

University of Rhode Island

DigitalCommons@URI

Open Access Master's Theses

2021

SEVERAL FACTORS AND THEIR ROLE IN CONTROL OF LEPIDOPTERA POPULATIONS

Alex K. Baranowski

Follow this and additional works at: <https://digitalcommons.uri.edu/theses>

SEVERAL FACTORS AND THEIR ROLE IN CONTROL OF LEPIDOPTERA POPULATIONS

BY ALEX BARANOWSKI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

UNIVERSITY OF RHODE ISLAND

2021

MASTER OF SCIENCE THESIS OF ALEX BARANOWSKI

APPROVED:

Thesis Committee:

Major Professor Evan Preisser

Steven Alm

Marian Goldsmith

Brenton DeBoef

Dean of Graduate School

UNIVERSITY OF RHODE ISLAND

2021

Abstract

A number of factors influence Lepidoptera populations. The larvae are at the mercy of their environment, susceptible to the effects of poor food quality, and highly vulnerable to predation. In this thesis I present three manuscripts dealing with a small snapshot of what dynamics are at play in the control of Lepidoptera numbers.

Host quality is a cornerstone of developmental success, with host suitability to the herbivore in question being affected by the plant's nutritional profile and its defenses against herbivory. Plant genotype influences plant suitability to herbivores; domesticated plants selected for properties such as high fruit yield are demonstrably vulnerable to herbivory. I conducted an experiment assessing the suitability of five *Vaccinium corymbosum* cultivars to the specialist notodontid *Datana drexelii*. In-situ weekly surveys of a managed blueberry patch for naturally occurring *D. drexelii* larval clusters complemented this work. Larval survival and pupal weight did indeed differ by cultivar. Larval occurrence on the managed blueberries also differed by cultivar. One especially important result was that the cultivar 'Jersey' yielded few naturally-occurring larvae and resulted in very high larval mortality and low pupal weight. The low *D. drexelii* preference for and performance on this cultivar suggest that this variety may be appropriate for areas where this pest is common. Cultivar-level variation in herbivore vulnerability highlights how understanding plant-pest interactions can help manage agricultural-state planning policy.

Natural enemies, in contrast to food plants, exert a top-down force on the populations of lepidopterans. Due to the high mortality caused by these enemies, the introduction of new enemies can have catastrophic effects on existing populations of lepidopterans. This is exemplified with the non-native generalist parasitoid *Compsilura concinnata* (Diptera: Tachinidae). This fly has been linked to the decline of *Hyalophora cecropia* and *Callosamia promethea*. Work done in the 1990s on these two saturniid species found that *C. concinnata* parasitized 81% of deployed *H. cecropia* and 68% of deployed *C. promethea*. In 2017 and 2018, we repeated this field experiment. In 2017, *C. concinnata* parasitized only 19% of *H. cecropia* larvae and 1% of *C. promethea* larvae; in 2018, parasitism rates were 3% and 0%, respectively. This suggests a shift in the role of this parasitoid in the population dynamics of these saturniid moths.

Finally, I deal with the mere risk of predation, which has demonstrable effects on the development and behavior of prey species. While prey responses to predators reduce the threat of consumption, the physiological costs of these responses can be considerable. *Actias luna* is a large saturniid native to Eastern North America with multiple natural enemies. *Actias luna* larvae were housed with the predator *Vespula maculifrons*, which were rendered non-lethal but able to move freely, as well as in a control (wasp-free) treatment. To rule out generic disturbance by an obnoxious insect, a third group of larvae were housed with a similarly-sized but harmless scavenging fly. Larvae in the wasp treatment died at a higher rate than those in the control treatments; and survival in the fly and control treatments did not differ. Larvae that died in the fly

and wasp treatments gained virtually no weight between the start of the experiment and their death, suggesting that they may have succumbed to starvation.

