30% shaded, and the upper tree crown was not always exposed to more light than the lower layers due to shadows cast by surrounding objects such as other trees. It was less common to find fall webworms occupying pecan trees with high light exposure, and those that did exclusively occupied the shadiest portion of the tree crown, often the north side of a tree. Because there was often little difference in the light environment between pecan hosts' tree crown layers in the field, the leaf toxicity that larvae experienced in tree crowns in the field was likely lower than the leaf toxicity of the tree crown leaves used in our lab experiment. Light exposure for lab persimmon leaves matched light exposure observed for persimmon leaves in the field. Lab light exposure was kept consistent for all persimmon and pecan experimental plants in order to avoid confounding light exposure and plant identity.

The fungal disease and detection results from our persimmon treatments suggest that leaf-inhabiting entomopathogenic endophytic fungi are present in some fall webworm tree hosts and pose a problem for the developing larvae, which has not been reported for this species and is consistent with what is known about leaf endophytes. Leaf endophytes can vary in their tree crown distribution depending on the light environment, with certain taxa preferring sun leaves over shaded leaves or vice versa (Unterseher et al., 2007). Although the specific identity and role of the sun leaf endophytic fungus detected in this work is unconfirmed, it is well established that some fungal endophytes secrete anti-herbivore chemicals (Tanaka et al., 2005; Schardl et al., 2007; Vega et al., 2008; Schardl et al., 2013). Based on the adverse fitness responses shown by fall webworms restricted to leaves originating from the upper tree crown, we suspect that this sun leaf endophyte is among them. It is possible fall webworm distribution in a tree crown is largely driven by the distribution of its leaf endophytic fungi, especially fungi that secrete anti-herbivore compounds.

We suggest that fall webworm tree crown distribution is determined mainly by leaf characteristics because the webbed shelters the larvae nest in provide protection against physical stressors. This has received less attention in prior studies featuring herbivore tree crown distributions, which instead tend to highlight herbivore hygrothermal sensitivity and non-social systems (Basset, 1991; Fortin & Mauffette, 2002; Hervieux, 2012; Pinault et al., 2012). For example, it is well established that the protective web shelter of a similar social moth called the eastern forest tent caterpillar (*Malacosoma distria*; Lepidoptera: Lasiocampidae) plays an important thermoregulatory function and provides multiple advantages such as shielding the larvae from high UV radiation as well as heat and water loss (Knapp & Casey, 1986; Joos et al., 1988; Costa, 1997; Ruf & Fiedler, 2002). The shelters of fall webworms play a similar role. Previous work established temperature and humidity within the web are temporally consistent, and the shelters are an important protection against predators (Sullivan & Green, 1950; Rehnberg, 2002, 2006). We confirmed that web temperature and humidity is spatially consistent. Visually, nests built in sun appeared thicker than nests built in shade. A thicker nest also implies greater predator protection, which may explain why we detected 5% more larvae per web in trees located in sunny areas relative to trees located in shade. Nevertheless, increased predator protection presumably comes at the expense of a greater energetic construction cost. These factors likely play a role in the vertical distribution of fall webworms, but it appears that predator and parasitoid escape is not the main determinant since lower and shadier levels of the tree crown were generally preferred over higher and sunnier levels.

Maternal oviposition preference is an important factor that influences the vertical distribution of herbivorous larvae, and it could be the main driver of vertical distribution in cases where juvenile mortality does not differ among tree crown levels. In the solitary oak leafminer moth (*Cameraria hamadryadella;* Lepidoptera: Gracillariidae*)*, juvenile mortality did not differ between tree crown levels, yet larvae were distributed more often in the lower tree crown. Importantly, in this case it was verified that natural enemies occurred at approximately equal densities in different levels of the tree crown and differences in crown leaves did not change larval mortality (Connor, 2006). In the lab, mothers of this species did not discern between foliage from the lower and upper tree crown, ovipositing equally on foliage originating from both places. Potted lab trees placed closer to the ground received more oviposition than those farther from the ground, suggesting that mothers oviposit based on crown height instead of crown leaf characteristics (Brown et al., 1997). In contrast, we verified that fall webworm juvenile mortality differed between sun and shaded crown areas due to leaf differences, and this effect was more pronounced in pecan than persimmon.

Although our work suggests female oviposition preference is not the main reason for fall webworm vertical distribution, there are other patterns in host occupancy driven by this factor. We found more walnut trees contained larvae than other hosts, even though it was clear there were fewer larvae per walnut tree compared to persimmon and pecan. This may be because mothers select walnut for reasons unrelated to larval success, which should be explored further. Importantly, a key part of the fall webworm life cycle is the death of the mother immediately after oviposition. She lays her entire clutch of 400 - 1000 eggs on a single tree, then perishes without leaving that tree (Sourakov & Paris, 2014). She cannot lay some of her eggs on one host, then fly away to finish on a different host. It is therefore unlikely that the lower larval abundances we detected for some hosts was because the mother elected to lay fewer eggs, for her priority should be to lay as many eggs as possible on the tree before she expires. Instead, the reason for differential larval abundance is more likely because conditions on some hosts are not as ideal for eggs to hatch or larvae to survive.

More work is needed regarding the distribution of the fall webworm's natural enemies across different tree species, rainfall regions, and crown levels to determine the extent to which natural enemies may alter distribution. Whereas it appears plant-level defense characteristics are more important for this social moth's vertical distribution based on the current work, follow-up is needed to determine the role of predators and parasitoids in horizontal fall webworm distribution. For example, outside of the native range, in Japan, it was determined that larval mortality was higher in later instars of black-headed fall webworms due to predation and parasitism, but instar-specific mortality from natural enemies has not yet been documented for red-headed fall webworms (Itô & Miyashita, 1968). We expected host hygrothermal structure to play a minor role in distribution and performance of social shelter-building herbivores due to the physiological benefits the herbivores derived from shelter, yet natural enemies do not necessarily play a minor role in social shelter-building herbivore population dynamics or influence them to a lesser extent than solitary herbivores. Social shelter-building herbivores derive predator and parasitoid protection from their groups and shelter unlike solitary herbivores, but their concentrated larval densities attract greater attention from natural enemies (Costa, 1997). This means top-down trophic pressure may be a powerful force in their ecology and evolution, even if it is less important than leaf defense for vertical distribution as we found in this case.

Future ecological assessment and management of fall webworms should target lower and shaded portions of tree crowns. For example, herbivore exclusion barriers and target spraying can be directed to these locations instead of wasting resources on unoccupied tree crown locations (Chouinard et al., 2016). Simply maximizing light exposure can reduce herbivory in these trees. Follow-up is also needed to see if the vertical distribution pattern we observed is common across other social shelter-building herbivores. If so, then this can be the default tactic for controlling known social shelter-building herbivores. If not, then at least accounting for both vertical and horizontal distribution will lead to more focused and effective population control priorities.

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Figures

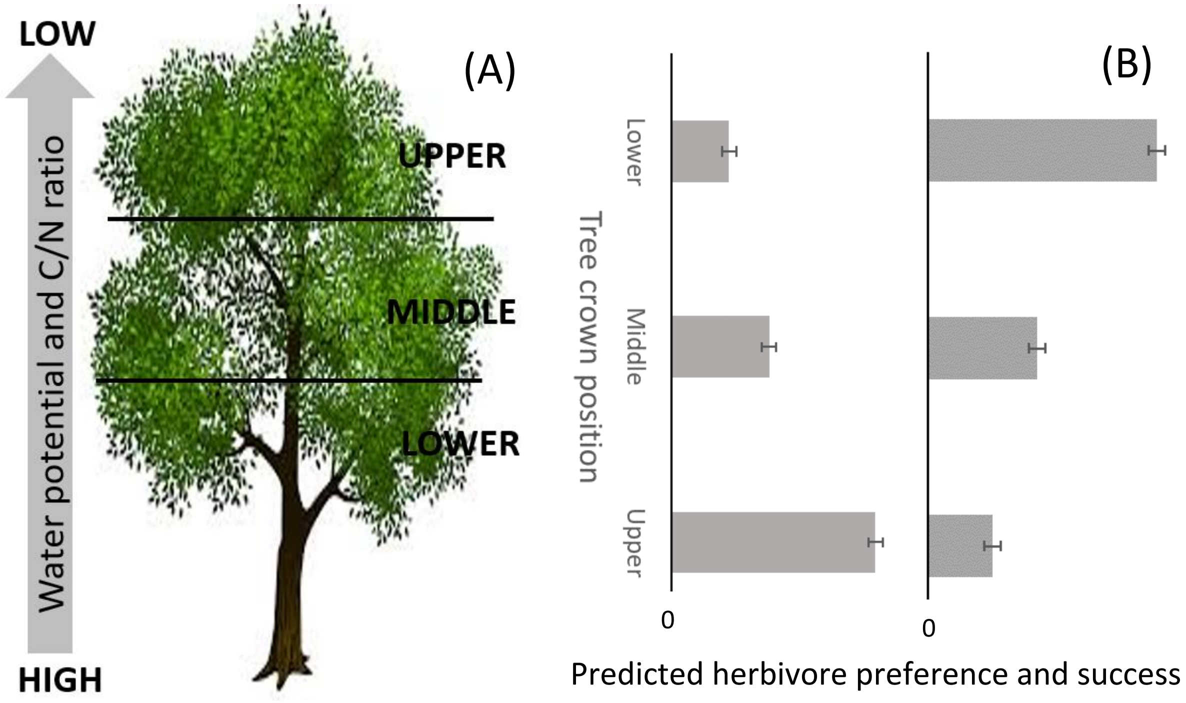


Fig. 1. Two predictions about preference and success of herbivorous insects in the lower, middle, and upper levels of the tree crown derived from A) foliage properties in the tree crown change with decreasing C/N and water potential. B) Left: If the herbivores are not sensitive to stress related to water, heat, or plant defense, then they should prefer higher portions of the tree crown since leaves higher up in the tree should contain more nitrogen. The depicted expectation is based on the Plant Stress Hypothesis. Right: Lower crown leaves should invest more in growth instead of defense, so they should be preferred according to the Plant GDB and Plant Vigor hypotheses

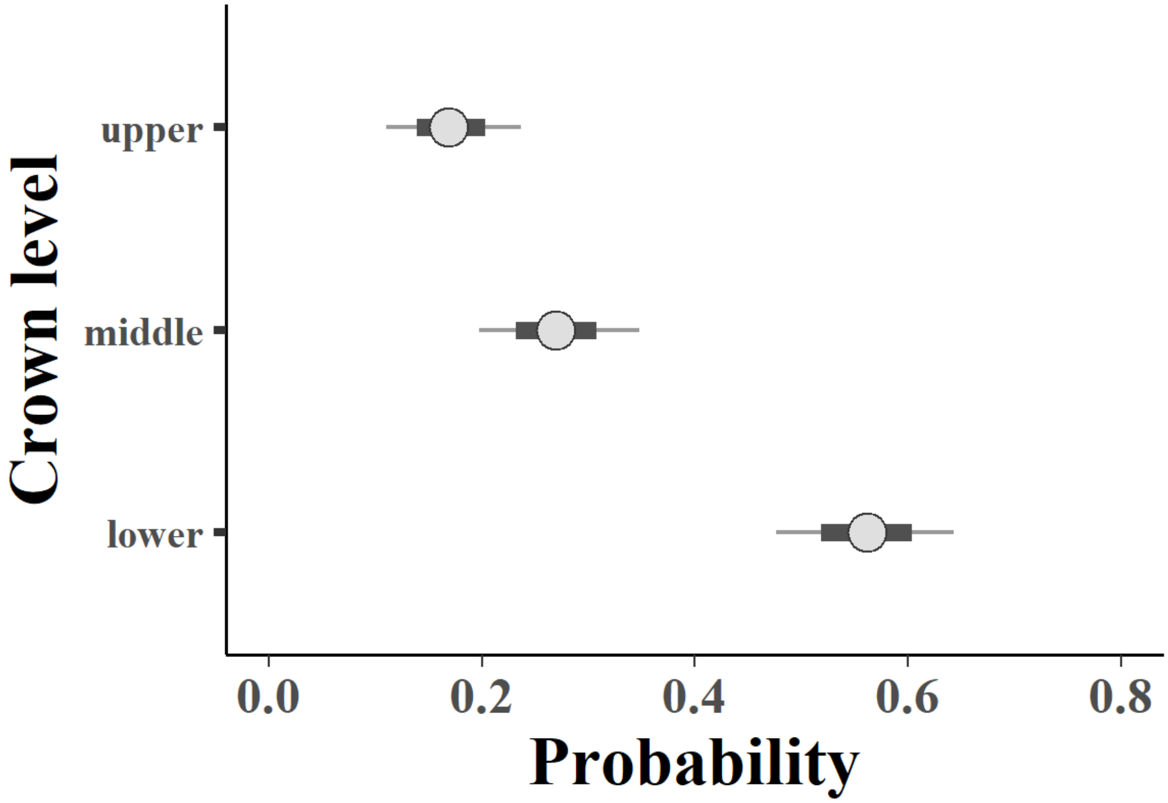


Fig. 2. Multinomial probability estimates for larval web locations, with thick credible intervals shown for the standard 95% range and thin confidence intervals for the whole posterior distribution. Fall webworms were most likely to build webs in the lower tree crown and least likely to build webs in the upper tree crown.

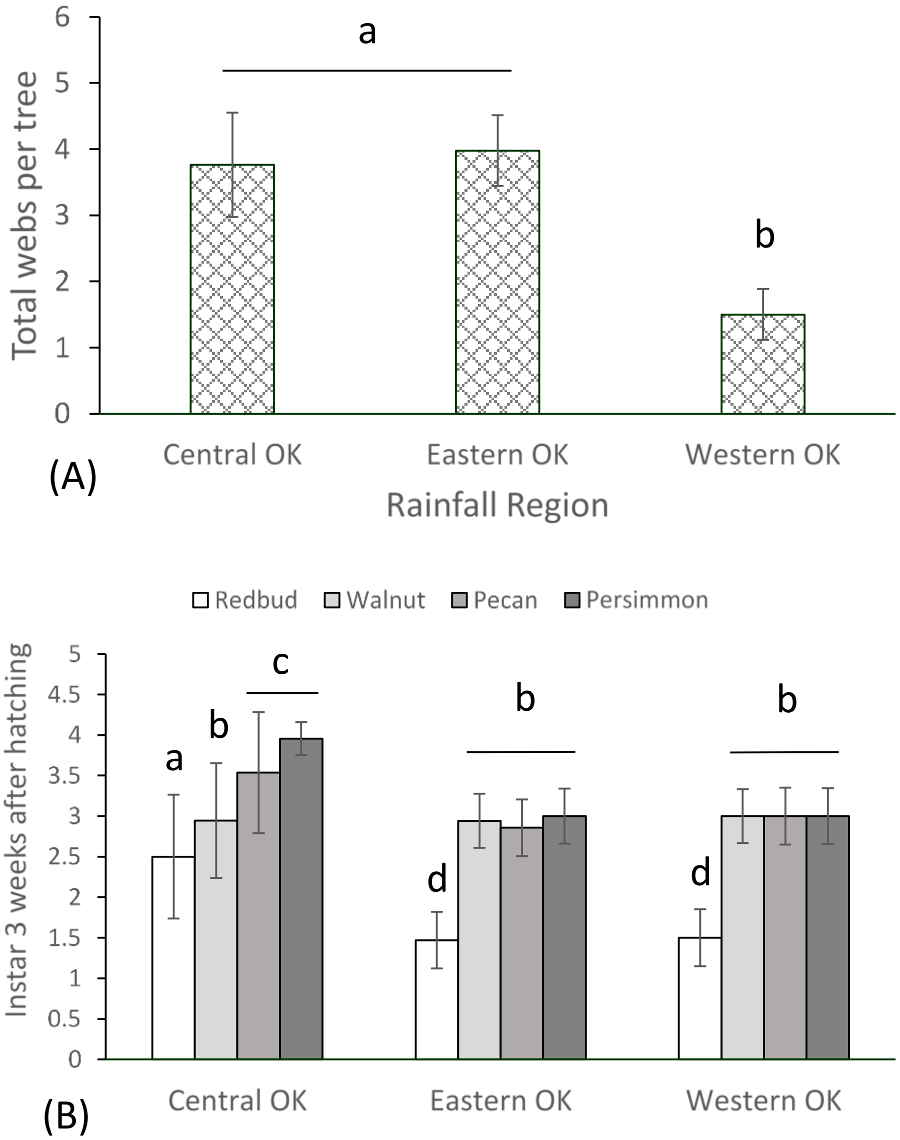
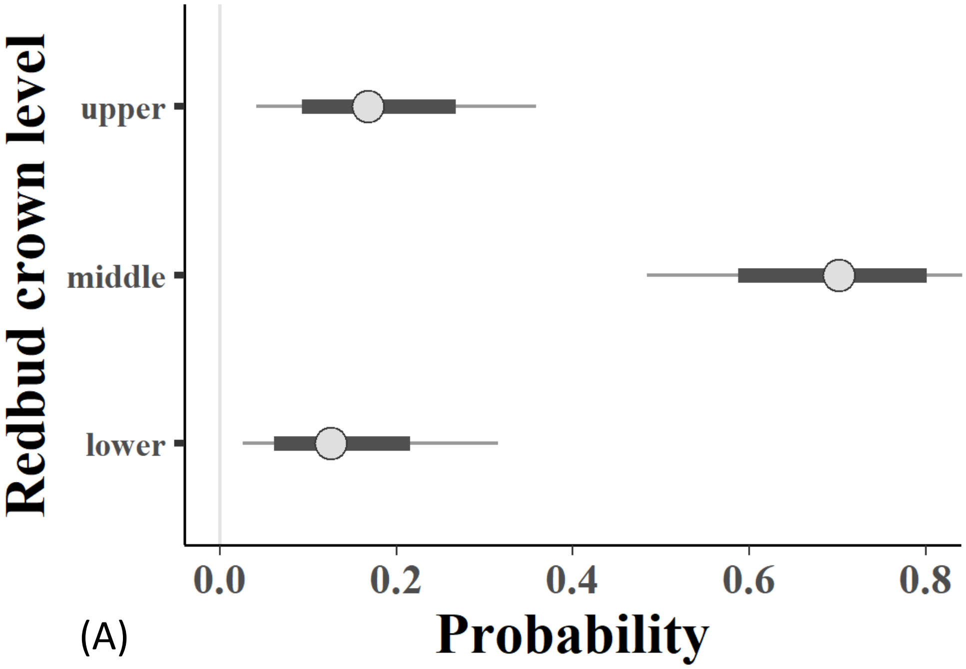
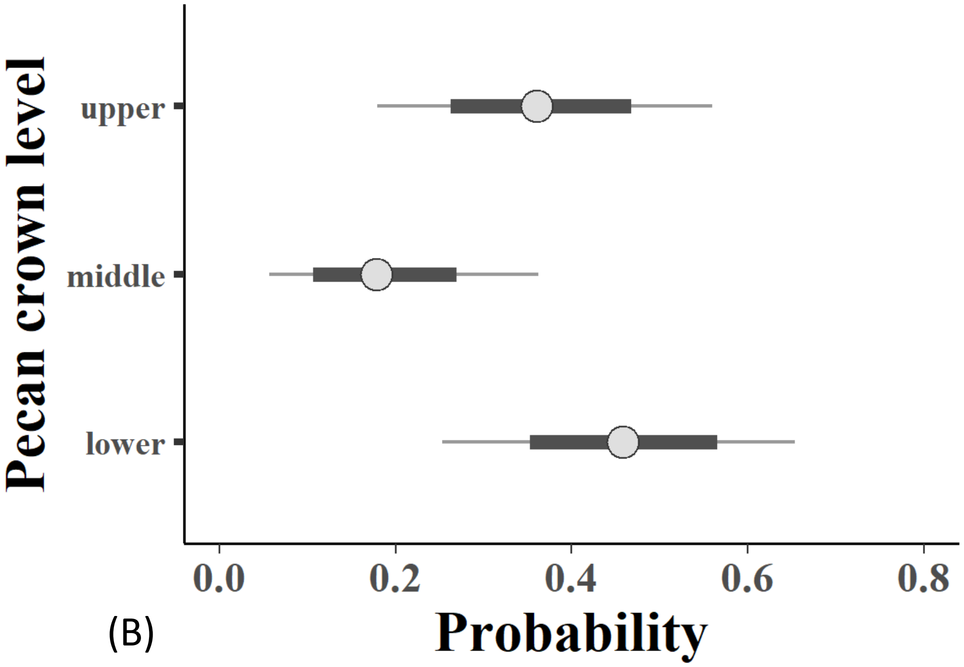


Fig. 3. Larvae in the field built more webs and matured differently on average depending on their rainfall region. Letters that differ denote statistically significant differences, and error bars represent standard deviations. A) Eastern and central Oklahoma host trees contained similar numbers of webs, but western Oklahoma host trees contained 40% fewer webs than trees in the other two regions on average ($F_{2,\,159}$ = 9.058; *p* < 0.001). B) Eastern and western larvae appeared to mature similarly, with redbud larvae taking twice as long to mature compared to larvae on other hosts ($F_{3,158}$ = 5.513; *p* = 0.005). Larvae from central Oklahoma matured the most quickly on persimmon and pecan followed by walnut, then redbud ($F_{3,\,158}$ = 6.256; *p* < 0.001).


Redbud crown level
upper
middle
lower
0.0
0.2
0.4
0.6
0.8
Probability

(A)


Pecan crown level
upper
middle
lower
0.0
0.2
0.4
0.6
0.8
Probability

(B)

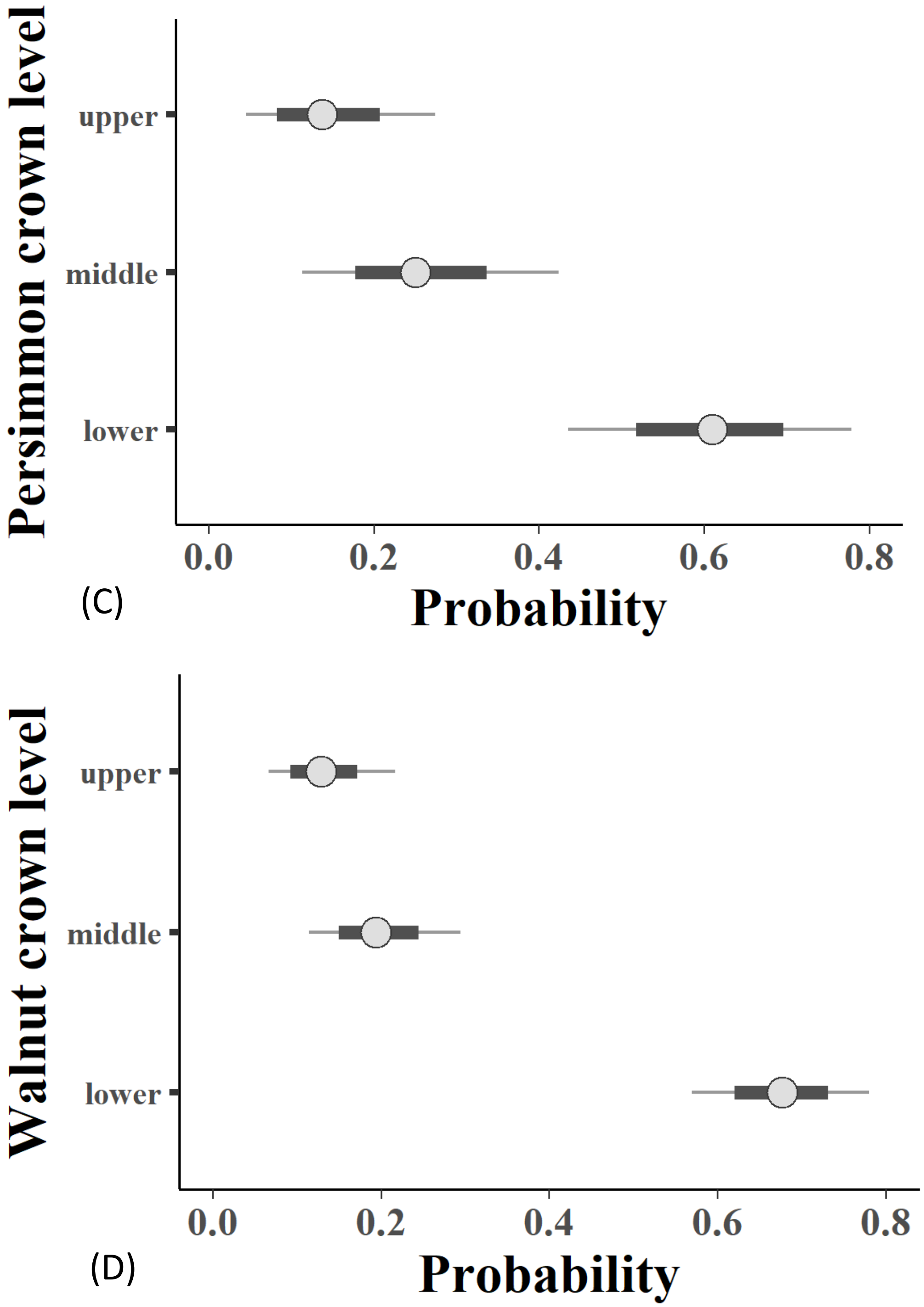


Fig. 4. Multinomial probability estimates for larval web locations in A) redbud B) pecan C) persimmon and D) walnut. Thick credible intervals are shown for the standard 95% range and thin confidence intervals are shown for the bounds of the whole posterior distribution. Probability is highest for the lower tree crown in walnut and persimmon and the middle tree crown in redbud.