Acknowledgements:

I would like to thank Dr. Steve Alm, Dr. Larry Englander, Dr. Marian Goldsmith, Dr. Brian Maynard, and Dr. Evan Preisser for continued encouragement and friendship in my academic journey. For support and friendship I want to thank Becca Christ, Kate Conroy, Gabby Grandstaff, Olivia Hinsey-Langlais, Casey Johnson, Lexi Johnson Corinne Proulx, Sara Tucker, and Liz Varkonyi. I want to thank Hannah Broadley, Dr. Jeff Boettner, Alana Russel, and Dr. Lisa Tewksbury for working on additional scientific projects with me. For supporting my additional, eccentric projects in my free time I want to thank Dr. Andrew Cary, Arielle Chaves, Michael Dodge Dr. Julia Kuzovkina, Dr. Alison Roberts, and Dr. Bill Steele. I wanted to thank Dr. Marian Goldsmith for helping me edit this manuscript. I also want to give a special thanks to my dog Riley and all of my other pets for their constant presence.

Preface:

This thesis is prepared in manuscript format. The first manuscript was published in *The Journal of Economic Entomology* in 2020. The second manuscript was published in *Agricultural and Forest Entomology* in 2019. The third manuscript was published in *Frontiers in Ecology and Evolution* in 2018.

Table of Contents

Abstract.....	ii
Acknowledgements.....	v
Preface.....	vi
Table of contents.....	vii
List of Figures.....	viii
List of Tables.....	ix
Manuscript 1 “ <i>Datana drexelii</i> (Lepidoptera: Notodontidae) oviposition and larval survival on highbush blueberry cultivars”	1
Manuscript 2 “Reduced <i>Compsilura concinnata</i> parasitism of New England saturniid larvae”	16
Manuscript 3 “Predator Cues Increase Silkworm Mortality”	31

List of Figures

Manuscript 1 Figure 1.....	9
Manuscript 1 Figure 2.....	10
Manuscript 3 Figure 1.....	40

List of Tables

Manuscript 2 Table 1.....	24
Manuscript 3 Table 1.....	41

Manuscript 1 was published in the Journal of Economic Entomology in 2020.

***Datana drexelii* (Lepidoptera: Notodontidae) oviposition and larval survival on
highbush blueberry cultivars**

Alex K. Baranowski¹, Steven R. Alm², and Evan L. Preisser¹

¹*Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881*

²*Department of Plant Sciences and Entomology, University of Rhode Island, Kingston, RI
02881*

Introduction

Herbivore fitness is influenced by host plant phenotype. Although wild plants experience strong selection for herbivore tolerance and/or resistance, domesticated plants are subjected to different pressures. Selection for high fruit yield in domesticated plants, for example, can reduce plant defense against herbivores (Sanchez-Hernandez et al. 2006, Turcotte et al. 2014, Hernandez-Cumplido et al. 2018). Larvae of *Lymantria dispar* L. (Lepidoptera: Erebidae) grow more quickly and have lower mortality when reared on domesticated versus wild-type *Vaccinium corymbosum* L. (Hernandez-Cumplido et al. 2018). Wild-type tomatoes (*Solanum lycopersicum* L.) produce more phenolic compounds than domesticated ones (Sanchez-Hernandez et al. 2006), and growth rate of the moth *Manduca sexta* L. (Lepidoptera: Sphingidae) is negatively correlated with such phenolics (Stamp and Yang 1996, Yang and Stamp 1996).

Vaccinium corymbosum (hereafter 'blueberry') is a deciduous ericaceous plant native to North America grown commercially for its fruits. As with other agricultural plants, blueberry has multiple cultivars that have been selected for yield, flavor, or pest/disease resistance (Lobos and Hancock 2015, Clift et al. 2017, Rodriguez-Saona et al. 2019). Cultivar-related differences in herbivore growth and mortality have been recorded in lepidopteran species such as *Streblote panda* (Hübner, 1820) (Lepidoptera: Lasiocampidae) (Calvo and Molina 2010), and tephritid flies such as *Bactrocera dorsalis* (Hendel), *Ceratitis capitata* (Wiedemann, 1824) (Follett et al. 2011) and *Rhagoletis mendax* (Curran) (Liburd et al. 1998).