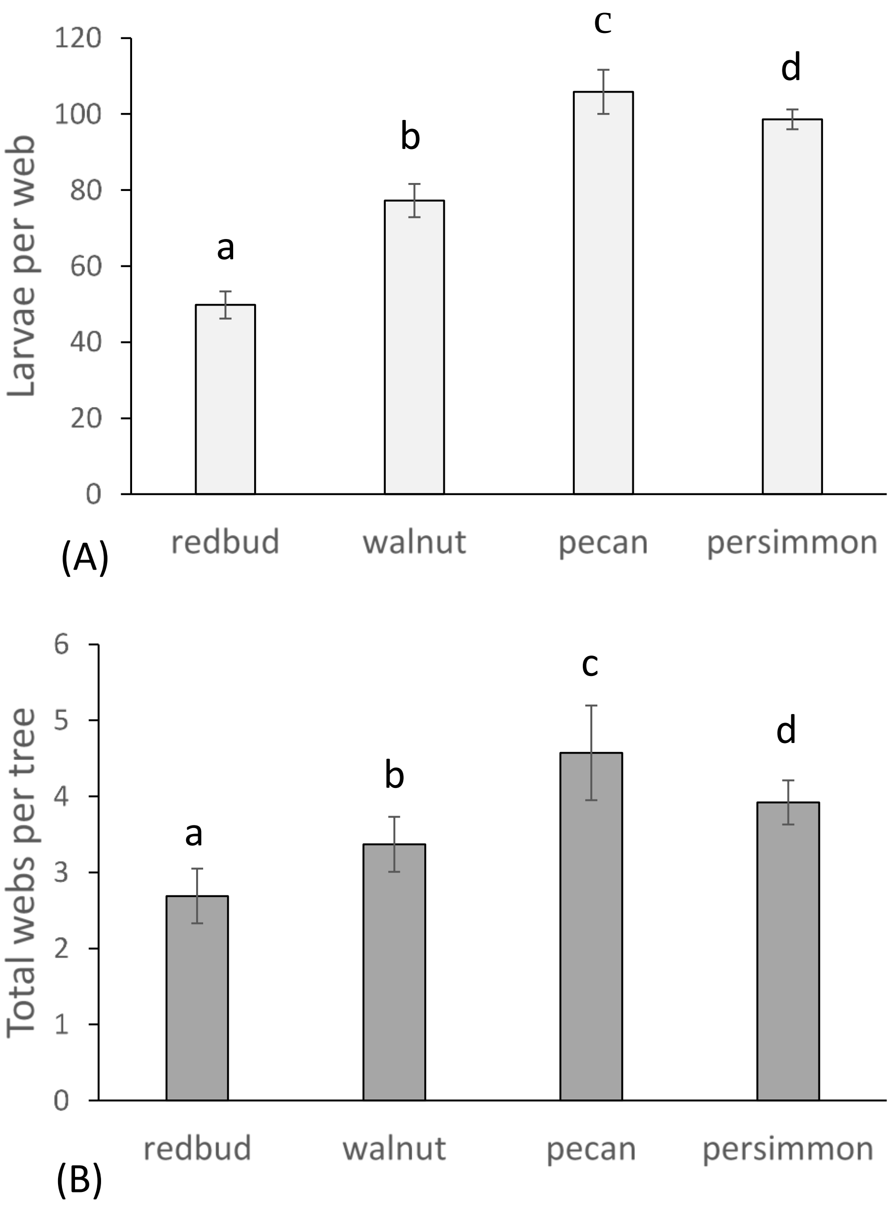


Fig. 5. Average larval abundance on different host taxa in the field. Letters that differ denote statistically significant differences, and error bars represent standard deviations. Pecan trees had the highest average A) larvae abundance per web ($F_{3,\,158} = 6.111$; $p = 0.001$) and B) web abundance per tree ($F_{3,\,158} = 5.083$; $p = 0.002$), regardless of rainfall region.

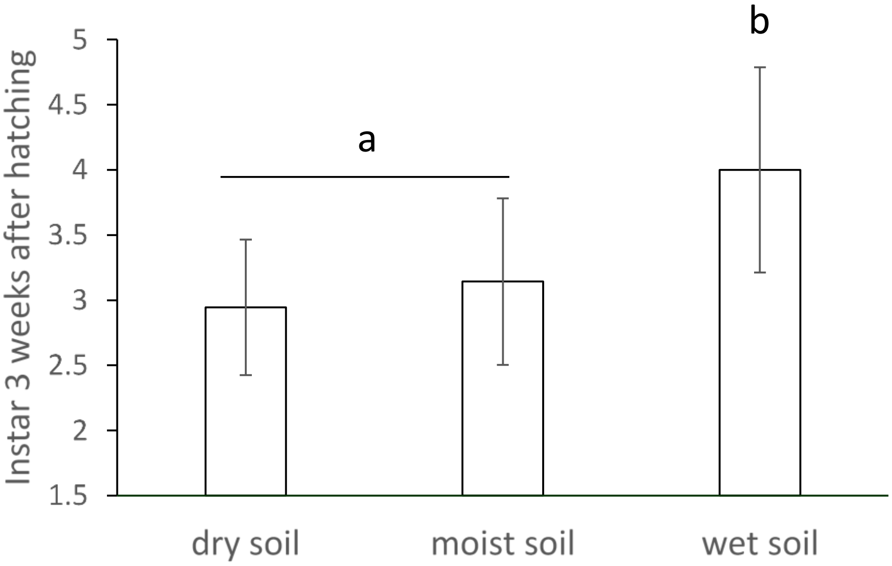


Fig. 6. Fall webworms on hosts exposed to high soil moisture matured to the next instar more quickly compared to larvae on hosts with lower soil moisture ($F_{2, 159}$ = 6.29; *p* = 0.002). Letters that differ denote statistically significant differences, and error bars represent standard deviations.

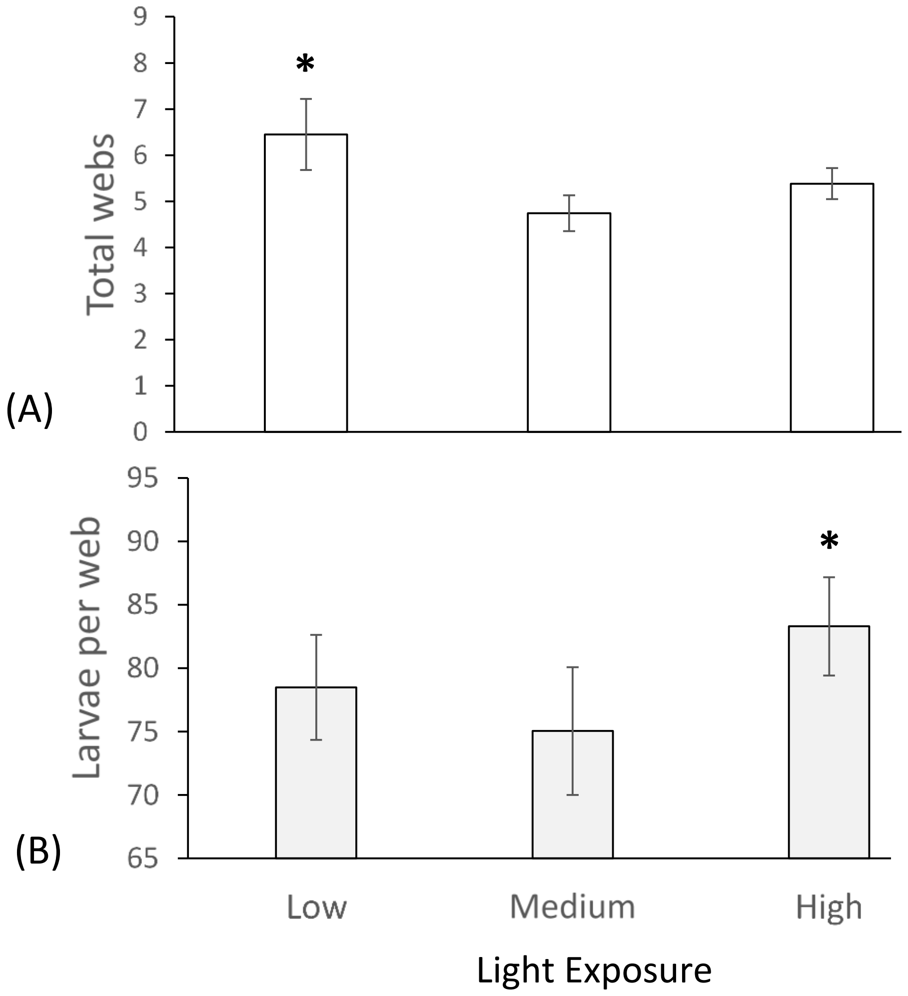
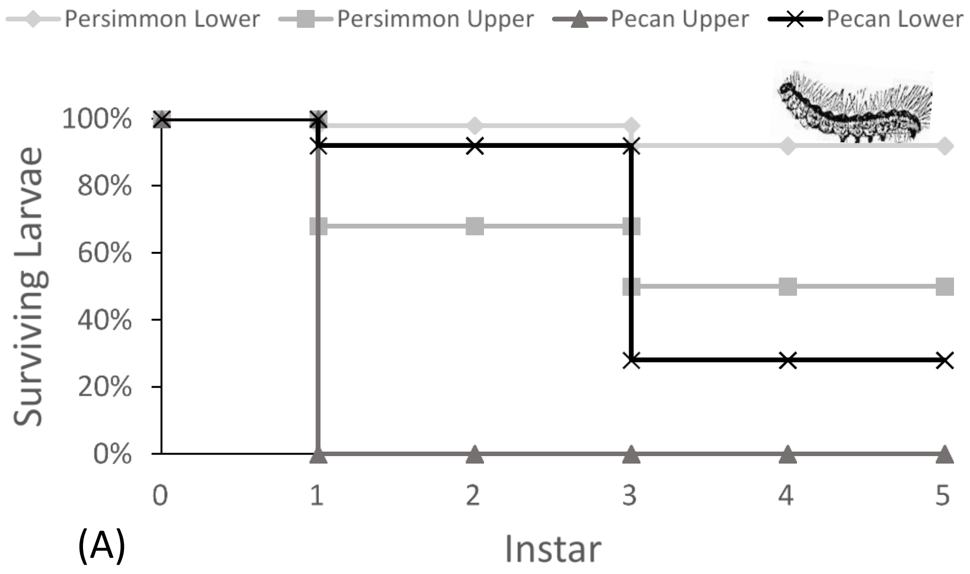
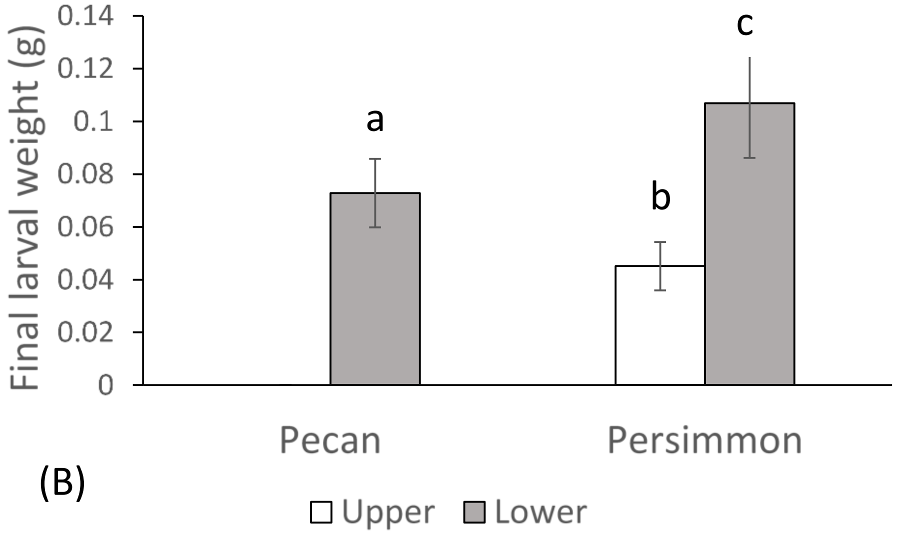


Fig. 7. Plotted averages of larval abundance on hosts in different levels of light. Low: more than 50% shade, medium 30%-50% shade, high less than 30% shade. Asterisks denote statistically significant differences, and error bars represent standard deviations. A) In low light, there were more webs per tree ($F_{2, 159} = 6.290$; $p = 0.002$). B) In high light, there were more larvae per web ($F_{2, 159} = 3.127$; $p = 0.047$).


Persimmon Lower
Persimmon Upper
Pecan Upper
Pecan Lower
100%
80%
60%
40%
20%
0%
Surviving Larvae
0
1
2
3
4
5
Instar
(A)


0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Final larval weight (g)
a
b
c
Pecan
Persimmon
(B)
□ Upper
▨ Lower

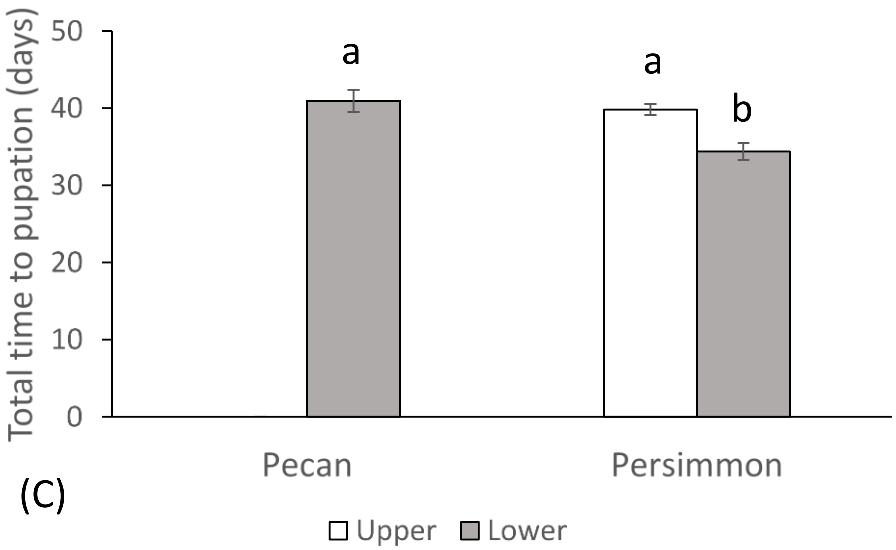


Fig. 8. Mortality and growth of fall webworms reared on leaves from the upper and lower tree crown for two common host plants, pecan and persimmon. These larvae were raised in a lab environment, free of predators and parasitoids. A) Kaplan-Meier survival plot shows mortality occurred during the first and third instars. No larvae fed pecan leaves from the upper tree crown survived beyond the first instar. Larvae fed persimmon and lower crown leaves had higher survival (df = 3; p = 0.03). B) Prior to pupation, the mass of larvae fed foliage from the lower crown ($F_{3, 596}$ = 96.808; p < 0.001) and persimmon ($F_{3, 596}$ = 425.484; p < 0.001) was higher on average. C) Larvae fed lower crown persimmon pupated ~6 days earlier on average than larvae on the other diets ($F_{3, 596}$ = 666.102; p < 0.001). Letters that differ denote statistically significant differences, and error bars represent standard deviation.

Table S1. Null results of MANOVA applied to fall webworm field data. Dependent variables included larval maturation measured in instars, tree height, tree age, total webs per tree, total larvae per web, total larvae per tree, and total larvae per crown level. Independent variables included a) crown level, including upper, middle, and lower, b) rainfall region, including eastern, central, and western Oklahoma c) tree height, d) tree age, e) nest height above the ground, f) soil temperature, g) soil moisture, and h) tree light exposure. Data were log-transformed prior to analysis.

	Num *df*	Den *df*	*F*-ratio	*P*
a) Crown Level				
Larval maturation	2	159	1.412	0.247
Larvae per web	2	159	1.559	0.214
b) Rainfall Region				
Larval maturation	2	159	0.514	0.599
Larvae per web	2	159	0.988	0.375
Tree height	2	159	0.131	0.877
Tree age	2	159	1.56	0.776
c) Tree Height				
Larval maturation	2	159	0.342	0.696
Larvae per web	2	159	1.33	0.586
Webs per tree	2	159	1.19	0.365
Larvae per tree	2	159	3.682	0.280

Larvae per crown level	2	159	0.487	0.720
d) Tree Age				
Larval maturation	2	159	0.676	0.431
Larvae per web	2	159	1.162	0.222
Webs per tree	2	159	3.183	0.144
Larvae per tree	2	159	3.269	0.212
Larvae per crown level	2	159	0.964	0.318
e) Nest Height				
Larval maturation	2	159	2.888	0.059
Larvae per web	2	159	1.264	0.352
Webs per tree	2	159	4.264	0.16
Larvae per tree	2	159	1.677	0.191
Larvae per crown level	2	159	2.416	0.093
f) Soil Temperature				
Larval maturation	2	159	0.259	0.772
Larvae per web	2	159	0.535	0.587
Webs per tree	2	159	0.508	0.730
Larvae per tree	2	159	0.199	0.820
Larvae per crown level	2	159	0.347	0.708
g) Soil Moisture				
Larvae per web	2	159	1.680	0.190
Webs per tree	2	159	0.592	0.555
Larvae per crown level	2	159	1.445	0.239

h) Light Exposure				
Larval maturation	2	159	0.595	0.553
Larvae per tree	2	159	2.975	0.060
Larvae per crown level	2	159	1.970	0.144

Table S2. Number of fall webworm host trees.

	redbud	walnut	persimmon	pecan
Total	15	83	32	32
East OK	7	29	9	9
Central OK	6	11	19	18
West OK	2	43	4	5

Chapter 2: Cross-resistance or trade-offs? Regional variation in drought and toxin tolerances of an herbivorous social insect

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Author Contributions

Amy Adams and Katie Marshall conceived the ideas and searched related literature; Amy Adams designed methodology, collected and analyzed the data, and led the writing of the manuscript; Amy Adams and Michael Patten conceived the statistical analyses. All authors contributed critically to the figures and drafts and gave final approval for publication.

Data Availability Statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Abstract

1. Experimental studies and population models often neglect multifactor stress. When stressors are combined, energetic constraints may lead to either tolerance trade-offs or cross-resistance. Consistent with the principle of allocation derived from Shelford's law, we hypothesized that there may be a trade-off between tolerance to chemical stress from host plant defences and tolerance to abiotic stressors such as heat and desiccation in the fall webworm (*Hyphantria cunea*).
2. We also hypothesized that instead of trade-offs, mechanisms protecting larvae against chemical stress may be linked to mechanisms protecting them against physical stress. This is consistent with principles of cross-tolerance and cross-talk. Local adaptation may modulate this. For example, populations from drought-prone areas may be specialized to tolerate desiccation combined with additional stressors better than other populations.
3. To test these hypotheses, we ran a multifactorial common garden experiment that exposed *Hyphantria cunea* larvae from different rainfall regions across Oklahoma, USA to one of these common tree hosts: persimmon (*Diospyros virginiana*), which has a lower leaf toxicity, and pecan (*Carya illinoinensis*), which has a higher leaf toxicity. Treatments also included abiotic conditions that mimicked summer and fall temperature and low and high rainfall in Oklahoma, USA.
4. As expected from Shelford's law, there was a trade-off between heat and toxin tolerance. This depended on rainfall region origin. Larvae from the intermediate rainfall region fed pecan leaves in hot, wet conditions had the highest mortality and lowest mass of any treatment, but this was not the case for larvae from the lowest and highest rainfall regions.

5. As expected from cross-talk and cross-tolerance, in dry conditions larvae fed pecan leaves had higher mass and survival than larvae fed persimmon leaves. This pattern did not exist among larvae from the highest rainfall region.
6. As conditions become drier and warmer due to climate change, more toxic tree hosts may be most suitable for *Hyphantria cunea* locally adapted to low rainfall. Those locally adapted to higher rainfall will better tolerate more toxic hosts as temperatures rise, provided they are not water stressed. This reinforces that patterns of response to multifactor stress depend on local adaptation.

Keywords: cross-talk, cross-tolerance, *Hyphantria cunea*, principle of allocation, Shelford's law of tolerance, trade-off, local adaptation, epigenetic inheritance

Introduction

The need for intraspecific multifactor stress experiments, particularly in the context of climate change, has outpaced supply over the past ten years. Approximately 70% of published climate change experiments investigated only one stress factor, and climate change population models tend to assume that biological responses are additive when stressors are combined (Collins, 2009; Matesanz & Ramírez-Valiente, 2019) even though biological stress responses may be interactive, especially when chemical stress is involved, which has been investigated in less than 2% of climate change experiments and has focused on anthropogenic pollution (Öncel et al., 2000; Li, 2012; Karl et al., 2014; Büscher et al., 2017). There is empirical evidence that intraspecific variation in stress response is a common phenomenon, but it is often ignored in climate change population models (Matesanz & Ramírez-Valiente, 2019). It is essential to account for multistressor effects and intraspecific variation in these effects to accurately predict which populations will respond to environmental changes positively, neutrally, or negatively.

Reciprocal transplant and common garden experiments can demonstrate if a population is ecologically specialized, or adapted to a specific range of conditions. This leads to higher fitness in those conditions but lowers success in alternative environments (Ferry-Graham et al., 2002; VanWallendael et al., 2019). Local adaptation is often neglected in distribution and population models (Hällfors et al., 2016), in part because local adaptation experiments have mainly featured self-fertilizing crop plant species (VanWallendael et al., 2019). For what is currently known across kingdoms, ~70% of populations are locally adapted (Hereford, 2009). Resource investment is limited in organisms, leading to local adaptation instead of expansive limits of tolerance (Ferry-Graham et al., 2002; VanWallendael et al., 2019).

Shelford’s law states that organisms’ distributions and abundances depend on their limits of tolerance (Shelford, 1931; Odum & Barrett, 1971; Lynch & Gabriel, 1987; Rana, 2013). A subsidiary of Shelford’s Law of Tolerance is commonly known as the principle of allocation. Organisms can have a wide tolerance range for one factor, but this means a narrow tolerance range for another factor due to constraints in energetic investment. Evidence for this has been found for toxin stress (Moe et al., 2013). For example, freshwater snails that can withstand high concentrations of cadmium have reduced heat tolerance (Salice et al., 2010), atrazine tolerance leads to reduced desiccation tolerance in salamanders (Rohr & Palmer, 2005), and fish tolerant of polychlorinated biphenyls are more sensitive to a variety of other stressors, including polycyclic aromatic hydrocarbons (Wirgin et al., 2011).

According to another subsidiary of Shelford’s law, when conditions are not favorable for one factor, limits of tolerance for other factors are reduced (Odum & Barrett, 1971; Rana, 2013). This pattern has been found for a variety of stress combinations and organisms. For example, low nitrogen in the soil leads to reduced drought resistance in plants (Saud et al., 2017), and in low pH soils, plants are less tolerant of aluminum toxicity (Delhaize & Ryan, 1995). There are also examples in vertebrate animals, such as how humidity and temperature increase susceptibility to viral disease in guinea pigs (Lowen et al., 2007) and mercury exposure reduces heat tolerance in swallows (Hallinger & Cristol, 2011). Invertebrate animals have also shown this phenomenon, such as reduced heat tolerance in yellow fever mosquitoes (*Aedes aegypti*) infected with fungal pathogens (Angleró-Rodríguez et al., 2017). Exposure to plant defence chemicals and high temperature reduces the body mass of herbivores (Shelford, 1926; Stamp & Yang, 1996; Yang et al., 1996; Lemoine & Burkepile, 2012; Lemoine et al., 2013).

Alternatively, it is possible that individuals more tolerant to one stress are more tolerant to another. This cross-resistance can occur through one of two mechanisms: cross-talk and cross-tolerance (Sinclair et al., 2013). In cross-talk, mechanisms that protect against one type of stress activate mechanisms that protect against other types of stress. In cross-tolerance, a mechanism that protects against one type of stress simultaneously protects against another stress. Instead of a trade-off in stress tolerance, overall stress tolerance improves. As with Shelford's law, this pattern has been found in a variety of systems. Among autotrophs, wounding increases salt tolerance in tomato plants (Capiati et al., 2006), whereas cross tolerance for salinity and heat exists in other plants such as wheat (Song et al., 2005). In tertiary consumers such as garter snakes, antioxidant enzymes increase tolerance to oxygen deficiency and cold (Hermes-Lima & Storey, 1993).

In invertebrates, cross-tolerance and cross-talk have been found for physical stress, such as withstanding temperature extremes, and chemical stress, such as withstanding pollution. Cold increased resistance to fungal infection in adult *Drosophila melanogaster* (Le Bourg et al., 2008). Yellow fever mosquitoes tolerant of copper, coal, or petroleum also have increased insecticide resistance (Poupardin et al., 2012).

Trade-offs in chemical and climate stress have been detected in solitary insect herbivores, but there is a lack of empirical evidence for this in social insect herbivores, which we aimed to rectify. Due to greater behavioral complexity exhibited by social herbivores, their physiological tolerances may differ from their non-social counterparts. For example, *Hyphantria cunea* use protective webs to regulate physical factors such as temperature, humidity, and predators (Sullivan & Green, 1950; Knapp & Casey, 1986; Joos et al., 1988; Costa, 1997; Roda et al., 2000; Rehnberg, 2002; Ruf & Fiedler, 2002; Rehnberg, 2006; Lemos et al., 2010; Eouzan et al., 2019). It is possible that relative to a solitary herbivore, the niche of a social shelter-building herbivore depends more on chemical stress from its plant host than physical climatic stress. Since larval *Hyphantria cunea* feed on the leaves of a range of deciduous trees, we were able to experimentally compare their performance on more toxic hosts and less toxic hosts in various climate conditions (Morris, 1963; Ge et al., 2019). The hosts we compared grow wild in Oklahoma, USA across a rainfall gradient (64 – 143 cm) where precipitation is lowest in the west and increases moving east (mesonet.org).

Across Oklahoma, USA, *Hyphantria cunea* are equally likely to occupy persimmon and pecan trees (Adams et al., 2020), even though the latter is more toxic. Naphthoquinones such as juglone are the main chemical plant defences of pecan trees, comprising ~20% of leaf weight (Hedin et al., 1980; Borazjani et al., 1985; Kim & Wetzstein, 2005; Solar et al., 2006; Stampar et al., 2006; Villarreal-Lozoya et al., 2007; Cosmulescu & Trandafir, 2011; Nour et al., 2013). Of the known plant defence chemicals in *Hyphantria cunea* host trees, juglone causes the most oxidative stress in the guts of a variety of lepidopteran larvae, particularly those from family Erebidae, and causes severe damage to epithelial gut tissue (Thiboldeaux et al., 1994; Thiboldeaux et al., 1998; Sun et al., 2007; Akhtar et al., 2012; Hu et al., 2018; Lv et al., 2018). Aside from 1) juglone and its relatives such as 1,4-naphthoquinone, the following phenolics are present and active in pecan tree foliage: 2) flavonoids such as catechin, epicatechin, rutin, quercetin, and myricetin and 3) phenolic acids such as vanillic, syringic, elagic, gallic, caffeic, p-coumaric, ferulic, sinapic, salycilic, and chlorogenic (Villarreal-Lozoya et al., 2007). In contrast, persimmon leaves use only one class of phenolics to defend against Lepidopteran larvae, the flavonoids. This includes catechin, astragalin, isoquercitrin, quercetin, kaempferol, and glucopyranoside (Kameda et al., 1987; Choi et al., 1996; Kotani et al., 2000; Chen et al., 2008; Sun et al., 2011).

We aimed to complete an intraspecific multifactor climate change experiment that included herbivores' response to plant defence toxins, a common source of chemical stress other than anthropogenic pollution. We used a factorial experimental design and common garden lab incubation to test the following questions: 1) Is there a trade-off between the tolerance of *Hyphantria cunea* to chemical stress, such as host plant defence toxins, and physical stress, such as heat and drought? And 2) Is there intraspecific variation in the stress tolerances of *Hyphantria cunea* based on the rainfall region from which they originated? We compared the following hypotheses: 1) there is a trade-off between chemical and physical stress according to Shelford's law 2) there is no trade-off; cross-talk or cross-tolerance should reduce insect herbivore susceptibility to chemical and physical stress and 3) local adaptation modulates relationships between stress tolerances. We predicted the following possibilities (Fig. 1): A) more toxic plant matter should reduce insect herbivore mass and survival in heat and drought and B) more toxic plant matter should improve insect herbivore mass and survival in heat and drought. High temperatures were considered more stressful because of the energetic cost of manufacturing more stress buffering proteins such as heat shock proteins, and hot conditions have been shown to reduce size, survival and fecundity in Lepidoptera (Carroll & Quiring, 1993; Neven, 2000; Shirai, 2000; York & Oberhauser, 2002; Mironidis & Savopoulou-Soultani, 2010). In an environment that does not allow for the herbivore to ingest much water, stress-buffering proteins may not be readily synthesized since they require a lot of water (Somero et al., 2016). We also expected insects descended from populations occupying lower rainfall environments to be ecologically specialized for drought, and therefore withstand chemical and heat stress better in low moisture lab treatments relative to insects descended from populations occupying higher rainfall environments.

Methods

Study Organisms

We used an herbivorous social insect called the fall webworm (*Hyphantria cunea*; Lepidoptera: Erebidae) to test if there is either codependence or trade-offs in chemical and climate stress tolerances, and if this varied geographically. *Hyphantria cunea* larvae have at least two colour morphs: red-headed and black-headed. In North America, *Hyphantria cunea* are an abundant nuisance moth species, ranging from southern Canada (latitude 50-55 °N) to the southern United States and parts of Mexico (latitude 19 °N). They spread to Europe and Asia during the 1940's, becoming invasive in those areas (Morris, 1963; Ge et al., 2019). The red-headed *Hyphantria cunea* is not as well-studied as their black-headed counterpart (Vega et al., 2008; Loewy et al., 2013; Murphy & Loewy, 2015; Vidal et al., 2020). Our study featured the red-headed *Hyphantria cunea* for this reason and because it is more abundant than the black-headed colour morph in Oklahoma, USA, which is where this study took place (Rebek, 2009).

In Oklahoma, first-generation red-headed *Hyphantria cunea* larvae feed for a period of approximately six weeks from mid-June through the first week or two of August, and second-generation larvae feed for a period of approximately six weeks from September through mid-October (Rebek, 2009; Adams et al., 2020). We confirmed through observations in the laboratory that red-headed *Hyphantria cunea* have up to five instars. They moult four times to accommodate an expanding head capsule width.

Potent carbon and hydrogen-based phenolics and terpenoids are the main chemical plant defences of red-headed host trees (Hedin et al., 1980; Borazjani et al., 1985; Choi et al., 1996;

Kim & Wetzstein, 2005; Solar et al., 2006; Villarreal-Lozoya et al., 2007; Zhang et al., 2009; Cosmulescu & Trandafir, 2011). Red-headed *Hyphantria cunea* are found on a wider range of tree species in Oklahoma urban areas. Outside of the urban areas they are found on native redbud (*Cercis canadensis*), black walnut (*Juglans nigra*), persimmon (*Diospyros virginiana*), and pecan or hickory (*Carya,* especially *C. illinoiensis*) trees (Rebek, 2009; Adams et al., 2020).

Foliage feeding lab experiment

A pilot foliage feeding experiment featuring larvae from only one region in Oklahoma took place a year before the summer 2019 common garden experiment. *Hyphantria cunea* eggs were collected from pecan and persimmon trees located in Cleveland County, Oklahoma, USA (35.2226° N, 97.4395° W) and hatched in the laboratory in summer 2017. Their descendants were used in the laboratory experiment in summer 2018. Over a period of six weeks, the duration of the *Hyphantria cunea* life cycle in summer, 400 larvae were raised in a multifactorial lab feeding experiment. Larvae were fed a diet of either pecan or persimmon leaves and exposed to one of the following treatments: hot and dry, hot and wet, cool and dry, and cool and wet. They were exclusively fed leaves from one host plant since in the field they are restricted to one tree for the duration of their larval life cycle. There were 50 larvae per treatment reared in climate-controlled incubators (Panasonic MIR-254-PE), and we followed rearing guidelines established by Loewy et al. (2013). Larvae were weighed with a Smart Weigh High Precision Digital Milligram Scale twice per week until they reached adulthood. “Hot” was defined as 34 °C, mimicking summer temperature in central Oklahoma, USA and matching other work featuring caterpillar heat stress (York & Oberhauser, 2002; Mech et al., 2018). “Cool” was defined as 23 °C, mimicking fall temperature in central Oklahoma, USA. Simulated rainfall in the “wet” treatments mimicked the maximum seasonal precipitation occurrence of central Oklahoma. This was applied by spraying 3.3 L of water per square meter daily. Simulated rainfall in the "dry" treatments mimicked the minimum seasonal precipitation occurrence of central Oklahoma and was applied by spraying 1.8 L of water per square meter daily. These values were determined from detailed environmental monitoring measurements provided by the Oklahoma Mesonet (mesonet.org). Wet and dry conditions were held constant across temperature treatments.

In summer 2019, this experiment was repeated using larvae from three differing rainfall origins. This time, 1-2 day old larvae in their first instar were collected from pecan or persimmon trees. They continued to be fed their larval host plant in the laboratory. The larvae were acclimated to the lab for the remainder of their first instar following transport, and treatments and measurements occurred starting with the second instar (Obernier & Baldwin, 2006). The larvae originated from one of the following regions: 1) western Oklahoma (35° N, 98° W), mean annual precipitation less than 64 cm, 2) central Oklahoma (35° N, 97° W), mean annual precipitation 64-102 cm, and 3) eastern Oklahoma (35 ° N, 95 ° W), mean annual precipitation 102-143 cm (mesonet.org).

Statistical Analyses

To compare the effects of temperature, moisture, and plant diet on larval mass, we used analysis of variance (ANOVA) using IBM SPSS Statistics software. We calculated Cohen's eta-squared (η^2) thresholds to measure effect size, with a low effect size defined as $\eta^2 = 0.02$, a medium effect size defined as $\eta^2 = 0.13$, and a large effect size defined as $\eta^2 = 0.26$ (Fritz et al., 2012).

To compare the effects of temperature, moisture, and plant diet on larval mortality, we created Kaplan-Meier survival plots using R (R Core Development Team, www.r-project.org). Stairstep plots were broken into larval instars. Log-rank tests determined if survival times between treatments significantly differed. Cox proportional hazards regression models were used to test if other independent variables affected larval survival.

Results

Mass

Larvae from the lowest rainfall region of western Oklahoma did not grow to a final mass beyond 150 mg (Fig. 2A). Larvae from the intermediate rainfall region of central Oklahoma (Fig. 2B) and the high rainfall region of eastern Oklahoma (Fig. 2C) had a maximum final mass of approximately 200 mg. Even though larvae from western Oklahoma had a smaller maximum size than larvae from other regions, patterns in relative mass for the various treatments generally matched central Oklahoma.

There was a significant interaction between origin location, plant diet, and moisture. Larvae from central Oklahoma reared on pecan leaves in dry conditions grew larger while larvae reared on persimmon leaves in wet conditions grew larger ($F_{7, 272} = 5.76$; $p < 0.001$; $\eta^2 = 0.17$). This was also the case for larvae from western Oklahoma ($F_{7, 187} = 3.18$; $p = 0.003$; $\eta^2 = 0.10$) except there was no statistically significant difference when it was hot and wet. There was also a significant interaction between origin location and temperature such that larvae from central Oklahoma reared at higher temperatures grew smaller ($F_{3, 272} = 2.65$; $p < 0.001$; $\eta^2 = 0.11$). Central Oklahoma larvae showed a higher mass difference at different temperatures than their western counterparts ($F_{3, 187} = 0.335$; $p = 0.799$; $\eta^2 = 0.0045$). Larvae from eastern Oklahoma did not show a significant difference in final mass between treatments ($F_{7, 206} = 2.00$; $p = 0.055$; $\eta^2 = 0.16$).

Mortality

Mortality was most likely to occur during the third instar across all experimental groups (Fig. 3 &4). The final instar was the next larval stage where mortality was most likely to occur, except in larvae from central Oklahoma. No mortality was detected in central Oklahoma larvae during the final instar except for a 2% mortality rate in the final instar of central Oklahoma larvae fed persimmon leaves in hot and wet conditions (Fig. 4B). Central and western larvae had a total of five instars while eastern larvae only had four instars.

In dry conditions, survival for western and central Oklahoma larvae ranked in this order: pecan hot, pecan cool, persimmon cool, persimmon hot (Fig. 3A & 3B). Log-rank tests revealed a significant interaction between dryness and plant diet, such that more toxic pecan leaves led to higher survival in dry conditions. The survival of central Oklahoma larvae for the different treatments was identical between 2018 and 2019 (Fig. 3B & 4B). In central Oklahoma, 90% or more of larvae survived dry conditions, except when it was hot survival decreased by 46% if the diet was persimmon leaves instead of pecan ($p = 0.001$). No central Oklahoma larvae died when fed pecan leaves in hot and dry conditions, which was the lowest mortality of any treatment. Hot conditions led to greater differences in mortality between the plant diets relative to cool conditions. If the larvae came from the central Oklahoma intermediate rainfall region, relative to larvae from the drier western Oklahoma region survival was 4% higher in the hot and dry pecan treatment, 9% higher in the hot and dry persimmon treatment, 11% higher in the cool and dry pecan treatment, and 13% higher in the cool and dry persimmon treatment.

In contrast to western and central Oklahoma, larvae from eastern Oklahoma survived better on persimmon in dry conditions ($p = 0.009$; Fig. 3C). Temperature did not significantly interact with the pecan diet when conditions were dry for eastern Oklahoma larvae. By the final instar mortality was similar between treatments, except survival was much higher for larvae on a persimmon diet in cool and dry conditions. If the larvae came from the central Oklahoma intermediate rainfall region, relative to larvae from the wetter eastern Oklahoma region survival was 33% higher in the hot and dry pecan treatment, 11% lower in the hot and dry persimmon treatment, 25% higher in the cool and dry pecan treatment, and 2% lower in the cool and dry persimmon treatment.

In wet conditions, larvae from western and central Oklahoma survived better on persimmon than on pecan (Fig. 4A & 4B), though this difference was more pronounced for central Oklahoma ($p < 0.001$). There was a significant interaction between pecan leaf diet and temperature in wet conditions among western larvae such that lower temperatures led to greater mortality in the final instar ($p = 0.002$). Higher temperatures caused mortality during the fourth instar for larvae from central Oklahoma, leading to higher overall mortality in hot and wet conditions.

For eastern larvae, survival was not always higher on persimmon in wet conditions (Fig. 4C). Eastern larvae survived the best on persimmon when it was cool, and pecan when it was hot ($p = 0.01$). Larval age interacted with plant species in wet conditions, as a persimmon leaf diet caused mortality in the fourth instar while a pecan leaf diet did not. This also happened in larvae from western Oklahoma.

Overall, survival and mass were correlated, though this relationship was weak for larvae from the highest rainfall region. When dryness was included in a treatment, *Hyphantria cunea* from western and central Oklahoma had significantly higher mass and survival when fed more toxic leaves. When conditions were wet, *Hyphantria cunea* from eastern Oklahoma had significantly higher survival when fed more toxic leaves at higher temperatures. Eastern larvae also grew larger on average in the pecan hot and wet treatment, but this was not statistically significant because standard deviations were 2-3 times larger in eastern Oklahoma larvae relative to larvae from lower rainfall regions (Fig. 2).

Discussion

We assessed for local adaptation to rainfall conditions and two contrasting phenomena: 1) stress response trade-offs suggested by Shelford's law of tolerance and 2) improved stress responses from cross-talk or cross-tolerance. Modality of response to multiple stressors differed based on source population. It seems local adaptation was modulating relationships between stress tolerances. Larvae from the highest rainfall region of eastern Oklahoma followed Shelford's law when conditions were dry, but when wet cross-talk or cross-tolerance for heat and toxin stress occurred. Larvae from the low and intermediate rainfall region followed Shelford's law through a heat and toxin stress response trade-off when conditions were wet, but there was cross-tolerance or cross-talk for toxin and desiccation stress. For *Hyphantria cunea* from central and western Oklahoma, mass and survival were strongly influenced by leaf toxicity as we expected for social insect herbivores, while the survival of eastern Oklahoma larvae depended more on temperature than other stress factors. Western Oklahoma *Hyphantria cunea* appeared to be less sensitive to heat and moisture stress relative to the other regions, which we expected because frequent droughts have likely influenced their genetics more than *Hyphantria cunea* from the other regions.

We posit that midgut microsomal oxidases and heat shock proteins may be key players in the cross-talk or cross-tolerance we detected for *Hyphantria cunea*. For example, at least five microsomal oxidases common in lepidopteran midguts have higher activity in higher temperatures (Ahmad et al., 1986). This aligns with survival results from the eastern *Hyphantria cunea*, where there appeared to be cross-talk or cross-tolerance occurring for heat and toxin tolerance. It is possible that midgut enzymes or heat shock proteins may differ for eastern *Hyphantria cunea* relative to those from lower rainfall regions, which would explain why the central and western *Hyphantria cunea* experience a trade-off in heat and toxin tolerance instead of cross-talk or cross-tolerance for these stressors. Cross-talk between transcription factors for heat shock proteins and proteins that mitigate oxidative stress have been observed in a variety of eukaryotes (Dayalan Naidu et al., 2015; Driedonks et al., 2015), so this may occur in *Hyphantria cunea* as well. Alternatively, cross-tolerance could be occurring because heat shock proteins such as HSP70 have been shown to protect caterpillars against heat and chemicals simultaneously (Augustyniak et al., 2017). That cross-tolerance mitigates chemically disrupted energy reserves instead of tissue damage, so cross-talk involving oxidase enzymes is more likely to be behind our results because leaf chemicals damage digestive tissue via oxidative stress (Thiboldeaux et al., 1994; Thiboldeaux et al., 1998; Sun et al., 2007; Akhtar et al., 2012; Hu et al., 2018; Lv et al., 2018).

To our knowledge, our study is the first to establish that cross-talk or cross-tolerance could occur for desiccation and plant chemical stress resistance in a lepidopteran species. In the model organism *Drosophila melanogaster* as well as other *Drosophila* species, desiccation resistance has also been found to be correlated with toxin resistance, though the genes and enzymes underpinning this are still under investigation. In *Drosophila*, desiccation resistance increases as metabolic rate decreases, so enzymes controlling metabolic rate have been proposed as key players in the cross-talk or cross-tolerance of desiccation and toxin resistance (Hoffmann & Parsons, 1993; Matzkin & Markow, 2009). It is possible that this is the case for other insects such as *Hyphantria cunea*. Lower metabolic rate extends larval development and increases body size in invertebrates (Kingsolver & Huey, 2008; Graham et al., 2013; Scholten et al., 2018). In our study, the *Hyphantria cunea* in the low and intermediate rainfall regions had extended larval development relative to *Hyphantria cunea* from the high rainfall region because they had an extra larval instar, so *Hyphantria cunea* from the high rainfall region may have higher metabolism. Protracted development could be why *Hyphantria cunea* from the high rainfall environment do not show signs of cross-talk or cross-tolerance for toxin and desiccation stress relative to *Hyphantria cunea* from drier environments.

We believe that local adaptation to rainfall region is the most likely reason for the variation we detected in our common garden lab experiment, in that mass and survival patterns of *Hyphantria cunea* descended from the intermediate rainfall region was consistent between the pilot study and the common garden lab experiment. In the first year we ran this experiment, we collected data from *Hyphantria cunea* born and raised in the laboratory. These insects were descended from webworms collected from the intermediate rainfall region in the summer one year before the experiment. There were multiple generations between the wild-caught and experimental *Hyphantria cunea* in this case. For the common garden experiment, data was collected from larvae taken from the wild early in their development. If epigenetic inheritance such as maternal effects determined larval responses to stress rather than local adaptation, then differences in mass and survival should have been detected between the two experiments. We did not have multiple generations of lab reared eastern and western descendants to compare to those taken directly from the wild, so we cannot infer how epigenetic inheritance may have affected the results for those regions. At least for central Oklahoma, maternal effects appeared to play a negligible role when it came to mass and survival. That is, unless the maternal effects can persist across more than two generations; the number of generations that maternal effects may persist in *Hyphantria cunea* is currently unknown. Epigenetic effects based on diet stress have been found to persist for up to two generations in other organisms, such as with humans descended from famine survivors (Wei et al., 2015). Nevertheless, it is also common for meiosis to reset epigenetic modifications in organisms, so a maternal effect might not persist beyond one generation (Bond & Finnegan, 2007). Future work should explore the role of maternal effects in the stress tolerances and population dynamics of this species and other insect herbivores.

As conditions become warmer and drier due to climate change, *Hyphantria cunea* may be more successful on more toxic trees. Although pecan trees may currently be a more suitable host for *Hyphantria cunea* in the summer season because of higher insect mass and survival, cooling temperatures and increasing precipitation currently make persimmon trees a more suitable host for the insects in the fall season. If the climate in the fall shifts to be more like the summer, then there should no longer be seasonal shifts in *Hyphantria cunea* performance on the different species. Populations from central and western Oklahoma will tolerate plant chemical stress well as the landscape becomes drier and warmer. Populations from eastern Oklahoma will tolerate plant chemical stress well as conditions become warmer, provided they are not simultaneously water stressed. We can expect climate change to reduce the performance of eastern *Hyphantria cunea* populations and promote outbreaks in the central and western populations based on our findings, which reinforces the importance of accounting for intraspecific variation in multifactor stress responses.