Members of the notodontid genus *Datana* (Walker, 1855) are defoliating pests of agricultural, silvicultural, and horticultural crops (Cutler and Harris 1979, Harris 1983). *Datana drexelii* (Hy. Edwards) (hereafter '*Datana*') is a native defoliating pest of ericaceous plants in the genera *Vaccinium* L. and *Gaylussacia* (Kunth). Females lay clusters of up to 200 eggs on suitable host plants; their gregarious nature, combined with the fact that larvae can reach six cm in length, make it an especially destructive pest (Wagner 2005). While this insect does not directly attack fruit, its defoliation of blueberry bushes reduces the following year's flowering and subsequent fruit crop (Lyrene 1992, Williamson and Miller 2000). We reared *Datana* larvae on different blueberry cultivars and measured their survival to and size at pupation. In addition, we repeatedly surveyed a patch planted with multiple blueberry cultivars for naturally-occurring clusters of *Datana* larvae. Together, the data reveal substantial differences in *Datana* preference for and performance on different blueberry cultivars.

Materials and Methods

Performance Assay: In June 2019, we mated adults from a lab colony of *Datana drexelii*, reared on wild-type *V. corymbosum*, in an outdoor emergence cage at the University of Rhode Island's East Farm research facility (Kingston, RI). We collected their eggs and assigned five each to 946 mL polypropylene cups (Pactiv LLC, Lake Forest, IL). Each cup was randomly assigned to one of five blueberry cultivars: 'Bluecrop', 'Blueray', 'Bluetta', 'Jersey', or 'Lateblue'. These particular cultivars were used because they were the most abundant and thus the removal of foliage from them would have done the least amount of damage to the plantings. There were 22-23 replicate cups per cultivar.

Eggs in a given cup generally hatched on the same day, although there were three cups where hatching occurred over a two-day period and one cup where hatching occurred over a three-day period. Host foliage from the appropriate cultivar was added to the cup immediately following emergence of the first hatchling. Larvae received four-leaf sections of foliage from current year's growth (indicated by soft, green bark), but no other leaf position standardization was done. Since a 2% bleach (=0.1% NaOCl) solution and air drying of foliage before being added to rearing containers kills pathogenic fungi and bacteria that can harm larvae (Trivedy et al. 2011), we treated foliage in this manner before feeding it to the larvae. No survival comparison was done between larvae fed uncleaned foliage and larvae fed cleaned foliage. Foliage was replaced every three days or as needed to ensure a constant food supply.

Four days after the last hatchling in a given cup eclosed, we weighed all hatchlings together and counted the number of larvae and unhatched eggs. The total number of hatched larvae was our starting number of larvae for a cup, regardless of how many eggs hatched. We used this data to calculate post-hatching survival. Larvae were subsequently counted and weighed together each week; we recorded the date each larva entered the prepupal phase. Prepupae were left in cups until all larvae in a cup reached such a state or died. When all prepupae had either died or become pupae, each pupa was sexed, weighed and then held in a 6L polypropylene bin (Sterilite Corp, Townsend, MA) of moist coconut coir for overwintering.

Preference Assay: In summer 2019, we conducted a six-week *Datana* survey of an East Farm blueberry patch enclosed in bird-proof netting that did not exclude insects.

The patch consisted of 240 bushes arranged in eight rows of 30 bushes. The cultivars represented (numbers of bushes in parentheses) were 'Bluecrop' (25), 'Bluegold' (5), 'Bluehaven' (15), 'Bluejay' (15), 'Blueray' (15), 'Bluetta' (15), 'Chandler' (5) 'Collins' (20), 'Darrow' (20), 'Earliblue' (30), 'Herbert' (15), 'Jersey' (15), 'Lateblue' (15), 'Northland' (15), and 'Reka' (15). Cultivars were arranged in five-bush groups within a given row.

Between July 16th and August 26th, we conducted 15 total censuses (with as many as nine days and as few as one day between censuses) for *Datana* larval clusters. We walked on both sides of each bush and scanned for larval clusters. We spent a minimum of thirty seconds per bush and longer if necessary and recorded the number of larval clusters on each bush before removing them from the bush. Following the final census, we measured the height and maximum width of each bush.