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Figures

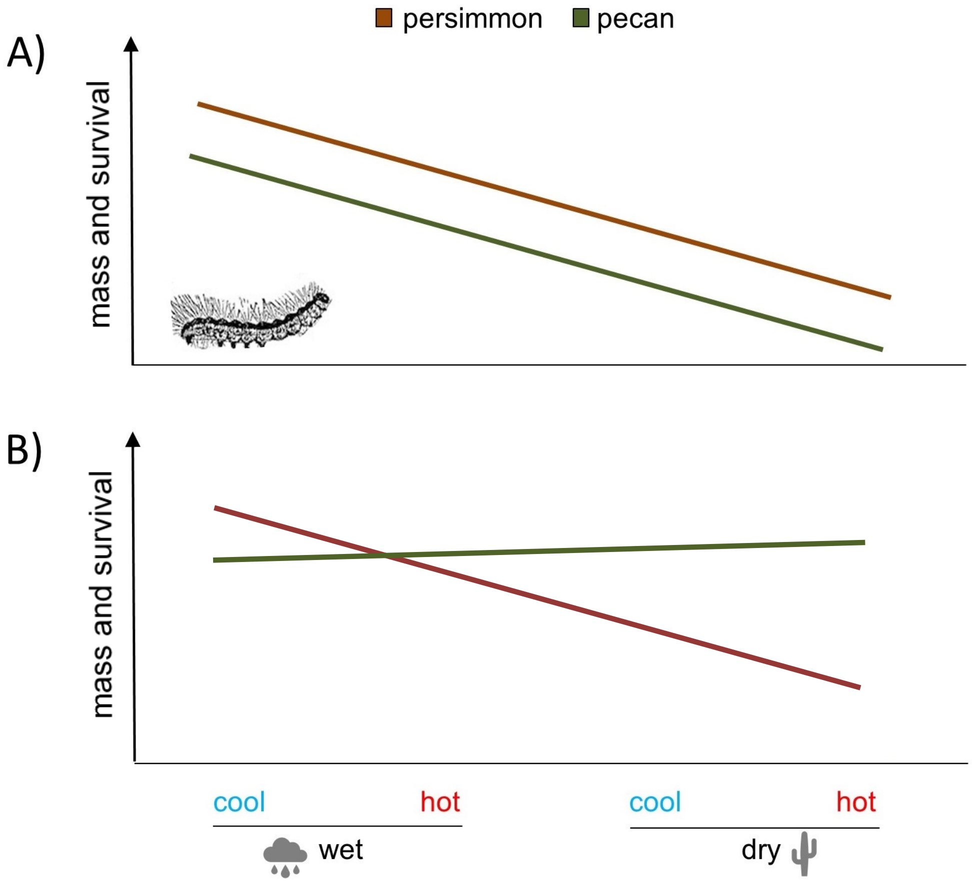
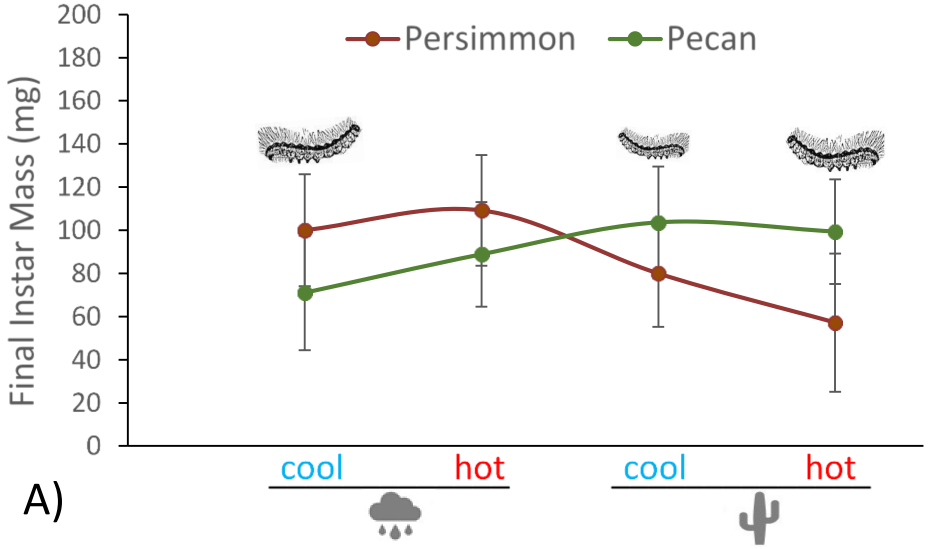
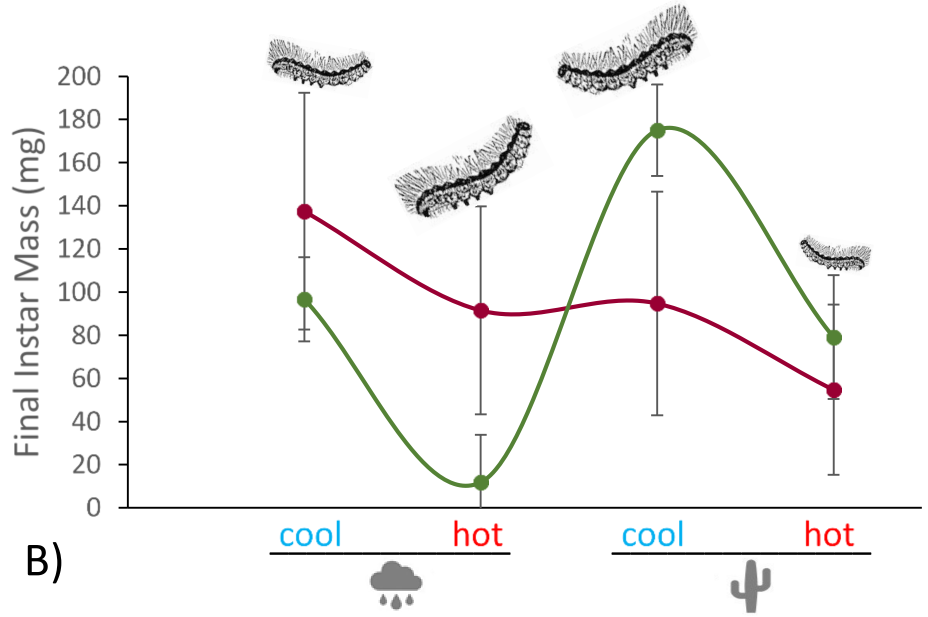


Figure 1. A) Predictions based on the hypothesis that there is a trade-off between physical stress (heat, drought) and chemical stress (plant chemical defences) tolerances. More toxic leaves, from pecan trees, should reduce insect herbivore performance. B) Predictions based on the hypothesis that cross-talk or cross-tolerance should improve insect herbivore performance under multifactor stress. More toxic leaves, from pecan trees, should improve insect herbivore performance.


Final Instar Mass (mg)
200
180
160
140
120
100
80
60
40
20
0
Persimmon
Pecan
cool
hot
cool
hot
A)


Final Instar Mass (mg)
200
180
160
140
120
100
80
60
40
20
0
cool
hot
cool
hot
B)

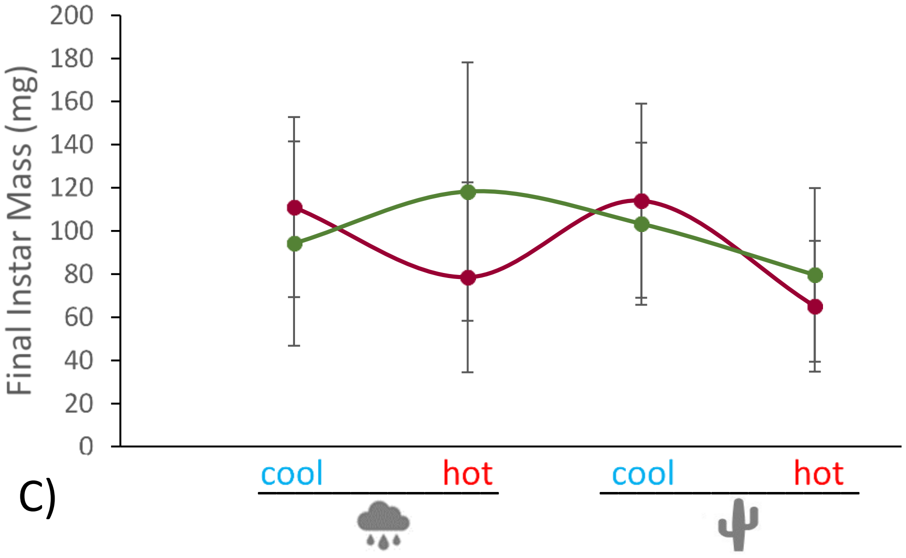
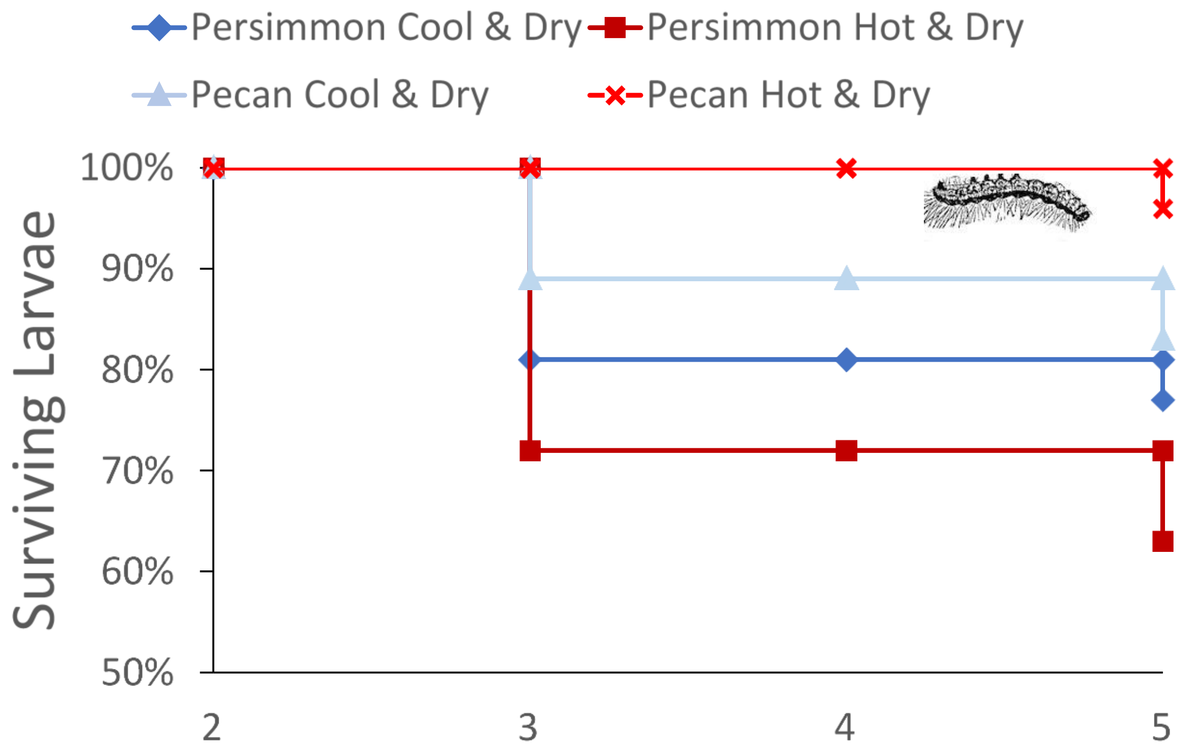
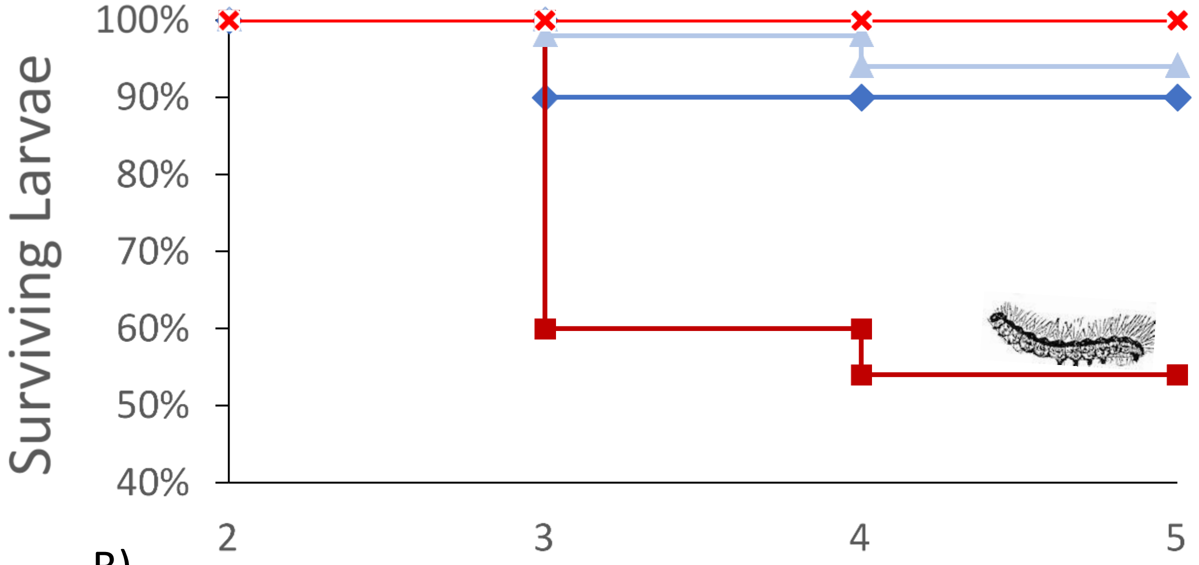


Figure 2. Final instar mass of larvae originating from A) western ($F_{7, 187} = 3.18$; $p = 0.003$; $\eta^2 = 0.10$) B) central ($F_{7, 272} = 5.76$; $p < 0.001$; $\eta^2 = 0.17$) and C) eastern Oklahoma ($F_{7, 206} = 2.00$; $p = 0.055$; $\eta^2 = 0.16$). If central and western Oklahoma were the rainfall regions of origin, pecan leaves resulted in the highest mass in dry conditions. Larvae from eastern Oklahoma did not show a significant difference in final mass between treatments, inconsistent with larvae originating from lower rainfall regions. Error bars denote standard deviation. Caterpillars denote significant differences; the larger the caterpillar, the greater the difference between leaf diets.


Persimmon Cool & Dry
Persimmon Hot & Dry
Pecan Cool & Dry
Pecan Hot & Dry
Surviving Larvae
100%
90%
80%
70%
60%
50%
2
3
4
5

A)


Surviving Larvae
100%
90%
80%
70%
60%
50%
40%
2
3
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B)

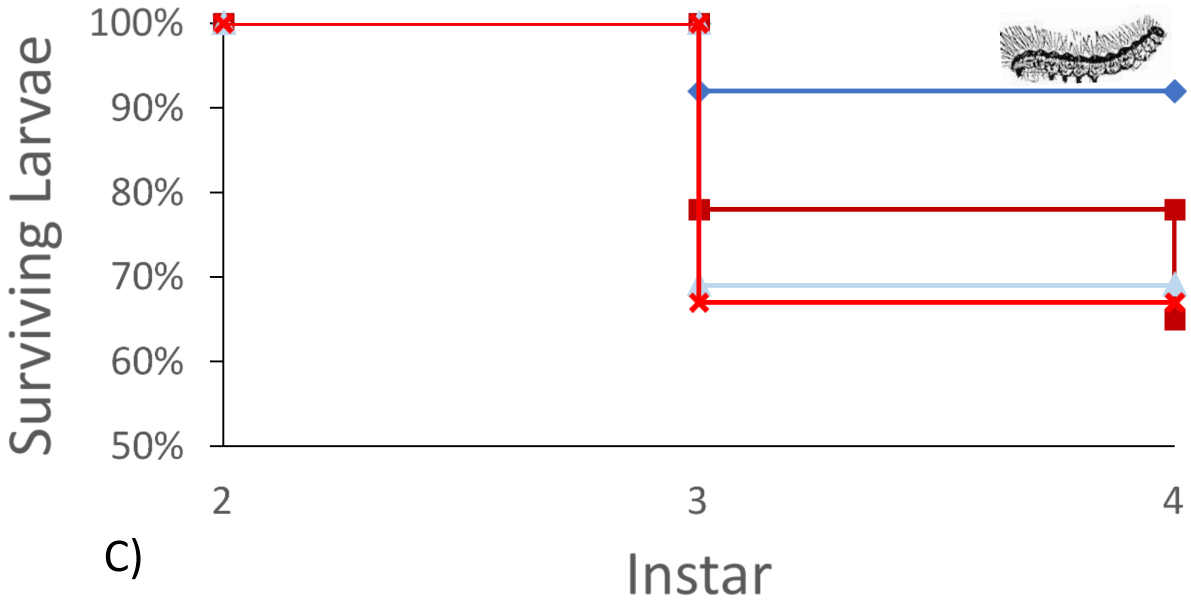
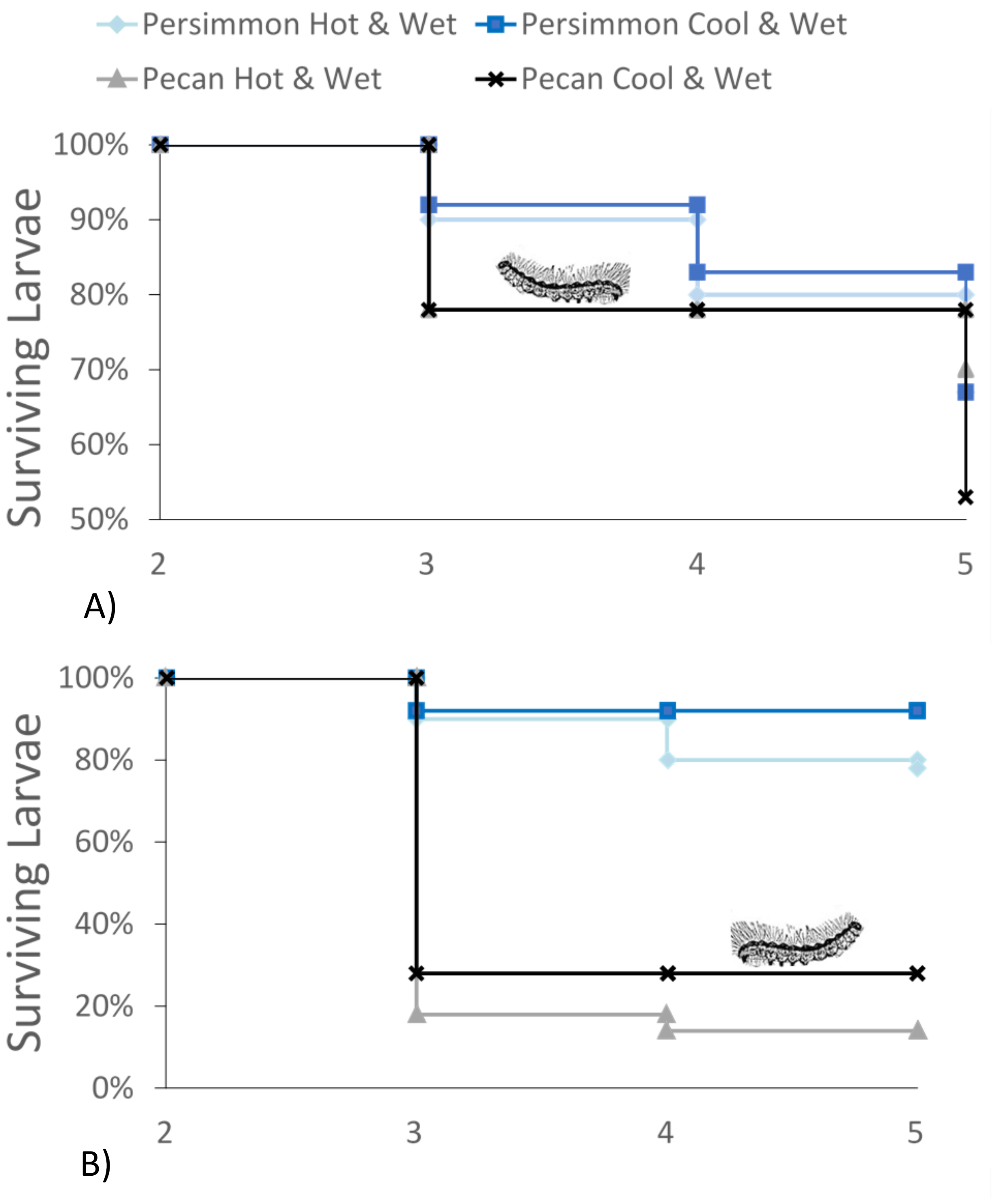


Figure 3. Kaplan-Meier survival plots for dry conditions, with larvae originating from A) western (*p* = 0.001) B) central (*p* = 0.001) and C) eastern Oklahoma (*p* = 0.009). P-values for the log-rank tests based on overall survival, not just survival at the final instar. Larvae from eastern Oklahoma did not develop a fifth instar. Western and central larvae survived better on pecan, especially in hot conditions. Eastern larvae survived better on persimmon, though by the final instar there was not a difference between the two diets in hot conditions.


Persimmon Hot & Wet
Persimmon Cool & Wet
Pecan Hot & Wet
Pecan Cool & Wet
Surviving Larvae
100%
90%
80%
70%
60%
50%
2
3
4
5
A)
Surviving Larvae
100%
80%
60%
40%
20%
0%
2
3
4
5
B)

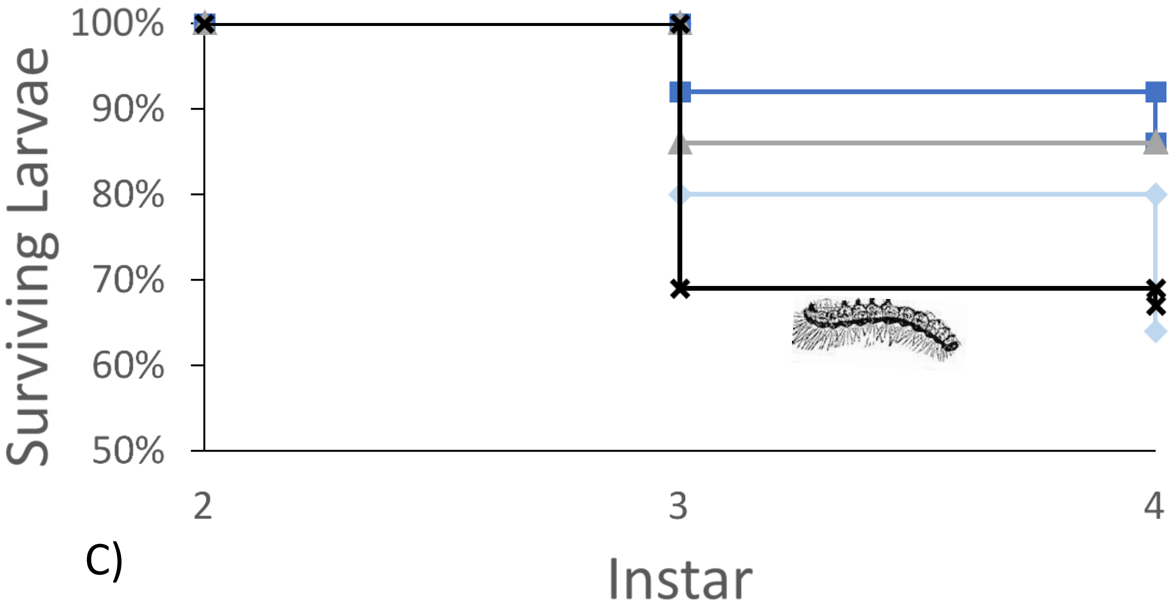


Figure 4. Kaplan-Meier survival plots for wet conditions, with larvae originating from A) western (*p* = 0.002) B) central (*p* < 0.001) and C) eastern Oklahoma (*p* = 0.01). P-values for the log-rank tests based on overall survival, not just survival at the final instar. Larvae from eastern Oklahoma did not develop a fifth instar. For larvae from the central intermediate rainfall region, most mortality occurred during the third instar, with very little in the final instars. Western and central larvae survived better on persimmon. For larvae from the low western and high eastern rainfall regions, most mortality occurred in the third or the final instar. Eastern larvae survived better on persimmon in cool conditions and pecan in hot conditions.