Statistical Analysis: For the performance assay, mean pupal weight and percent survival (average per cup) to pupation were analyzed using analysis of covariance (ANCOVA), with 'cultivar' as the main effect and 'hatch date' and, since we were concerned about sex-mediated performance differences, 'number of female pupae per cup' as covariates. We excluded 15 cups in which only a single larva hatched, leaving a total of 98 cups (=replicates), since these data points skew survival percentages. When the ANCOVA revealed a significant main effect, we used Tukey's HSD tests ($\alpha=0.05$) to differentiate between treatment.

For the preference assay, we summed the total number of larval clusters counted per bush over the fifteen censuses. Prior to analysis, we removed data from two cultivars, 'Bluegold' and 'Chandler', only represented by a single five-bush cluster

within the patch; all other cultivars were each represented by between three and six five-bush clusters. During our surveys, we noticed that small (~0.5 m in height) recently-planted bushes had virtually no larval clusters regardless of their cultivar. We addressed this bias by excluding bushes below the 10th percentile in height (0.7 m) from the analysis; this excluded 27 bushes from five cultivars but only two of 108 larval clusters. The final 203-bush data set was analyzed using ANCOVA, with 'cultivar' as the main effect and 'row', 'column', and 'bush height' as covariates. Data was square-root transformed prior to analysis to attain normality. The model was initially run using all covariates; non-significant covariates were then removed and the model re-run. When the ANCOVA revealed a significant main effect, we used Tukey's HSD tests ($\alpha=0.05$) to differentiate between treatments.

All analyses were performed using JMP 9.0.0 (SAS 2010).

Results

Performance Assay: Larvae reared on 'Lateblue' pupated at nearly three times the weight of larvae reared on 'Blueray', 'Bluetta', and 'Jersey' (0.375 g versus 0.127 g, respectively; $F_{4,91} = 3.18$, $P = 0.017$; Fig. 1A). Survival to pupation was also higher on 'Lateblue' than on 'Bluecrop', 'Bluetta', and 'Jersey' (16.9% versus 5.1%; $F_{4,91} = 3.62$, $P = 0.009$; Fig. 1B). Hatch date affected survival, with later-hatching larvae having higher mortality ($F_{1,91} = 7.84$, $P = 0.006$). The number of female pupae per cup was correlated with both weight at and survival to pupation (both $P < 0.001$).

Preference Assay: We found a total of 108 *Datana* larval clusters over the six-week course of the survey. The distribution of larval clusters over time was as follows:

14 on July 16th, ten on July 18th, 35 on July 22nd, one on July 23rd, five on July 25th, three on July 26th, one on July 29th, two on July 30th, two on July 31st, 11 on Aug. 1st, 12 on Aug. 2nd, one on Aug. 6th, two on Aug. 11th, eight on Aug. 20th, and one on Aug. 26th.

Cultivars differed in *Datana* colonization ($F_{12,161} = 2.96$, $P = 0.001$; Fig. 2), with 'Bluehaven', 'Collins', and 'Darrow' having more *Datana* clusters (1.00/bush, 0.75/bush, and 0.85/bush, respectively) than either 'Jersey' or 'Earliblue' (0.13 and 0.07 per bush, respectively; Tukey's HSD at $\alpha = 0.05$). No covariates affected *Datana* colonization (all $P > 0.1$).

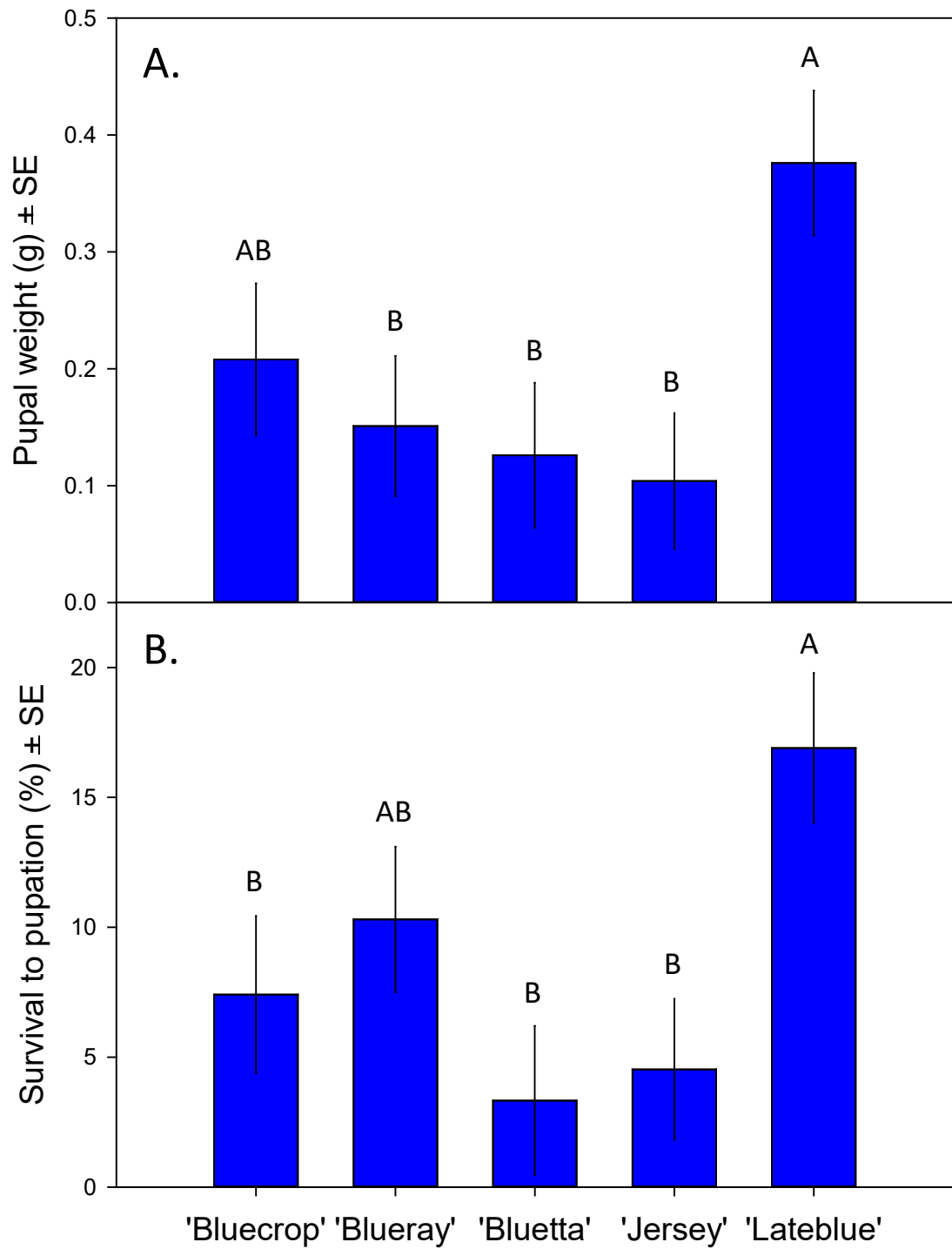


Figure 1: Figure 1. Mean (+ SE) *Datana drexelii* pupal weight (A) and mean (+ SE) *D. drexelii* survival to pupation (B) when reared on five different *Vaccinium corymbosum* cultivars. Bars with different uppercase letters are significantly different (Tukey's HSD at $\alpha = 0.05$).