Chapter 3: Regional variation in predator defense, inbreeding, and eclosion behaviour of a social insect herbivore

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Data availability statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Understanding behavioural response to stress is an increasingly important aim in ecology, particularly as climate change accelerates extinctions and behaviour is likely the first response to climate change. Consequently, we assessed for regional variation in intraspecific behavioural interactions of the social insect herbivore *Hyphantria cunea*. We also tested if positive intraspecific behavioural interactions increased with increasing stress as claimed by the Stress Gradient Hypothesis, or with increasing genetic relatedness as claimed by kin selection. Findings are reported from common garden experiments that included descendants of insects extracted from the following regions in the USA: western Oklahoma, which receives less than 64 cm annual rainfall, central Oklahoma, which receives 64-102 cm annual rainfall, and eastern Oklahoma, which receives 102-143 cm annual rainfall. Assistive eclosion behaviour was detected in adults, and it was more likely to occur following dry larval conditions and if insects were siblings. Eastern *H. cunea* adults were less likely to assist conspecifics during eclosion and had more inbreeding relative to those from drier regions. There were also pronounced differences in the defensive emesis produced by larvae based on rainfall origin. Emesis was darker and produced in larger volumes by larvae descended from wetter regions, while larvae descended from drier regions were more likely to use alternative predator defense strategies. Accounting for such regional variation in behavioral stress responses can improve population management, especially in the context of a changing climate. We conclude that although the Stress Gradient Hypothesis and kin selection were both supported by our findings, populations will evolve to cooperate when stressed regardless of kin relatedness.

Keywords: Stress Gradient Hypothesis, local adaptation, altruism, reciprocity, inbreeding, kin selection, emesis, eclosion, *Hyphantria cunea*

Introduction

Population resilience and persistence in stressful environments typically depend more on behavioural interactions than other factors (Cahill et al. 2013; Ockendon et al. 2014). For example, stress shifts behavioural interactions positively, towards cooperation, according to the Stress Gradient Hypothesis (Bertness and Callaway 1994). The main tests of this hypothesis have featured interspecific plant competition (Maestre et al. 2005; Kawai and Tokeshi 2007; Holmgren and Scheffer 2010; Armas et al. 2011), so we set out to conduct a novel study of intraspecific cooperation in animals. Understanding behavioural response to stress is an increasingly important aim in ecology, as climate change and habitat destruction in the Anthropocene are accelerating biological extinctions (Collins 2009; Schimel et al. 2013; Büntgen et al. 2020).

The Stress Gradient Hypothesis has not often been extended to account for evolutionary consequences (O'Brien et al. 2017). If increasing positive interactions increases fitness in stressful conditions, then populations in more stressful conditions may locally evolve adaptive behaviours such as conspecific assistance. So far, evidence for this response has mainly been collected for plant-microbe mutualistic interactions (Johnson et al. 2010; Rúa et al. 2016), not for one species distributed across locations that vary in stress intensity.

Because the Stress Gradient Hypothesis usually is tested in the context of interspecific behavioural interactions, it has not traditionally been associated with theoretical concepts in sociobiology such as kin selection described by Hamilton (1963). According to Hamilton's rule, for positive behavioural interactions to occur between kin, $rB > C$, where r represents the genetic relatedness of the individuals, B represents the reproductive benefit gained from the beneficiary of the behaviour, and C represents the cost of the behaviour to the benefactor. Here, genetic relatedness is the key determinant of positive behavioural interactions. Genetic relatedness is not factored into the Stress Gradient Hypothesis; instead, the benefits an organism will derive from cooperating with another simply need to outweigh the costs.

Hyphantria cunea moths engage in cooperative social behaviour in addition to naturally occurring both across a precipitation gradient and on plants with varying phenolic concentrations in Oklahoma, USA (Adams et al. 2020). This led us to use *H. cunea* from Oklahoma in a common garden experiment to test the following hypotheses: 1) According to the Stress Gradient Hypothesis, stress increases the probability of positive intraspecific behavioural interactions regardless of the genetic relatedness of individuals, and 2) According to Hamilton's rule of kin selection, genetic relatedness increases the probability of positive intraspecific behavioural interactions. Water availability and plant diet were manipulated to introduce stress in this study, and we compared behaviours between siblings that were familiar with each other, siblings that were not familiar with each other, and non-siblings.

The moths we used were descended from source populations from one of the following regions in the United States: 1) western Oklahoma (35° N, 98° W), mean annual precipitation less than 64 cm, 2) central Oklahoma (35° N, 97° W), mean annual precipitation 64-102 cm, and 3) eastern Oklahoma (35 ° N, 95 ° W), mean annual precipitation 102-143 cm (mesonet.org). Because of this, we were able to test if there was regional variation in *H. cunea* behaviour, possibly caused by local adaptation or epigenetic inheritance. Based on the Stress Gradient Hypothesis, we expected moths descended from the more stressful dry region of western Oklahoma to have a higher probability of cooperative behaviour relative to moths from wetter regions.

Methods

Evaluated Behaviours

To successfully test the kin selection hypothesis, we needed to determine if *H. cunea* are capable of kin recognition as adults. As larvae, they nest together in protective webbed shelters of hundreds of full siblings and likely communicate through chemical signals (Morris 1963; Fitzgerald and Costa 1986; Crump et al. 1987; Underwood and Shapiro 1999; Ruf et al. 2001; Fitzgerald and Pescador-Rubio 2002). Although larval nest recognition occurs, chemical communication is not well understood in *H. cunea* (Fitzgerald 2015), and it is not clear if this recognition persists through the adult stage. We evaluated two behaviours in adult moths to determine if kin recognition occurred: helpful eclosion, and inbreeding.

Helpful eclosion behavior has not previously been described in *H. cunea* literature. It is generally accepted that sociality does not persist in this species after the larval stage, but we discovered that sociality persists past the larval stage in Oklahoma populations of *H. cunea*. We used red-headed *H. cunea*, which are not as well studied as their black-headed counterparts even though they are more common in Oklahoma, USA (Rebek 2009; Murphy and Loewy 2015; Adams et al. 2020). While establishing breeding stock in the laboratory for various experiments run 2016-2020, we observed that larvae will pupate together in groups of three or more individuals. The moths in these groups will eclose together, and those that emerge first will scrape at the posterior end of other pupae in the group as the moths within attempt to free themselves. We found that red-headed *H. cunea* from Oklahoma can successfully free their heads and thoraxes from their pupae, but they struggle to free their abdomens without external help, such as assistance from other moths. Sometimes a moth will perish because it only partially eclosed. We tested to see if the probability of helpful eclosion was higher among siblings than non-siblings, which would support the kin selection hypothesis. We also tracked inbreeding to see if there was evidence of inbreeding avoidance, which would provide additional evidence of kin recognition.

Aside from testing for regional variation and stress effects on adult eclosion and inbreeding behaviours, we evaluated how these factors influence predator defense behaviour in larvae. Predator defense is the main benefit Lepidoptera derive from sociality (Costa and Pierce 1997). Regurgitation is an especially costly predator defense, and all larvae in a group benefit when an individual regurgitates (Higginson et al. 2011; Daly et al. 2012). We noticed that the regurgitant, or defensive emesis, varied between larvae in terms of quantity and coloration. Initially emesis variation seemed to be driven by source population of western, central, and eastern Oklahoma (Fig. 1), but we quantified and analyzed this experimentally. We only compared the predator defense of full siblings, so we did not evaluate the kin selection hypothesis during the larval stage, but we were able to use emesis production as a metric of cooperative behaviour to test the Stress Gradient Hypothesis.

Specimens

In June 2019, 1-2 day old *H. cunea* were collected from eastern, central, and western Oklahoma. Stock populations were raised and bred at room temperature on a laboratory bench following a rearing protocol established by Loewy et al. (2013). Larvae were collected from either pecan (*Carya illinoiensis*) or persimmon (*Diospyros virginiana*) trees, and for the rest of their lives we fed them leaves from the plant species they were originally found on. We chose to focus on pecan and persimmon in our research because pecan leaves contain more phenolics, which cause digestive stress in Lepidoptera, than persimmon trees (Hedin et al. 1980; Thiboldeaux Robert L. et al. 1994; Thiboldeaux R. L. et al. 1998; Villarreal-Lozoya et al. 2007;

Sun et al. 2011; Akhtar et al. 2012; Lv et al. 2018), so we could evaluate how the insects responded to different levels of plant chemical stress.

If a moth came from a certain source population, it was only allowed to breed with others from that source population. We were able to determine if a source population influenced the next generation by doing this. For example, we allowed a moth from western Oklahoma mating access only to other moths from western Oklahoma. Siblings were prevented from mating with each other at this point. The offspring of these moths were used in common garden laboratory experiments starting in September 2019.

Emesis Experiment

We triggered predator defense behaviour in five-week-old larvae by laterally pinching each of them gently for 20 seconds. We measured a total of 864 larvae raised at 23 °C, mimicking fall temperature in central Oklahoma. Descendants of western, central, and eastern Oklahoma source populations each made up one third of this total, or 288 larvae per source population. These were broken into the following treatments, with 72 larvae per treatment: pecan leaf diet in wet conditions, pecan leaf diet in dry conditions, persimmon leaf diet in wet conditions, and persimmon leaf diet in dry conditions. Approximately 1200 more larvae were raised in these conditions and pinched, but measurements were not recorded. These were our backup insect specimens, which we could use to replace any of the original sample size that died during our eclosion and inbreeding experiments later. Simulated rainfall in the “wet” treatments mimicked the maximum seasonal precipitation occurrence of central Oklahoma. This was applied by spraying 3.3 L of water per square meter daily. Simulated rainfall in the “dry” treatments mimicked the minimum seasonal precipitation occurrence of central Oklahoma and was applied by spraying 1.8 L of water per square meter daily. These values were determined from detailed environmental monitoring measurements provided by the Oklahoma Mesonet (mesonet.org). Simulated rainfall did not continue past the larval stage.

To test if source population, plant diet, and water availability affected emesis coloration, we photographed the emesis each caterpillar produced and extracted its gray value, or pixel brightness, in ImageJ (Rueden et al. 2017). A lower gray value depicts darker coloration (Ferreira and Rasband 2012). We standardized the photography setting by pinching each insect on white sheet of copy paper on a table surface 76 cm above ground level, and this was in a windowless room lit by a 1600 lumen LED light fixture oriented 2.44 m above ground level. We positioned an iPhone 7 camera 30 cm above the insect, at a 180° angle that made the phone align parallel to the table surface. The table was cleaned with diluted Lysol between each photo shoot.

In other insects that regurgitate liquid such as emesis or honey, a darker pigmentation often indicates a higher phenolic concentration (Ortego et al. 1997; Calcagno et al. 2004; Can et al. 2015). We used a Folin-Denis assay to estimate the percentage of phenolics in *H. cunea* emesis (Swain and Goldstein 1964; Appel and Maines 1995) and compared that to the ImageJ gray values to verify if darker emesis indicated a higher concentration of phenolics. If so, measuring plant-derived chemicals in emesis through the photographic method could be more time and cost efficient than measuring through chemical assays. Knowledge of the phenolic content would also allow us to determine if plant defense chemistry contributes to variation in *H. cunea* predator defense behaviour, or if there is some other metabolic mechanism driving it instead.

Eclosion Experiment

To test if adult moths could recognize their kin and if this influenced whether they helped other moths eclose, after the larvae pupated, we separated them into 144 total groups each comprised of six moth individuals and assigned one of the following conditions: 1) the control, where full siblings that nested together were grouped so they would eclose together and 2) full siblings that were separated upon hatching and therefore did not nest together were grouped so they would eclose together and 3) individuals from the same region that were not siblings and did not nest together were grouped so they would eclose together. To test if stress affected assistive eclosion probability, 72 groups were exposed to wet conditions as larvae while the other 72 were exposed to dry conditions as larvae. There were 48 groups per condition per rainfall source population, for a total of 288 moths per rainfall source population. The three rainfall source populations were eastern, central, and western Oklahoma, so there were 864 moths total in this experiment. Half were raised on pecan, the other half on persimmon. We set video cameras to constantly record during the insects' second week as pupae so we would not overlook the start of eclosion. We used the footage to count occurrences of newly emerged adults helping their peers eclose.

Inbreeding Experiment

We tested if siblings would breed with each other or not by transferring them to a breeding chamber that allowed them to fly around, and the size and conditions of this chamber matched the breeding guidelines established by Loewy et al. (2013). We grouped moths in the breeding chambers as follows: siblings that nested together, siblings that did not nest together, and non-siblings. We then timed how long it took moths to begin mating attempts and counted the cases of copulation. Moths did not attempt to breed with each other until they were moved to the breeding chamber and were able to fly around in it.

Statistical analyses

To compare the effects of plant diet, source population, and water availability on emesis coloration, we ran analysis of variance (ANOVA) with Tukey's post hoc tests using IBM SPSS Statistics software. We calculated Cohen's eta-squared (η^2) thresholds to measure effect size, with a low effect size defined as $\eta^2 = 0.02$, a medium effect size defined as $\eta^2 = 0.13$, and a large effect size defined as $\eta^2 = 0.26$ (Fritz et al. 2012).

The probability of a newly eclosed adult moth helping others eclose and the probability of an adult moth breeding with a sibling were each estimated with a Bayesian multinomial model with flat priors (i.e., no assumptions about probability of helpful eclosing per treatment). Results from a JAGS model implemented in R (R Core Development Team, www.r-project.org) using the *rjags* package (Plummer 2012) with 3 Markov chains, a burn in of 1,000, and posterior distributions built from 10,000 iterations were plotted using package *bayesplot* in R.

Results

Defensive Emesis

We verified that darker *H. cunea* emesis is inversely correlated with a higher concentration of phenolics (Fig. 2). If the ImageJ gray value, or pixel brightness, increased by 10, then there was a decrease in the phenolic concentration by ~4%. Larvae fed pecan leaves produced darker emesis than larvae fed persimmon leaves, and larvae from eastern Oklahoma produced darker emesis than larvae from central Oklahoma ($F_{3, 144} = 9236.61$; $p < 0.001$; $\eta^2 = 0.98$). Water availability did not play a significant role in emesis coloration (Suppl. Table 1).

Helpful Eclosion

There was an effect of larval water availability, source population, and sibling status on helpful eclosion behaviour in *H. cunea* (Fig. 3). There was no difference between moths raised on a pecan diet and those raised on a persimmon diet. If the moths were descended from the source populations of western and central Oklahoma, they were 2-3 times more likely to help each other eclose if as larvae they had been raised in dry conditions relative to wet conditions. This was also the case for eastern Oklahoma moths, except there was no difference between wet and dry for siblings that had nested together as larvae. The probability of helpful eclosion was highest in moths descended from western Oklahoma, followed by central Oklahoma, then eastern Oklahoma. If larvae were raised in dry conditions, the probability of helpful eclosion among western moths was nearly twice as high as central moths and three times as high as eastern moths.

We found that siblings helped each other eclose more often than non-siblings. Western and central non-siblings were more likely to help each other eclose than eastern non-siblings. If larval conditions were wet, then eastern non-siblings almost never helped each other. There were only certain conditions in which siblings that nested together as larvae differed from siblings that were separated from each other as larvae. If the source population was eastern Oklahoma and larval conditions were wet, then siblings that shared a larval nest helped each other eclose more often than siblings that did not nest together. Larval nesting and dryness interacted in central Oklahoma siblings such that there was a higher probability of helpful eclosion among those that nested together relative to those that did not. In western Oklahoma descendants, the probability of helpful eclosion was ~2% higher among siblings that shared a larval nest, but overlapping credible intervals indicate that the nesting condition of siblings did not matter as much to the western moths relative to those from the other source populations.

Inbreeding

Inbreeding only occurred in *H. cunea* from the highest rainfall region of eastern Oklahoma. Non-siblings began mating attempts with each other within the first 24 hours after eclosion while siblings delayed mating attempts with each other until 24 hours after eclosion. Siblings from western and central Oklahoma never mated with each other no matter how much time passed.

Larval water availability affected adult inbreeding (Fig. 4). There was no difference between moths raised on a pecan diet and those raised on a persimmon diet. For siblings that nested together as larvae, wet larval conditions caused inbreeding to be ~10 times more likely to occur relative to dry larval conditions. For siblings that did not nest together as larvae, wet larval conditions caused inbreeding to be ~13 times more likely to occur relative to dry larval conditions. Siblings that did not nest together as larvae were 2% more likely to breed with each other following dry larval conditions and 5% more likely to breed with each other following wet larval conditions relative to siblings that nested together as larvae, though nearly identical overlapping credible intervals indicate that larval nesting did not affect inbreeding.

Discussion

We assessed if intraspecific behavioural interactions were locally adapted to rainfall environment in *H. cunea* and if positive intraspecific behavioural interactions increased with increasing stress, as claimed by the Stress Gradient Hypothesis (Bertness and Callaway 1994), or with increasing genetic relatedness as claimed by kin selection (Hamilton 1963). We found that the Stress Gradient, kin selection, and local adaptation hypotheses were all supported, but support for each depended on the type of stress involved. For example, plant defense chemistry did not affect *H. cunea* behaviour after the larval stage, whereas low rainfall did affect *H. cunea* behaviour throughout the life cycle. We successfully linked regional genetics and kin selection to the Stress Gradient Hypothesis in an intraspecific animal system. Our study was also a unique evaluation of the Stress Gradient Hypothesis in that we assessed both juvenile and adult behaviours instead of focusing on just one life stage. The hypothesis was clearly supported for adults.

Predator Defense

The Stress Gradient Hypothesis implies that source populations with higher stress (in our system, a more severe drought history) should have the strongest cooperative predator defense behaviour, but instead larvae from this source population reacted sluggishly and did not produce emesis. One might be tempted to conclude that the Stress Gradient Hypothesis was not supported at the juvenile stage because of this. However, there is meta-analytic evidence indicating biotic factors such as predation play a greater role in population resilience than abiotic factors (Ockendon et al. 2014). We propose that follow-up studies should focus on evaluating the Stress Gradient Hypothesis in the context of biotic stressors such as predation pressure. If water availability was solely determining emesis production, then why did larvae from eastern Oklahoma produce darker emesis? *H. cunea* from the wettest region of eastern Oklahoma had more toxic emesis than the other regions, so it is possible danger from predators and parasitoids was greater in the east relative to the west. If this is the case, then the Stress Gradient Hypothesis would be supported by predator defense in juveniles, and the mechanism of this support would be through predation instead of drought stress.

Although water availability did not play a role in the phenolic concentration of emesis, it may affect the volume of liquid regurgitated. Precipitation differs between western, central, and eastern Oklahoma, and this was reflected in the *quantity* of liquid regurgitated from larvae from these source populations. This predator response might be locally adapted to the point of fixation because eastern *H. cunea* would produce anterior and defensive liquid and central *H. cunea* would produce anterior defensive liquid even if they were raised in dry conditions. Instead of conserving their water by defensively biting or thrashing, they reacted by regurgitating. Drier environments may affect regurgitation onset and volume in subsequent attacks, which has yet to be tested, but we found it did not affect the initial predator defense response. We posit that local adaptation is probably the mechanism responsible for this result because it is common for meiosis to reset epigenetic modifications in organisms, so a maternal effect might not persist beyond one generation (Bond and Finnegan 2007). In addition to that, there is strong support for locally adapted toxin, heat, and desiccation tolerances in *H. cunea* based on these tolerances matching between field-collected and third generation laboratory moths (Adams et al. 2020). We cannot rule out epigenetic inheritance completely because the persistence of maternal effects across generations has not been tested in *H. cunea*, but for now we think local adaptation is more likely.

Helpful Eclosion

A key implication of the Stress Gradient Hypothesis is that populations will evolve so that kin relationships play a reduced role in determining positive behavioural interactions. We observed a prominent difference in helpful eclosion between eastern Oklahoma *H. cunea* non-siblings and non-siblings from the other regions. The fact that non-siblings from this wetter region almost never helped each other eclose following ideal precipitation conditions indicates that kin selection mattered more in this source population relative to the others. Meanwhile, non-siblings from the other regions tended to help each other even in ideal conditions. Although genetic relatedness of kin is an important determinant of positive behavioural interactions, our results showed that the importance decreases as populations adapt to cooperate in more stressful conditions. Organisms will become more likely to generally help non-related individuals.

Kin Recognition

For the first time, it has been verified that kin recognition persists in *H. cunea* beyond the larval stage. This allowed us to conclude that both stress and kinship increased positive behavioural interactions in this species. We would not have detected a higher probability of helpful eclosion in siblings and a lower probability of siblings mating relative to non-siblings if kin recognition was not occurring. Inbreeding avoidance in adult moths was an especially good indication of kin recognition in this study.

A possible explanation for western and central siblings never breeding with each other while eastern siblings eventually did may be because inbreeding depression is less of an issue in the east. There is meta-analytic support for inbred individuals being more sensitive to environmental stress (Fox and Reed 2011). Hence, in the central and western regions of Oklahoma where there is greater environmental stress from lower levels of precipitation, there should be greater selection against individuals that cannot recognize kin well.

Also, the difference in kin recognition following dry larval conditions relative to wet larval conditions may be linked to chemical communication. Siblings were able to recognize each other even if they were separated as larvae and later reunited as adults, probably because their pheromones matched. Olfaction may be the primary way siblings recognize each other, which has been observed in other social insects (Fitzgerald and Costa 1986; Crump et al. 1987; Underwood and Shapiro 1999; Ruf et al. 2001; Fitzgerald and Pescador-Rubio 2002; Guédot et al. 2006; Ostwald et al. 2019). Drier environments may retain insect pheromones better than wet environments (Steadman et al. 2020), or it is possible that scents stick better to the specialized cuticular hydrocarbons insects produce to protect themselves against desiccation stress (Blomquist et al. 2020). Regardless, siblings' scents may have been faint and more difficult to detect following wet conditions. Future studies should elucidate chemical communication in *H. cunea*.

Altruism vs Reciprocity

Although the helpful eclosion and emesis behaviours in *H. cunea* are positive behavioural interactions since one partner's presence is benefitting the others, prior to this study it was unknown if the interactions were cases of altruism or reciprocity because the relative fitness costs and benefits were unclear without knowledge of kin recognition and mating. Both Hamilton's (1963) rule of kin selection and the Stress Gradient Hypothesis posit that there must be fitness benefits and costs for assistive social behaviour to occur, and the benefits must outweigh the costs of the behaviour. When costs to an individual's fitness outweigh benefits, this is true altruism. In Hamilton's rule, inclusive fitness (the benefit of the behaviour multiplied by the coefficient of relatedness, r) is proposed to govern interactions that appear altruistic; although the cost appears to outweigh the benefit at the time the behaviour is performed, there is an indirect net fitness benefit because the individual's genes will be propagated through kin that were assisted. This is mainly used to explain why a caste system of non-reproductive individuals that assist reproductive individuals has evolved in ants, wasps, bees, and termites (West and Gardner 2010). Alternatively, reciprocity that initially appears altruistic can occur between unrelated individuals if there is a direct personal fitness benefit that occurs later, such as in primates when a low-ranking individual grooms a high-ranking individual to eventually receive either protection or mating opportunities from the high-ranking individual (Schino and Aureli 2009).

In *H. cunea*, inclusive fitness benefits from kin reciprocity govern larval emesis and adult helpful eclosion, though in locations of lower rainfall there may also be direct personal fitness benefits driving selection for adult helpful eclosion. A larva benefits other larvae in the nest by regurgitating in response to a predator even though the emesis costs water and energy to produce, so it raises its inclusive fitness because its full siblings are more likely to survive to reproductive maturity. In other social Lepidoptera, cheating has been documented through delayed regurgitation in larger groups, and it has not yet been tested if this occurs in *H. cunea* and if the coefficient of relatedness reduces cheating (Daly et al. 2012). In terms of helpful eclosion, adult *H. cunea* do not feed, instead relying on energy acquired as larvae (Loewy et al. 2014). Energy reserves are limited and mainly go toward reproduction, so the cost of helpful eclosion is a depletion in these reserves. Kin are more likely to be helped than unrelated individuals, so the benefit of raising inclusive fitness through helping siblings outweighs the benefit of raising personal fitness through helping prospective mates. We observed that mating does not occur until after the first flight in Oklahoma *H. cunea*, otherwise the personal fitness benefit of easy mating might make helpful eclosion more common between unrelated individuals.

We suggest two ways that helpful eclosion may raise personal fitness in western *H. cunea* populations, thus leading to less discrimination between kin and strangers, which does not operate on eastern *H. cunea* populations. First, increased danger from predators may raise the cost of helpful eclosion in the east relative to the west. Eastern *H. cunea* may need to spend more of their energy reserves on evading predators as adults than western and central *H. cunea*, so they cannot afford to help individuals that are not kin. Future work should compare predators between the different regions to account for this possibility. Second, a higher level of dryness may make pupal shells tougher and more difficult to remove in the west, making it more likely for an individual moth to require help with eclosion, which raises the behavioural benefit in those populations relative to the east. High generosity may raise personal fitness because it increases the likelihood of encountering another moth that will return the favor. We derived this idea from our observations of moths that attempted to help others eclose even if their own abdomens were not yet freed. Pupal thickness, hardness, and molecular composition should be compared in future work to account for this possibility.

Management Implications

H. cunea are a native herbivore of trees in North America and a destructive invasive pest in other countries (Morris 1963), damaging trees of both agricultural and aesthetic value, so understanding how they will respond behaviourally to environmental stress can improve management tactics. Here, we found that increasing drought intensity, which will be exacerbated by climate change, promotes local adaptation in stress-buffering beneficial interactions in this species. We can expect this to occur regardless of host plant toxicity. Populations already occupying regions of low rainfall are currently better equipped to react to stress favorably than populations from higher rainfall regions. This reinforces that behavioural interactions are key determinants of biological success (Cahill et al. 2013; Ockendon et al. 2014).

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Figures and Tables

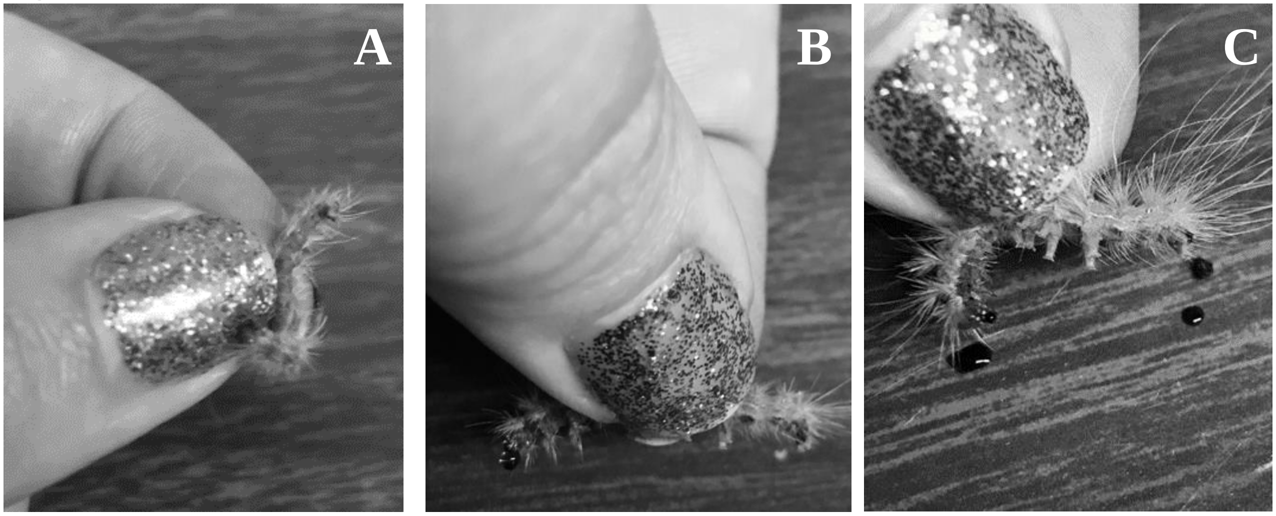

Fig. 1. Initial observations of *Hyphantria cunea* predator defense. The behavior differed depending on the rainfall region of origin. A) Larvae from western Oklahoma, where rainfall is low, defended themselves by biting. B) Larvae from central Oklahoma, where rainfall is moderate, orally secreted one drop of emesis. C) Larvae from eastern Oklahoma, where rainfall is high, secreted up to two drops of anterior liquid and up to two drops of posterior liquid. Emesis from eastern Oklahoma was visibly darker brown than emesis from central Oklahoma. Larvae from eastern Oklahoma thrashed violently when pinched while larvae from the other two regions moved more slowly or held still.

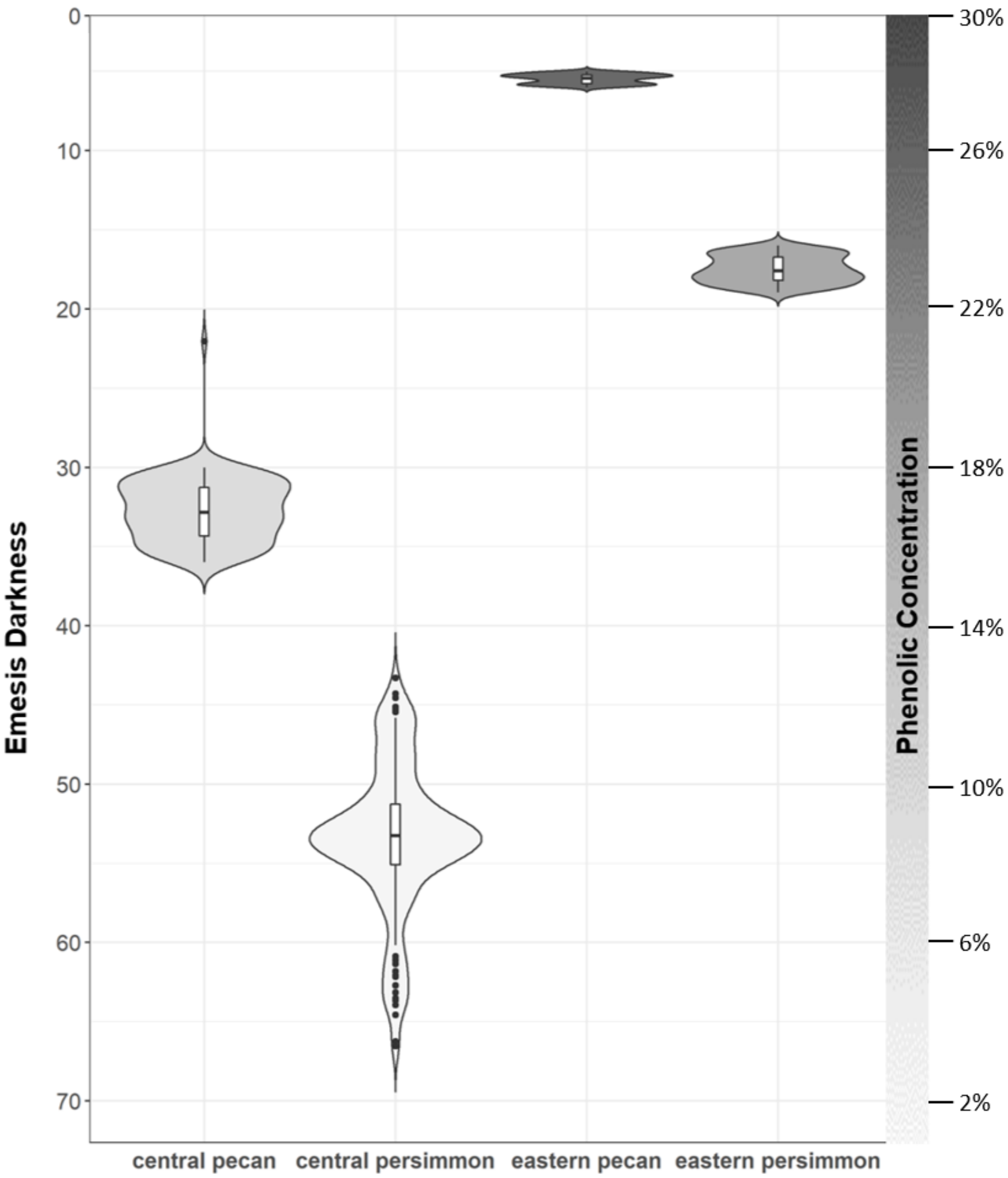

Fig. 2. Violin plots for the emesis, or defensive vomit, of larvae fed a diet of either pecan or persimmon leaves and descended from different rainfall source populations: central Oklahoma, where rainfall is moderate, and eastern Oklahoma, where rainfall is high. Western Oklahoma, where rainfall is low, was also included the study but larvae from this region defended themselves by biting instead of producing emesis. Lower pixel brightness values indicate darker emesis. The darker the emesis, the higher the phenolic concentration.

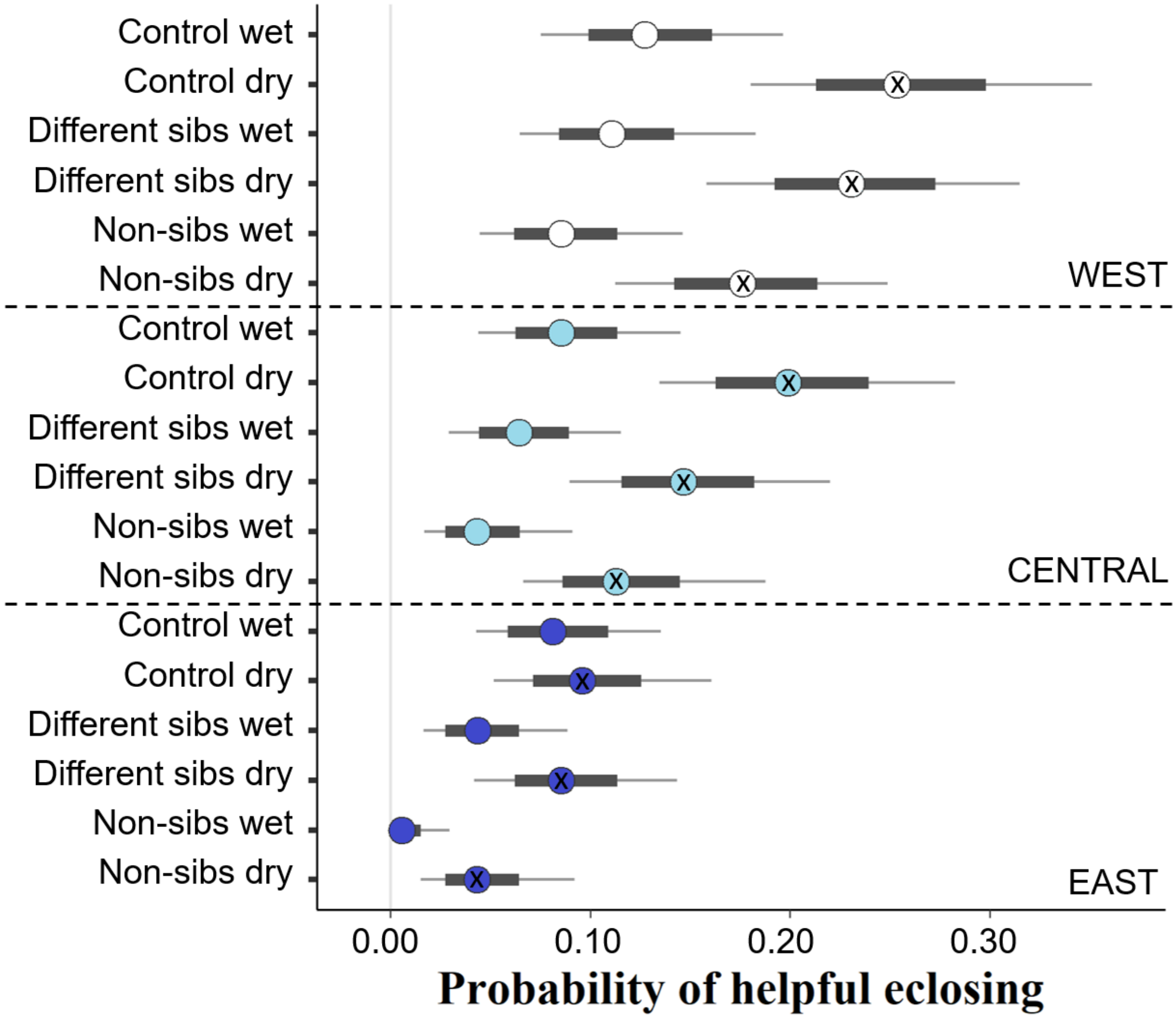

Fig. 3. Multinomial probability estimates for helpful eclosion, with thick credible intervals shown for the standard 95% range and thin credible intervals for the whole posterior distribution. *Hyphantria cunea* moths that had been raised in dry conditions as larvae are marked by the letter x. “Different sibs” refers to siblings that were separated just after hatching, and the “control” refers to siblings that nested together as larvae. Western Oklahoma has the lowest rainfall environment shown in white, central Oklahoma has a moderate rainfall environment shown in light blue, and eastern Oklahoma has the highest rainfall environment shown in dark blue. Siblings were more likely to help each other than non-siblings.

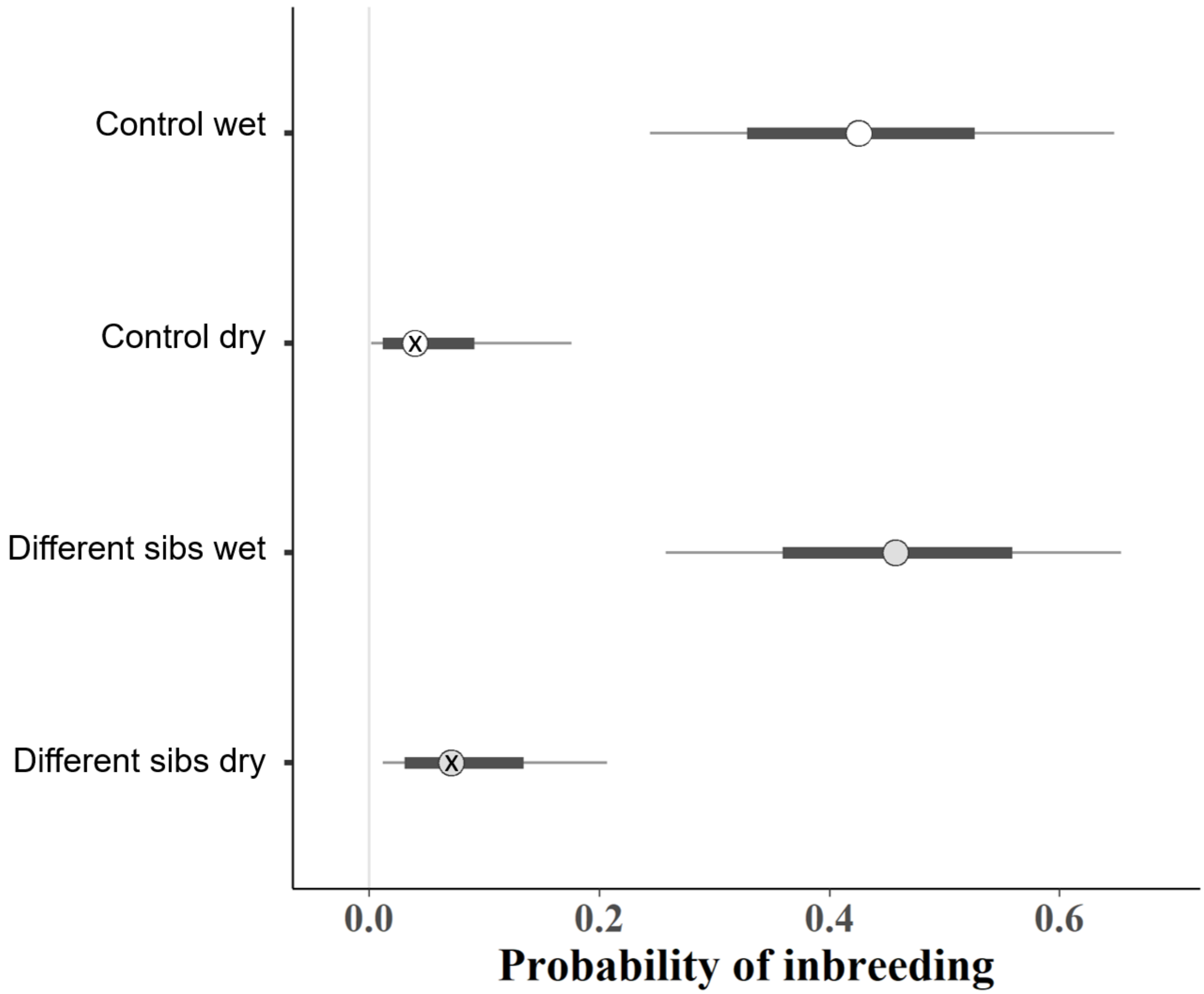

Fig. 4. Multinomial probability estimates for inbreeding, with thick credible intervals shown for the standard 95% range and thin credible intervals for the whole posterior distribution. Only *Hyphantria cunea* descended from the eastern Oklahoma source population bred with their siblings. Moths that had been raised in dry conditions as larvae are marked by the letter x. “Different sibs” refers to siblings that were separated just after hatching, and the “control” refers to siblings that nested together as larvae.

Table S1. Results from ANOVA post-hoc Tukey tests. Exposing Oklahoma *Hyphantria cunea* to wet and dry environments as larvae did not significantly affect emesis coloration ($p > 0.05$).

Region	Plant Diet	T-value	P-value
East	Pecan	-0.287	0.775
East	Persimmon	-0.507	0.612
Central	Pecan	0.506	0.613
Central	Persimmon	0.624	0.534

Chapter 4: Interspecific benefits, intraspecific conflicts: differential meta-analytic support for the stress gradient hypothesis

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Data availability statement
The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Understanding how stress shifts organism interactions is critically important for successfully predicting and managing population declines and extinctions. According to the Stress Gradient Hypothesis (SGH), ecological interactions between organisms shift positively as environmental stress increases. This hypothesis is contentious due to conflicting evidence, so we adopted a meta-analytic approach to determine if it is widely supported across a variety of contexts, including different kingdoms, ecosystems, habitats, interactions, stressors, and life history stages. We developed an extensive list of Boolean search criteria to search the published ecological literature and successfully detect studies that both directly tested the hypothesis, and those that were relevant but never mentioned it. We found that the SGH is well supported by studies that feature bacteria, plants, terrestrial ecosystems, interspecific negative interactions, adults, survival instead of growth or reproduction, and drought, fire, and nutrient stress. We conclude that the SGH is indeed a broadly relevant ecological hypothesis that is currently held back by cross-disciplinary communication barriers. More SGH research is needed beyond the scope of interspecific plant competition, and more SGH research should feature multifactor stress, especially in the context of increasing global stress.

Keywords: Stress Gradient Hypothesis (SGH), interspecific, intraspecific, positive interactions, negative interactions, climate change, meta-analysis

Introduction

Species interactions form the bedrock of ecology, affecting community and evolutionary dynamics including structure, function, coexistence, and productivity (Callaway 1995; Bertness & Leonard 1997; Doebeli & Dieckmann 2000; Barraclough 2015; Molloy & Hertweck 2017). In the context of environmental stress such as climate change, shifting species interactions have a greater role in population declines and extinctions than physiological climatic tolerances (Cahill *et al.* 2013; Ockendon *et al.* 2014). Understanding the nature of these shifts in species interactions is critically important for successful conservation and ecological management, especially because global stress is accelerating at this stage in the Anthropocene (Hodson & Marvin 2010; Schimel *et al.* 2013; Allen *et al.* 2015; Newman 2019; Büntgen *et al.* 2020).

Competitive and predatory antagonism have traditionally garnered more attention than positive interactions (Elton 1946; Stachowicz 2001; Beaudrot *et al.* 2020), leading Bertness and Callaway (1994) to urge community ecologists to follow evolutionary biologists in accounting for positive interactions such as mutualism. Even though greater theoretical incorporation and research investment in positive interactions began after the start of the 21st century (Hoeksema & Bruna 2000; Traveset & Richardson 2014; Valdez *et al.* 2020), a great proportion of climate change studies still focus on predation or competition (Harley 2011; Ockendon *et al.* 2014; Lancaster *et al.* 2017; Romero *et al.* 2018). The Stress Gradient Hypothesis (SGH) is helping to shift this focus. According to the SGH, ecological interactions between organisms become more positive as environmental stress increases (Maestre *et al.* 2009; Malkinson & Tielbörger 2010).

Although the SGH has been investigated and debated by plant community ecologists over the past two decades, it is not as well known outside of plant community ecology (Daleo & Iribarne 2009; Barrio *et al.* 2013; Dangles *et al.* 2013; Beaudrot *et al.* 2020). It is not clear how well supported the hypothesis is in various contexts, including the nature of the organism or interaction. Studies that explicitly address the SGH tend to feature interspecific competition among adult macroscopic plants and sessile organisms (Maestre *et al.* 2005; Kawai & Tokeshi 2007; Holmgren & Scheffer 2010; Armas *et al.* 2011; Fugère *et al.* 2012). Other ecological stress studies may indicate support for or against the SGH without acknowledging its existence, and it is unclear how much the nature of the ecosystem and abiotic or biotic stress matters (Lortie & Callaway 2006; Maestre *et al.* 2006).

We adopted a meta-analytic approach to broadly address whether ecological interactions between organisms shift in a positive direction as environmental stress increases as claimed by the SGH. Specifically, we asked 1) Does the SGH extend across kingdoms, beyond Plantae? 2) Is the shift proposed by the SGH more common for interspecific or intraspecific interactions? 3) Is the shift proposed by the SGH more common for certain ecosystems, habitats, response variables, and types of stress? 4) Does the SGH apply more to organisms in the juvenile phase or the adult phase of their life cycles? Other systematic reviews on this topic have been restricted to plants or plant-like organisms. To our knowledge, this will be the first systematic review and specifically the first meta-analysis that attempts to answer these questions.

Methods

Search inclusion

We searched for peer-reviewed primary publications to examine if, and in which direction, stress shifted ecological interactions. Data were included regardless of the age of the study, and studies fell in the time range of 1900 – 2019. We tracked experimental and observational studies, as this is a good common practice for meta-analyses (Côté & Jennions

2013). We also wanted to see if the type of study influenced the outcome, and if there was a deficit of either type of study for this topic.