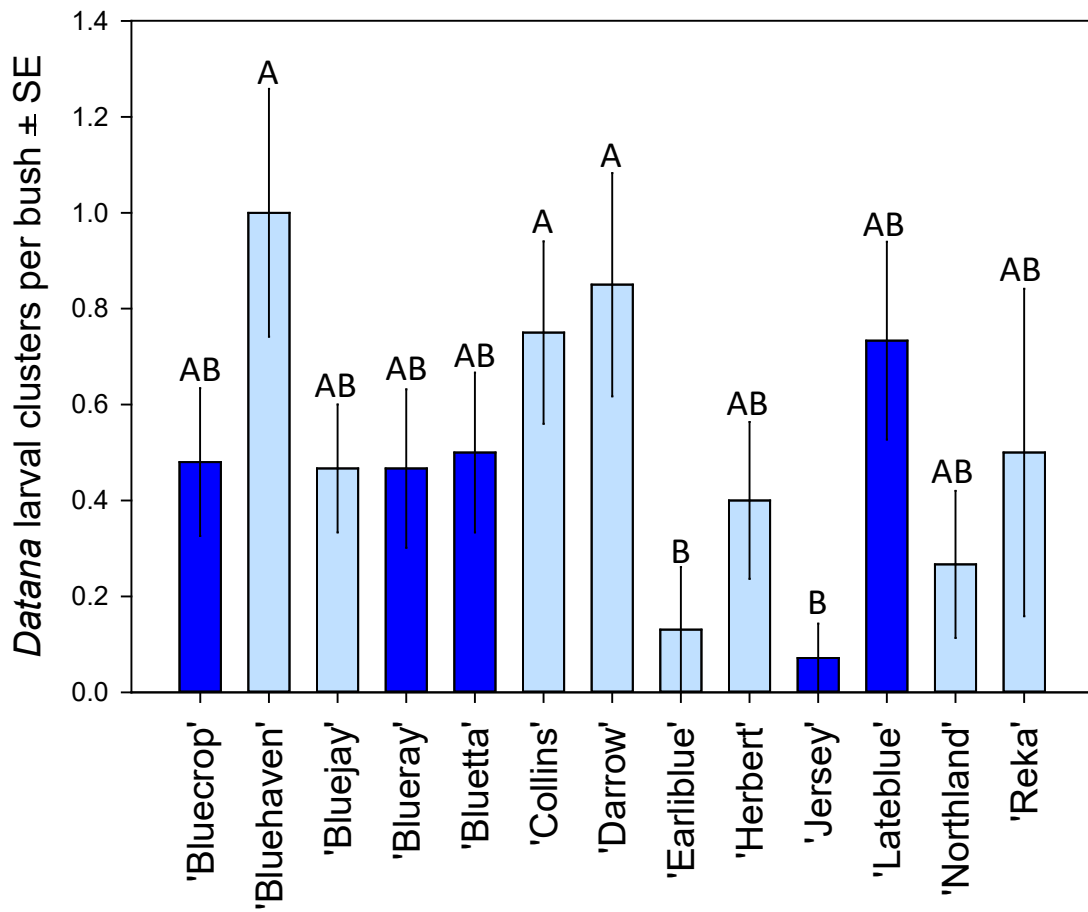


Figure 2: Mean + (SE) *D. drexelii* larval clusters counted per bush for 13 *V. corymbosum* cultivars over the course of six weeks and fifteen censuses. Cultivars in dark blue were included in the performance assay (Fig. 1). Bars with different uppercase letters are significantly different (Tukey's HSD at $\alpha = 0.05$).

Discussion

Datana larval performance in the lab did not overlap with field observations. Despite high larval performance on 'Lateblue', larval occurrence on it was not the highest on this cultivar in the field survey. High *Datana* densities on 'Bluehaven', 'Collins', and 'Darrow', cultivars not included in our performance assay, suggest they may be particularly suitable to this pest. In contrast, both occurrence and performance

were significantly lower for 'Bluetta' and 'Jersey' than other tested cultivars (Figs. 1, 2). This implies that 'Bluetta' and 'Jersey' may have antixenotic and antibiotic effects on *Datana*.

Mortality in the performance assay occurred mostly within a week of hatching, when larvae were small and inconspicuous. Our high early larval mortality across treatments could indicate that either cultivated blueberry is unsuitable for this species (comparison to wild-type blueberry is needed to determine this), or perhaps that the unnaturally low early instar densities could be reducing the feeding ability, and thus survival, of hatchlings (Dave Wagner, pers. corr). It is also possible that the quality of the cut blueberry used in the experiment may have diminished more quickly than the foliage was replaced (within 24hrs instead of 3 days), malnourishing larvae. If this high hatchling mortality also occurred in the field survey, we could have missed some oviposition events when all larvae died prior to reaching a detectable size. Because of this, the patterns in our field survey data may result from some combination of female oviposition preference and plant resistance to early-instar larval feeding. Studies on the survival of early-instar larvae are needed to parse out these different scenarios. While most larval clusters contained a similar number (10-20 individuals) of small 2nd-3rd instar larvae, we failed to detect some clusters until they contained 4th-5th instar larvae. The laboratory-based oviposition choice tests necessary to isolate the role of female preference may be complicated by this species' habit of readily ovipositing on container walls and other artificial objects.