We noticed an ecological study may provide support for or against the SGH, yet never acknowledge the existence of the hypothesis because it is not widely known beyond the field of plant community ecology. While this reduced possible bias in our sample of studies, we found it necessary to develop an extensive list of Boolean search terms to successfully gather data for as many stressors and interactions as possible (Supp. Table 1). We excluded theoretical data from modeling papers in order to evaluate the real-world effects of stress. We also excluded molecular studies because ecological interactions between organisms were not usually incorporated into such work.

The ecological interactions we considered included 1) positive interactions, defined as those where participants in the interaction do not harm each other and at least one participant benefits, such as: mutualism, pollination, commensalism, and decomposition, and 2) negative interactions, defined as those harming one or more participant in the interaction, such as: parasitism, amensalism, competition, and predation. We differentiated pollination from other mutualisms because the interaction itself is short-lived and coevolution between participants is particularly high (Kiester *et al.* 1984; Lunau 2004; Bronstein *et al.* 2006). Decomposition was differentiated because benefits tend to be indirect and coevolution between participants is relatively low (Mack & D'Antonio 2003; Kreuzer *et al.* 2004; Lohmann *et al.* 2009).

In addition to these ecological interactions, we examined a range of abiotic and biotic stressors (Supp. Table 1). Certain biotic variables could be either a predictor or a response variable in this meta-analysis, it depended on the study. For example, the predatory, competitive, or parasitic interaction in a study could respond to a stressor such as heat. In other cases, predators, competitors, or parasites did not experience stress, but acted as a source of stress for the participants in a different interaction such as a mutualism.

Extraction

We extracted sample sizes, standard deviations, and means for control and manipulated treatments (Appendix S1). Studies were excluded if any of these values were not reported or could not be determined, which led us to narrow down the 10287 relevant studies we originally found to 1662 total studies that could be used in the final analysis. We quality checked data three times to ensure accurate summary statistics were recorded for the included studies, and we excluded a study if the accuracy was suspect. For example, studies that reported standard deviations equal to zero were excluded.

Since our main objective was to assess an ecological hypothesis, the data came from tables and figures presented in peer-reviewed published primary literature that we searched using the advanced search tool in Web of Science. This database was chosen because it has the widest coverage of journals that contain published primary literature and a wide range of publication years. We stored the PDF files of relevant literature in Open Science Framework (OSF).

Because our Boolean search criteria covered such a wide range of predictors and interactions (Supp. Table 1), a search would sometimes yield 1000 or more studies. This happened for 18 of 94 combinations of search terms. When this happened, we searched based on title (TI) instead of topic (TS) to reduce the sample size to more manageable quantities. For example, 2480 studies were retrieved using the search criterion TS = ((“global change” OR “climate change” AND cooperat*)). After substituting TI for TS before the phrase, 178 papers were returned.

We found eleven types of performance responses for the 1662 relevant studies we gathered, including: abundance, biodiversity, biomass, defense, growth, immunity, metabolism, water potential, nutrition, reproduction, and survival (Fig. 1A). These responses came from organisms in five kingdoms: Animalia, Bacteria, Chromista, Fungi, and Plantae (Fig. 1B). We also found 14 stressors, including drought, elevation, fire, flood, heat, light, nutrients, chemicals, shading, wind, human disturbance, competition, parasitism, predation (Fig. 1C). Observational studies did not greatly outnumber experimental studies (Fig. 1D). The number of studies we found for each of the following variables are summarized in Fig. 1: E) aquatic vs terrestrial habitat, F) interspecific vs intraspecific interactions, G) adult vs juvenile organisms, H) commensalism, competition, decomposition, mutualism, pollination, parasitism, predation, and I) grassland, agricultural, coastal, desert, forest, freshwater, laboratory, marine, montane, and wetland ecosystems.

Analysis

We calculated Hedges' *g** effect sizes by dividing the difference between control and treatment means by the pooled standard deviation. The Hedges' *g** effect size statistic is interpreted similarly to Cohen's *d*, but its incorporation of the pooled standard deviation reduces bias and makes it a better estimate, especially for small sample sizes. It is also negligibly biased for larger sample sizes. This makes it the ideal effect size statistic to report in a meta-analysis, provided enough of the included studies report the sample sizes and summary statics necessary to calculate it. Sample sizes and the control and treatment standard deviations allowed us to calculate the pooled standard deviation: $S_p = \surd\ [((n_1-1)\ s_1^2 + (n_2-1)\ s_2^2\ )\ /\ ((n_1-1)+(n_2-1))$.

Hedges' *g** was estimated per study (i.e., per row of data) with a Bayesian model developed in JAGS and run via package 'rjags' in R (Plummer 2016). Individual estimates and their associated uncertainties were fed hierarchically into another JAGS model that estimated Hedges' *g** for each subset of data, such as kingdom Plantae or terrestrial habitat. At each step, posterior distributions of estimates were generated via 10,000 Monte Carlo iterations of three Markov chains. Standard trace plots revealed excellent mixture in parameter space, so we had no need to thin or otherwise subsample the posteriors.

Results

Kingdoms

Overall, stress decreased positive ecological interactions and increased negative ecological interactions, as shown by a pooled effect size of –0.2 and a narrow distribution (Fig. 2). This was more apparent among observational studies, while experimental work more often captured reduced negativity in ecological interactions. Distributions were narrow and non-overlapping for observational and experimental studies. There were clear differences among kingdoms; two even exhibited a pattern opposite to the overall trend.

Plantae and Bacteria both had positive effect sizes, indicating a shift towards more positive and less negative ecological interactions under stress; this was more pronounced for Bacteria (Fig. 2). The tail end of the distribution for Plantae overlapped zero whereas the distribution for Bacteria was entirely positive. Effect sizes were negative for three of the five biological kingdoms represented in the data: Chromista, Fungi, and Animalia, and there was greater uncertainty of estimate among Chromista, Fungi, and Bacteria relative to Animalia and Plantae.

Ecosystems and habitat

There were pronounced differences in ecological stress responses based on the type of habitat and ecosystem (Fig. 3). Effect sizes for aquatic and terrestrial habitat differed by an order of magnitude, with aquatic systems exhibiting greater negativity and variation. Marine, coastal, and freshwater ecosystems were decisively negative, ranging from –1.7 to –0.5 effect sizes, while terrestrial ecosystems ranged from –1.4 to 1.8 effect sizes. Freshwater ecosystems showed less negativity relative to the saline ecosystems.

Among terrestrial ecosystems, grasslands had the most negative effect size (–1.4), followed by deserts (–0.8), while the remainder were positive. Evidence was strongest for increased positive and decreased negative ecological interactions among wetland and agricultural ecosystems. Effect sizes did not differ greatly between forest (0.75) and laboratory (0.7) ecosystems, but effect sizes for these were approximately three times greater than montane ecosystems (0.25).

Interaction type

Certain ecological interactions became more positive or less negative under stress while others showed the opposite pattern (Fig. 4). The effect size was small and positive for interspecific interactions (0.3), while the effect size for intraspecific interactions was large and negative (–1.2). Direct interactions had a relatively narrow distribution and a negative effect size (–0.2), while indirect interactions varied more and were half as negative (–0.1) relative to direct interactions.

Stress reduced negative ecological interactions, except in the case of predation. Parasitism had the highest positive effect size (1.75) of any interaction type, and even though the distribution was broad it did not overlap zero. Competition had the second highest positive effect size (0.1) with a narrow distribution that did not overlap zero. In contrast, stress intensified predatory interactions, indicated by a negative effect size (–0.2).

Positive ecological interactions weakened in response to stress, except in the case of pollination. Overall, mutualisms had the most negative effect size (–1.2) out of all interaction types, followed by commensalisms (–0.6). Pollination deviated greatly from other mutualisms, as it had the third most positive effect size (0.5) that did not differ greatly from the effect size of decomposition (0.3). Although the effect sizes of pollination and decomposition were positive, broad distributions that extend below zero indicate stress sometimes weakened these interactions.

Response type

Stress responses varied depending on the age of the organism and the particular type of biological response to stress (Fig. 5). Juvenile organisms had a more negative effect size relative to adult organisms, and although the adult effect size was negative, the distribution extended slightly above zero. Biodiversity and water potential among organisms varied widely in both directions, but effect sizes for other response types were clearly positive or negative. Positive effect sizes, listed in order of highest to lowest magnitude, included defense, abundance, survival, and nutrition. These measurements generally supported the stress gradient hypothesis. Negative effect sizes, listed in order of highest to lowest magnitude, included biomass, metabolism, immunity, growth, and reproduction. These measurements generally opposed the stress gradient hypothesis.

Stress type

Drought and nutrient stress, especially when combined, shifted ecological interactions positively (Fig. 6). Even though the distribution was broad for drought and nutrient stress, it never overlapped zero and the positive effect size (5.5) was higher than any other evaluated in the meta-analysis. In comparison, other stressors that shifted ecological interactions positively, such as chemicals, fire, and wind, had smaller effect sizes closer to one and zero.

When biological agents were a source of stress in a system, ecological interactions shifted negatively. The sole exception to this was when drought was involved. The effect size of drought alone did not differ from the effect size of drought combined with predation. Fire shifted ecological interactions positively, but this was neutralized if predators were also a source of stress.

There were more stressors that were either neutral or shifted ecological interactions negatively. Neutral stressors included flooding, elevation, and human disturbance. Heat and shading had the most negative effect sizes, followed by the biotic stressors of competition, parasitism, and predation. Light stress also shifted ecological interactions negatively and did not greatly differ from parasitism and predation.

Discussion

According to the Stress Gradient Hypothesis, ecological interactions shift in a positive direction with increasing environmental stress. In this meta-analysis, we shed light on how well the SGH was supported in various contexts. The SGH is controversial among ecologists, in part because of contradictory evidence (Maestre *et al.* 2009; Malkinson & Tielbörger 2010; He *et al.* 2013). Although context-dependency has been acknowledged for the SGH, stressors, responses, ecosystems, habitat, and interactions have not been assessed as specifically as they were in this meta-analysis. Ours is the first to examine the SGH in the context of organisms from different kingdoms and life history stages and to compare intraspecificity and interspecificity.

Kingdoms

Although the SGH is explicitly tested most often in studies of plant community ecology, we found that greater support for it existed among bacteria than for any other kingdom. This was especially striking because the quantity of SGH relevant studies featuring bacteria was not far off from the quantity of SGH relevant studies featuring plants. As we extracted from the ecological microbiology literature, we noticed that the SGH was usually not mentioned or described even when results supported it. Although plant community ecologists proposed the hypothesis and have diligently tested and debated it over the years, this finding demonstrates that it may be especially relevant to bacteriologists.

We conclude that the SGH is indeed a broadly relevant ecological hypothesis that is currently held back by cross-disciplinary communication barriers. It was generally not supported in studies featuring kingdoms other than Plantae and Bacteria, but there were also fewer SGH relevant studies featuring other kingdoms to begin with so we encourage support of SGH studies targeting these kingdoms. Because life history strategies differ dramatically even within kingdoms, future work comparing finer taxonomic distinctions than the kingdom level would be useful. We urge ecologists who test the SGH to consider communicating their findings in journals, conferences, and other platforms that will reach a wide audience of ecologists.

Ecosystems and habitat

Even though famous examples of SGH support feature marine invertebrates (Bertness & Leonard 1997; Bertness *et al.* 1999), overall we found that the SGH was not supported in aquatic contexts. The mechanism behind the difference in aquatic and terrestrial SGH support is likely tied either to trophic cascades, or to the rarity of evolutionary transitions between aquatic and terrestrial systems (Vermeij & Dudley 2000). We believe the former to be more directly involved, considering the sizable differences that exist between aquatic and terrestrial food web function and structure (Wiegert & Owen 1971; Chase 2000).

Ecosystems that are typically nutrient-rich were far more likely to show interactions that shifted positively. Nutrient deficiency does not typically characterize wetlands, agroecosystems, laboratories, and forests; hence, we posit that the mechanism driving the SGH is tied to nutrient availability or acquisition in these systems. Nutrient stress alone or in combination with other stressors shifted interactions positively, supporting this idea. This is consistent with other SGH systematic reviews that have featured plants only, which suggested that moderate nutrient limitations best promote the SGH while nutrient limitations in the harshest of conditions will shift organism interactions negatively (Holmgren & Scheffer 2010). We compared categories of stressors and ecosystems, but follow-up work that singles out given stressors and establishes a standardized continuous format for comparing the magnitude of the stress across studies may provide clearer insight into the mechanisms driving the patterns we detected.

Interaction type

We compared intraspecific and interspecific interactions and found that SGH support is more likely for interspecific interactions, which aligns with theory differentiating intraspecific and interspecific interaction types. For example, according to the principle of competitive exclusion, long-term coexistence is not possible if niches overlap (Hardin 1960). Intraspecific competition is stronger than interspecific competition (Adler *et al.* 2018). In addition, according to principles in sociobiology, positive intraspecific interactions are more likely in stressful conditions if organisms are kin related due to indirect fitness benefits. If stress produces more costs than benefits in terms of direct fitness for individuals, and if there is little to no indirect fitness advantage for individuals, intraspecific interactions will shift negatively (Wilson & Wilson 2007). According to our findings, this happens often. We did not evaluate how the duration of an interaction may affect the patterns we detected, so we suggest it as a future research direction, especially since we found positive interactions were more likely to shift in a negative direction except for the shorter-term positive interaction of pollination.

Response type

Our meta-analysis is the first to demonstrate that study design matters a great deal when it comes to the SGH. We found that the metric of performance plays a huge role in conclusions about the SGH. Metrics related to survival are more likely to support the SGH while metrics related to growth and reproduction are more likely to oppose the SGH. Because of this key finding, we suggest that experimental work assessing the SGH should be as comprehensive as possible in how participants are evaluated in an ecological interaction. This finding reinforces the importance of accounting for trade-offs in survival, growth, and reproduction when evaluating the SGH, as selection pressures on these factors, especially at different life history stages, may oppose each other (Schluter & Smith 1986; Schluter *et al.* 1991; Cox *et al.* 2010; Brouwer *et al.* 2019).

Stress Type

Finally, we emphasize the need for more multifactor stress studies, as the only multifactor stress studies in our meta-analysis were cases of bifactor stress. We accounted for as many stressors as possible in our search phrases, so we attribute this scarcity to a lack of published data. More work is needed to show how three or more stressors may interact to shift ecological interactions (Adams *et al.* 2020). Only 30% of published climate change studies incorporate multifactor stress, which can be interactive rather than additive (Collins 2009; Matesanz & Ramírez-Valiente 2019) as we found when drought and nutrient stress and fire and predator stress were combined. There are not enough multifactor stress experiments out there to effectively evaluate support for or against the SGH, and this is urgently needed in the context of climate change to accurately predict organism resilience and extinction for improving ecosystem and conservation management.

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Figures and Tables

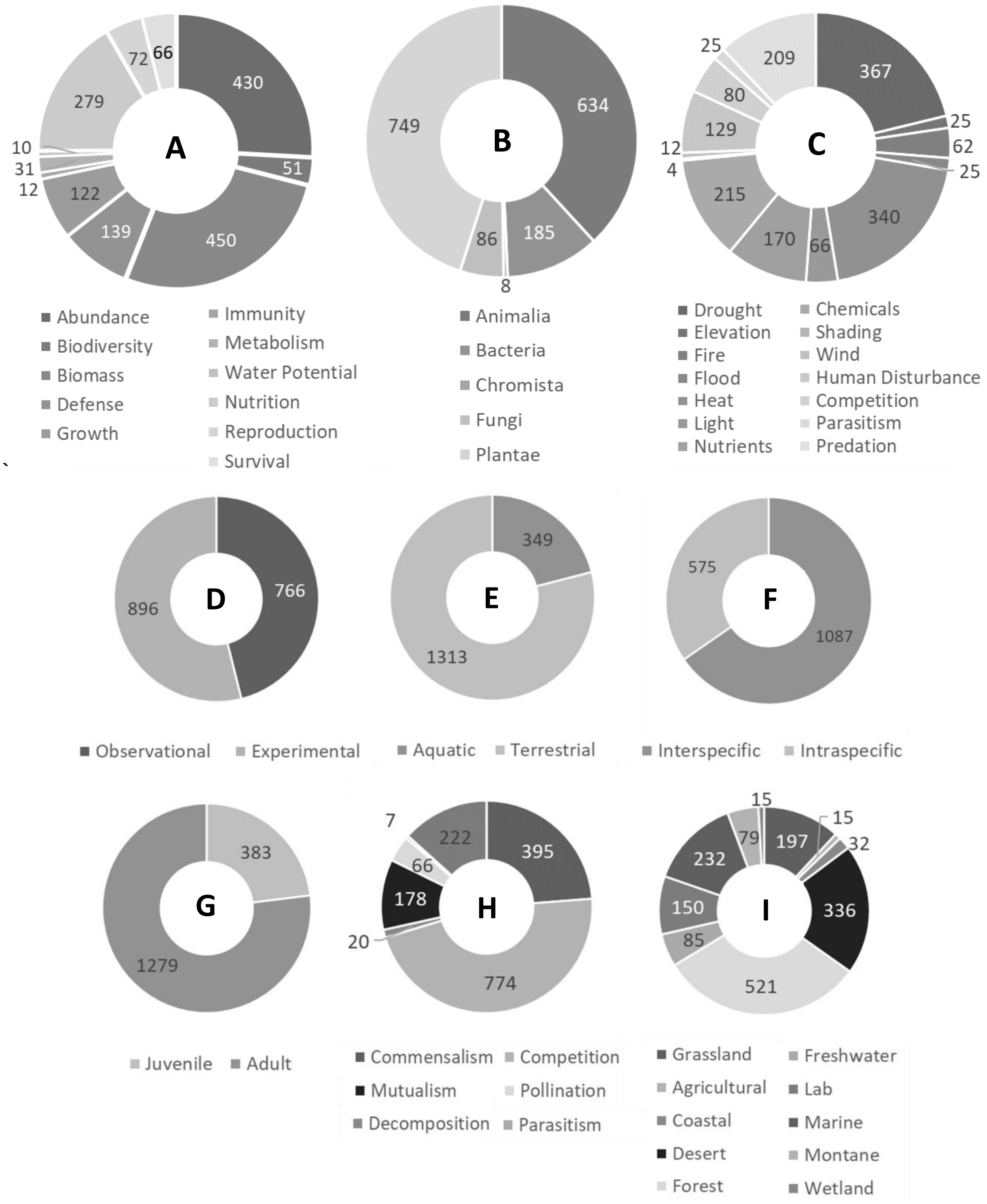

Fig. 1. The number of studies included in the final analysis that featured A) different performance responses, B) different Kingdoms, C) different stressors D) observational vs experimental studies, E) aquatic vs terrestrial habitat, F) interspecific vs intraspecific interactions, G) adult vs juvenile organisms, H) different ecological relationships, and I) different ecosystems.

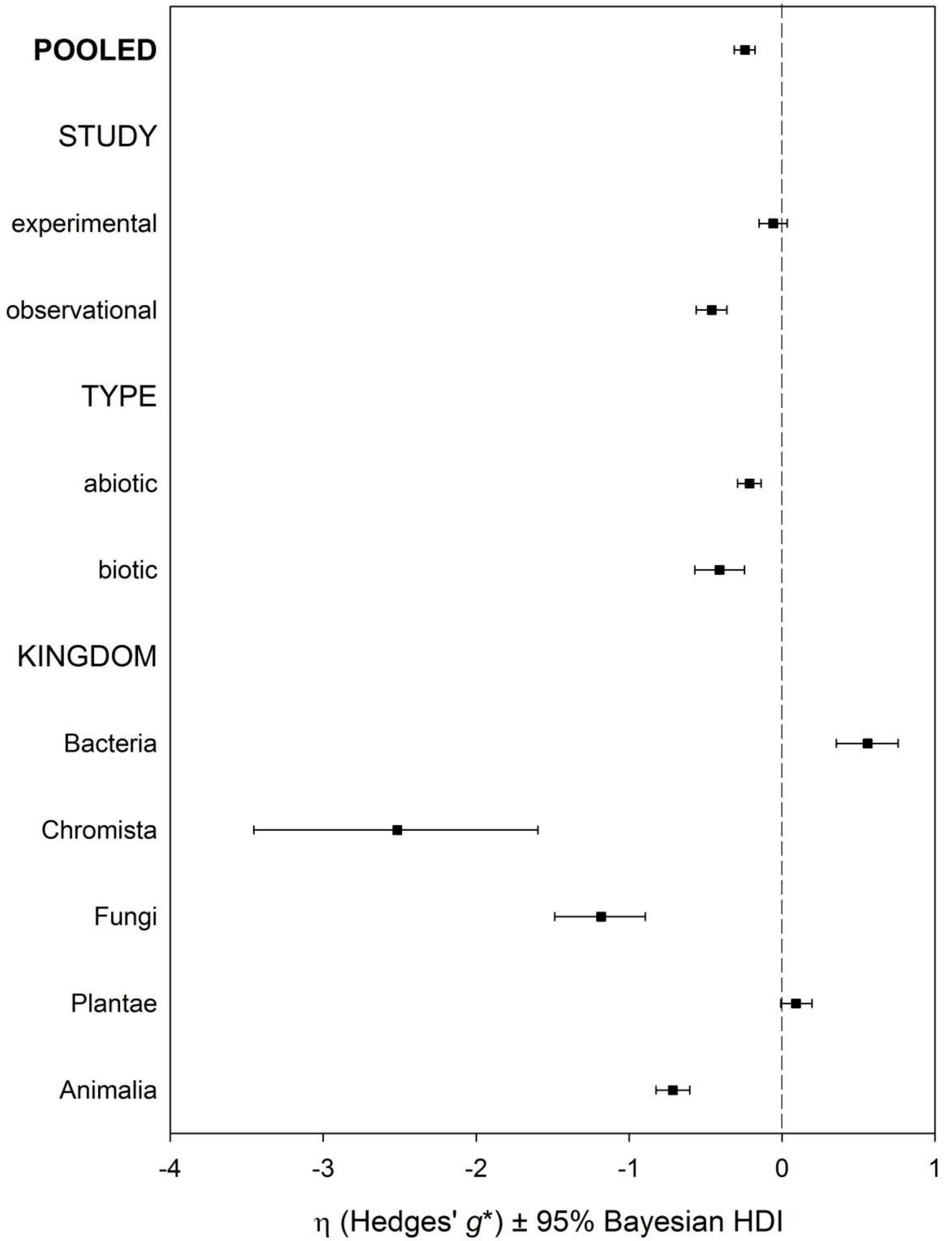

Fig. 2. Hedges' g* effect sizes (+95% Bayesian highest density intervals) of biological response to stress across different Kingdoms (Bacteria, Chromista, Fungi, Plantae, Animalia), stress types (abiotic and biotic), study types (experimental and observational), and all species and systems (pooled). Positive effect sizes reflect increases in positive ecological interactions and decreases in negative ecological interactions as expected from the stress gradient hypothesis (SGH), while negative values reflect the opposite of that.

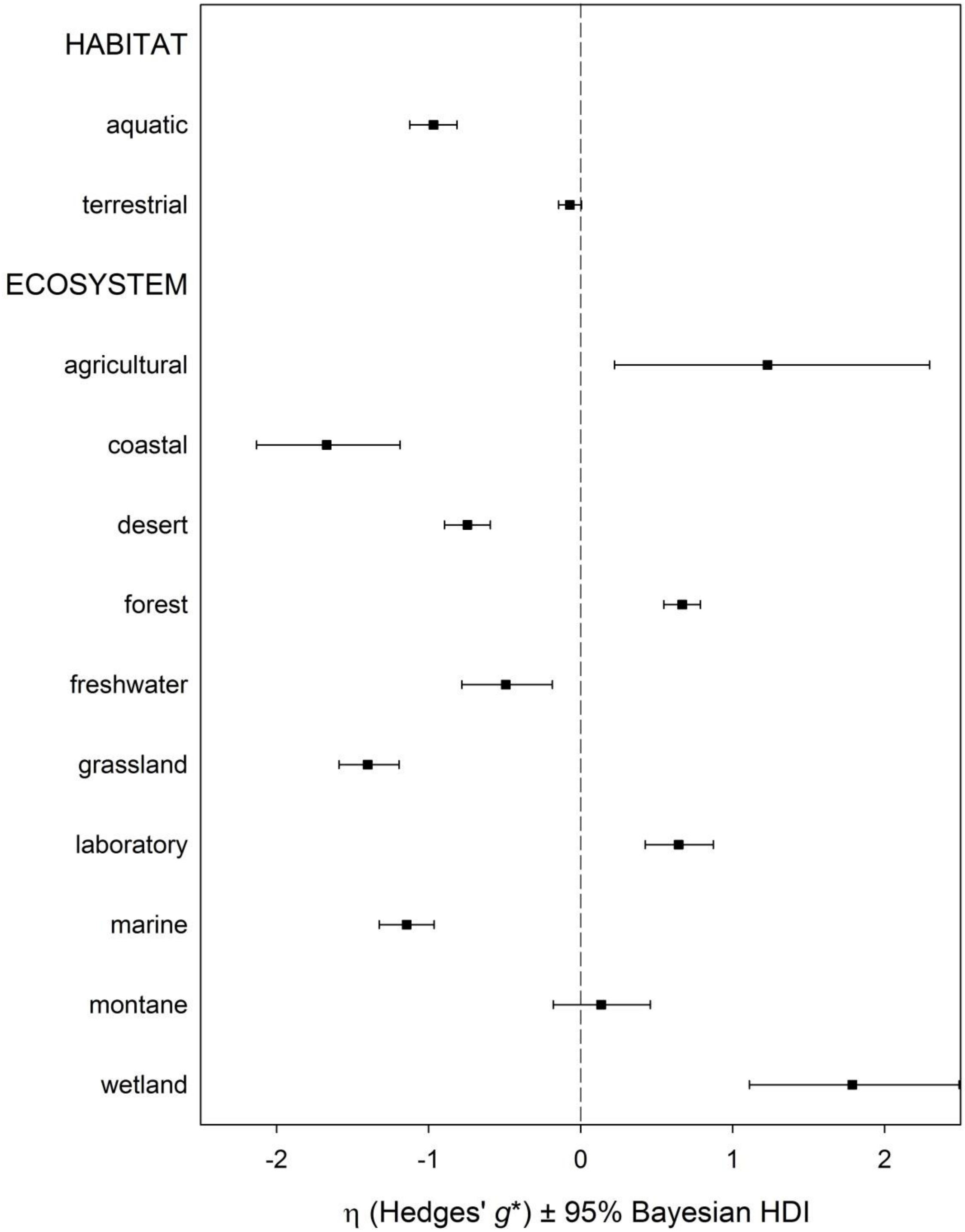

Fig. 3. Hedges' g* effect sizes (+95% Bayesian highest density intervals) of biological response to stress across different ecosystems (agricultural, coastal, desert, forest, freshwater, grassland, laboratory, marine, montane, wetland) and habitats (aquatic and terrestrial). Positive effect sizes reflect increases in positive ecological interactions and decreases in negative ecological interactions as expected from the stress gradient hypothesis (SGH), while negative values reflect the opposite of that.

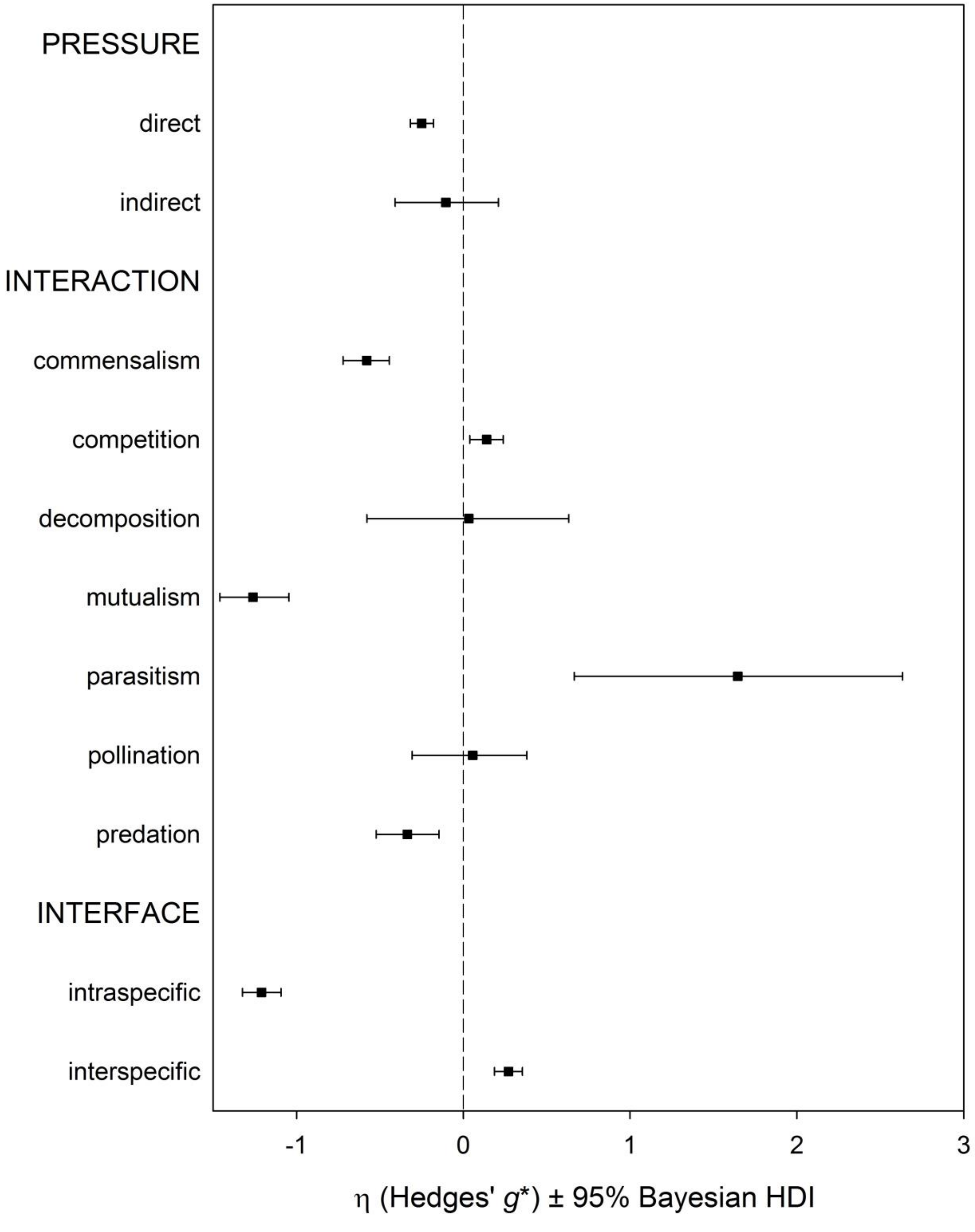

Fig. 4. Hedges' g* effect sizes (+95% Bayesian highest density intervals) of biological response to stress across different types of ecological interactions (intraspecific, interspecific, commensalism, competition, decomposition, mutualism, parasitism, pollination, predation, direct, and indirect). Positive effect sizes reflect increases in positive ecological interactions and decreases in negative ecological interactions as expected from the stress gradient hypothesis (SGH), while negative values reflect the opposite of that.

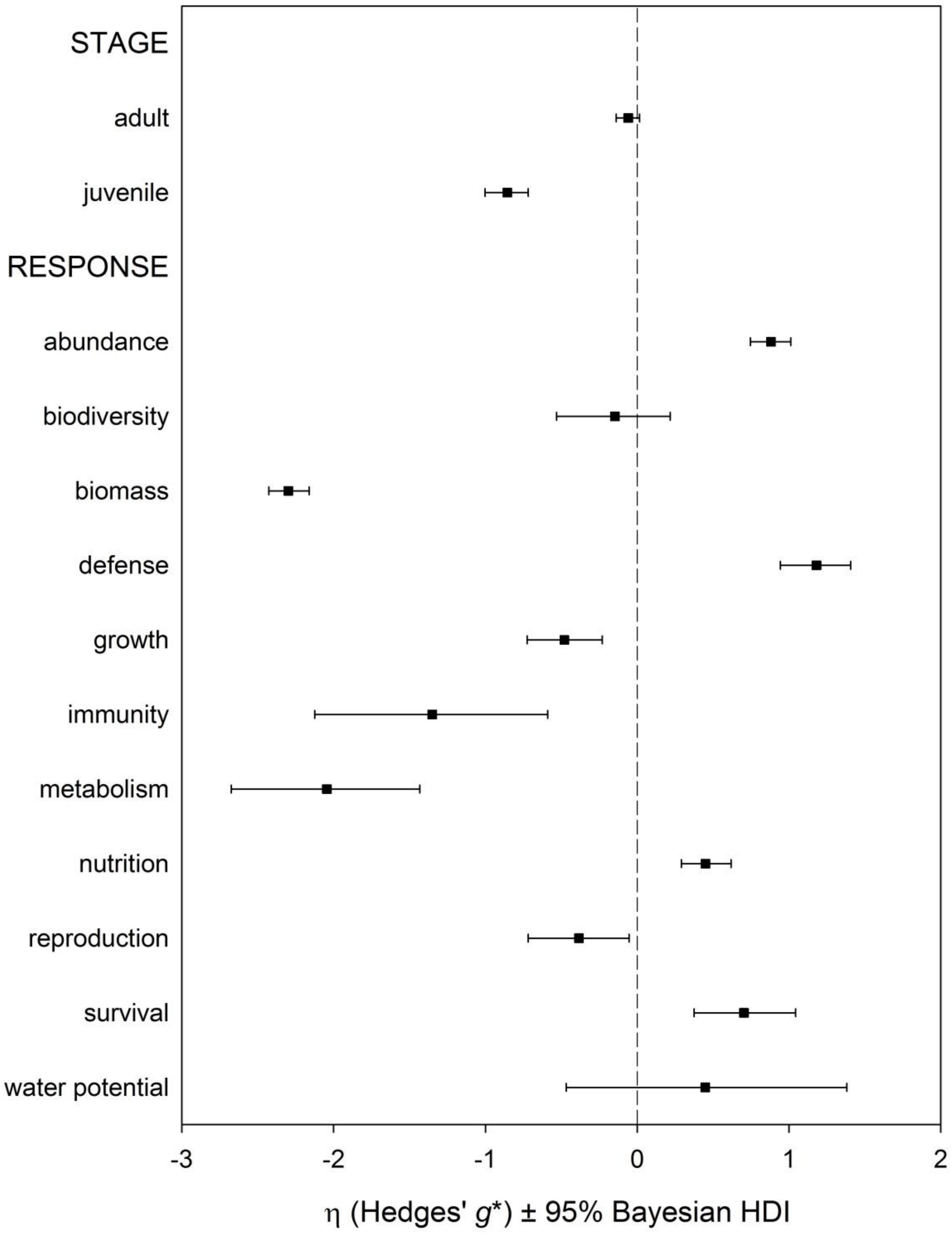

Fig. 5. Hedges' g* effect sizes (+95% Bayesian highest density intervals) of biological response to stress for different response types (abundance, biodiversity, biomass, growth, immunity, metabolism, nutrition, reproduction, survival, water potential) and life history stages (adult vs juvenile). Positive effect sizes reflect increases in positive ecological interactions and decreases in negative ecological interactions as expected from the stress gradient hypothesis (SGH), while negative values reflect the opposite of that.

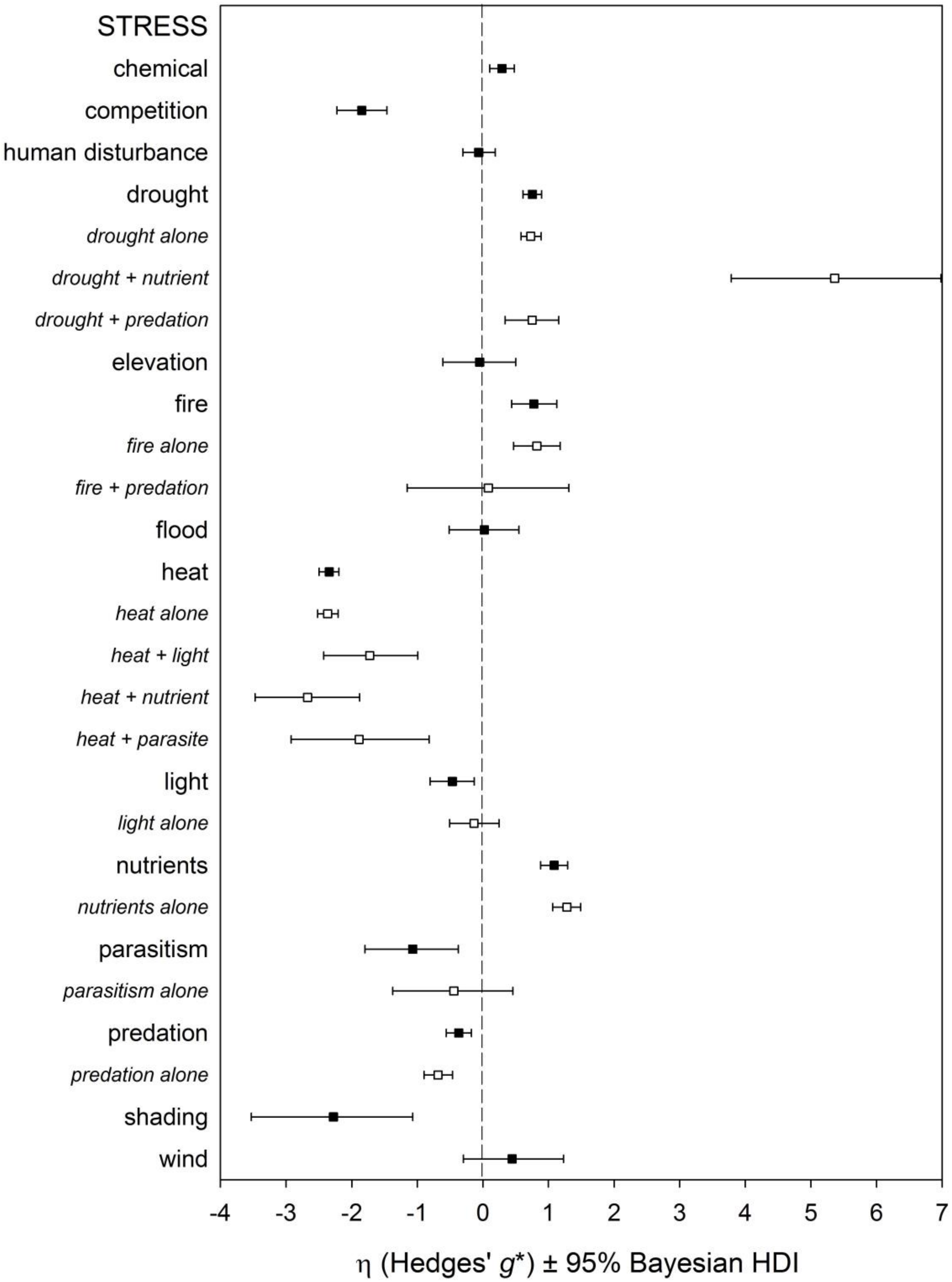

Fig. 6. Relative influence of various stressors on Hedges' g* effect sizes (+95% Bayesian highest density intervals). Filled in plots were derived from all studies that included a given stressor, regardless if another stressor was present or not. Plots not filled in were derived from studies featuring a given stressor by itself, or in combination with a specific other stressor. Positive effect sizes reflect increases in positive ecological interactions and decreases in negative ecological interactions as expected from the stress gradient hypothesis (SGH), while negative values reflect the opposite of that.

Appendix S1: The Excel data file that contains the complete compilation of studies is available from the corresponding author upon reasonable request.

Supp. Table 1. Search criteria for the meta-analysis. 94 total combinations of predictor and response phrases were entered in an advanced search in Web of Science. If a combination yielded more that 1000 studies, then the search was completed for title (TI) instead of topic (TS) to make the sample size more manageable. 1662 studies representing 51 of the original search combinations (bold and *italicized*) were included in the final analysis.

Topic or Title	Predictor Phrase	Response Phrase	Yield	Picked
TS =	(("stress gradient" OR "environmental gradient")	AND mutuali*)	43	0
TS =	(("stress gradient" OR "environmental gradient")	AND parasit*)	38	0
TS =	(("stress gradient" OR "environmental gradient")	AND amensal*)	1	0
TS =	(("stress gradient" OR "environmental gradient")	AND commensal*)	6	0
TS =	(("stress gradient" OR "environmental gradient")	AND neutral*)	121	0
TS =	***(("stress gradient" OR "environmental gradient")***	***AND compet*)***	***724***	***34***
TS =	(("stress gradient" OR "environmental gradient")	AND predat*)	141	0
TS =	***(("stress gradient" OR "environmental gradient")***	***AND decomp*)***	***65***	***48***
TS =	***(("stress gradient" OR "environmental gradient")***	***AND pollinat*)***	***31***	***46***
TS =	***(("stress gradient" OR "environmental gradient")***	***AND facilitat*)***	***497***	***4***
TS =	(("stress gradient" OR "environmental gradient")	AND cooperat*)	8	0
TS =	*(("stress gradient" OR "environmental gradient")*	*AND "positive interac*")*	*289*	*8*
TS =	***(("stress gradient" OR "environmental gradient")***	***AND "negative interac*")***	***37***	***12***
TS =	***(("stress gradient" OR "environmental gradient")***	***AND intraspec*)***	***123***	***117***
TS =	***(("stress gradient" OR "environmental gradient")***	***AND interspec*)***	***177***	***38***
TS =	(("stress gradient" OR "environmental gradient")	AND altrui*)	1	0

TS =	(("stress gradient" OR "environmental gradient")	AND alloparent*)	0	0
TS =	***(("stress gradient" OR "environmental gradient") AND "drought" OR "rainfall" OR "flood")***		***23***	***16***
TS =	***("environmental stress"***	***AND mutuali*)***	***34***	***10***
TS =	("environmental stress"	AND parasit*)	262	0
TS =	("environmental stress"	AND amensal*)	0	0
TS =	("environmental stress"	AND commensal*)	26	0
TS =	("environmental stress"	AND neutral*)	174	0
TS =	("environmental stress"	AND compet*)	631	0
TS =	***("environmental stress"***	***AND predat*)***	***311***	***45***
TS =	***("environmental stress"***	***AND decomp*)***	***87***	***19***
TS =	("environmental stress"	AND pollinat*)	47	0
TS =	***("environmental stress"***	***AND facilitat*)***	***516***	***23***
TS =	("environmental stress"	AND cooperat*)	61	0
TS =	***("environmental stress"***	***AND "positive interac*")***	***95***	***11***
TS =	("environmental stress"	AND "negative interac*")	10	0
TS =	("environmental stress"	AND intraspec*)	107	0
TS =	***("environmental stress"***	***AND interspec*)***	***118***	***14***
TS =	***("environmental stress"***	***AND altrui*)***	***6***	***6***
TS =	("environmental stress"	AND alloparent*)	0	0
TS =	(("global change" OR "climate change")	AND mutuali*)	513	0
TI =	(("global change" OR "climate change")	AND parasit*)	72	0
TS =	(("global change" OR "climate change")	AND amensal*)	3	0
TS =	***(("global change" OR "climate change")***	***AND commensal*)***	***53***	***23***
TI =	(("global change" OR "climate change")	AND neutral*)	26	0
TI =	***(("global change" OR "climate change")***	***AND compet*)***	***119***	***10***
TI =	(("global change" OR "climate change")	AND predat*)	72	0
TI =	(("global change" OR "climate change")	AND decomp*)	57	0
TI =	(("global change" OR "climate change")	AND pollinat*)	52	0

TI=	***(("global change" OR "climate change")***	***AND facilitat*)***	***67***	***18***
TI =	***(("global change" OR "climate change")***	***AND cooperat*)***	***178***	***178***
TS =	***(("global change" OR "climate change")***	***AND "positive interac*")***	***266***	***40***
TS =	(("global change" OR "climate change")	AND "negative interac*")	80	0
TI =	***(("global change" OR "climate change")***	***AND intraspec*)***	***22***	***7***
TI =	***(("global change" OR "climate change")***	***AND interspec*)***	***15***	***15***
TS =	(("global change" OR "climate change")	AND altrui*)	105	0
TS =	(("global change" OR "climate change")	AND alloparent*)	0	0
TS =	***(("global change" OR "climate change") AND temperature)***		***76***	***32***
TS =	("stress gradient hypothesis" AND temperature		0	0
TS =	("stress gradient hypothesis" AND ("drought" OR "rainfall" OR "flood*")		85	0
TS =	***("stress gradient hypothesis"***	***nutrient)***	***55***	***12***
TS =	***("stress gradient hypothesis"***	***climate)***	***122***	***8***
TS =	***("stress gradient hypothesis"***	***plant*)***	***390***	***8***
TS =	***("stress gradient hypothesis"***	***microb*)***	***139***	***139***
TS =	***("stress gradient hypothesis"***	***animal*)***	***19***	***2***
TS =	***("biotic stress"***	***AND mutuali*)***	***71***	***71***
TS =	***("biotic stress"***	***AND parasit*)***	***131***	***131***
TS =	("biotic stress"	AND amensal*)	0	0
TS =	("biotic stress"	AND commensal*)	3	0
TS =	***("biotic stress"***	***AND neutral*)***	***21***	***6***
TS =	***("biotic stress"***	***AND compet*)***	***93***	***44***
TS =	("biotic stress"	AND predat*)	27	0
TS =	("biotic stress"	AND decomp*)	8	0
TS =	("biotic stress"	AND pollinat*)	15	0
TS =	***("biotic stress"***	***AND facilitat*)***	***128***	***8***
TS =	("biotic stress"	AND cooperat*)	16	0
TS =	***("biotic stress"***	***AND "positive interac*")***	***15***	***15***
TS =	***("biotic stress"***	***AND "negative interac*")***	***17***	***17***
TS =	***("biotic stress"***	***AND intraspec*)***	***22***	***18***

TS =	***("biotic stress"***	***AND interspec*)***	***31***	***12***
TS =	("biotic stress"	AND altrui*)	0	0
TS =	("biotic stress"	AND alloparent*)	0	0
TS =	***(disturbance***	***AND mutuali*)***	***378***	***16***
TI =	***(disturbance***	***AND parasit*)***	***54***	***54***
TS =	(disturbance	AND amensal*)	4	0
TS =	***(disturbance***	***AND commensal*)***	***158***	***143***
TI =	***(disturbance***	***AND neutral*)***	***74***	***6***
TI =	***(disturbance***	***AND compet*)***	***133***	***15***
TI =	***(disturbance***	***AND predat*)***	***134***	***9***
TI =	***(disturbance***	***AND decomp*)***	***91***	***53***
TS =	***(disturbance***	***AND pollinat*)***	***340***	***6***
TI =	***(disturbance***	***AND facilitat*)***	***51***	***51***
T I=	***(disturbance***	***AND cooperat*)***	***26***	***5***
TS =	***(disturbance***	***AND "positive interac*")***	***237***	***32***
TS =	***(disturbance***	***AND "negative interac*")***	***74***	***5***
TS =	(disturbance	AND intraspec*)	501	0
TI =	***(disturbance***	***AND interspec*)***	***12***	***2***
TS =	(disturbance	AND altrui*)	24	0
TS =	(disturbance	AND alloparent*)	2	0
		TOTAL	**10287**	**1662**