Our work could be extended to comparisons of *Datana* interactions with cultivated versus wild-type blueberry, as well as with other *Vaccinium* species. Selective breeding for pest resistance (Lobos and Hancock 2015) and the incorporation of several related *Vaccinium* species into *V. corymbosum* cultivars (Lobos and Hancock 2015) may alter the cultivar's suitability to *Datana*. Both 'Lateblue' and 'Jersey', cultivars on which larvae did the best and worst, are pure *V. corymbosum*, but 'Bluecrop' is 4% *Vaccinium angustifolium* (Aiton, 1789) and 'Bluetta' is 28% *V. angustifolium*. Some cultivars are only 42% *V. corymbosum* and contain genes from up to five other species (Lobos and Hancock 2015). Intrageneric variation in herbivore susceptibility has been described for other *Vaccinium* (Ieri et al. 2013) species as well as for genera ranging from *Asclepias* L. (Waterbury et al. 2019) to *Quercus* L. (Rieske and Dillaway 2008).

In summary, there were blueberry cultivar-related differences in occurrence and performance of this blueberry defoliator. This information could prove useful for cultivar selection in areas where this pest becomes a major problem, and highlights how understanding plant-pest interactions can help reduce the need for costly chemical or mechanical (hand-removal of larval clusters) treatments.

References Cited

- Calvo, D., and J. M. Molina. 2010.** Differences in foliage affect performance of the lappet moth, *Streblote panda*: Implications for species fitness. *Journal of Insect Science* 10: 177.
- Clift, A. D., G. Murdoch, and S. Mansfield. 2017.** Blueberry cultivars differ in susceptibility to the elephant weevil, *Orthorhinus cylindrirostris* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 110: 2259-2262.
- Cutler, B. L., and M. K. Harris. 1979.** Foliage consumption and damage by the walnut caterpillar on pecan in Texas USA. *J. Econ. Entomol.* 72: 315-318.
- Follett, P. A., F. T. Zee, R. T. Hamasaki, K. Hummer, and S. T. Nakamoto. 2011.** Susceptibility of low-chill blueberry cultivars to mediterranean fruit fly, oriental fruit fly, and melon fly (Diptera: Tephritidae). *J. Econ. Entomol.* 104: 566-570.
- Harris, M. K. 1983.** Outbreak biology of walnut caterpillar *Datana integerrima* in Texas USA. *Southwest. Entomol.* 8: 231-240.
- Hernandez-Cumplido, J., M. M. Giusti, Y. Zhou, V. Kyryczenko-Roth, Y. H. Chen, and C. Rodriguez-Saona. 2018.** Testing the 'plant domestication-reduced defense' hypothesis in blueberries: the role of herbivore identity. *Arthropod-Plant Interactions* 12: 483-493.
- Ieri, F., S. Martini, M. Innocenti, and N. Mulinacci. 2013.** Phenolic distribution in liquid preparations of *Vaccinium myrtillus* L. and *Vaccinium vitis idaea* L. *Phytochem. Anal.* 24: 467-475.

- Liburd, O. E., S. R. Alm, and R. A. Casagrande. 1998.** Susceptibility of highbush blueberry cultivars to larval infestation by *Rhagoletis mendax* (Diptera: Tephritidae). *Environ. Entomol.* 27: 817-821.
- Lobos, G. A., and J. F. Hancock. 2015.** Breeding blueberries for a changing global environment: a review. *Frontiers in Plant Science* 6: 782.
- Lyrene, P. M. 1992.** Early defoliation reduces flower bud counts on rabbiteye blueberry. *HortScience* 27: 783-785.
- Rieske, L. K., and D. N. Dillaway. 2008.** Response of two oak species to extensive defoliation: Tree growth and vigor, phytochemistry, and herbivore suitability. *For. Ecol. Manage.* 256: 121-128.
- Rodriguez-Saona, C., C. Vincent, and R. Isaacs. 2019.** Blueberry IPM: past successes and future challenges, pp. 95-114. In A. E. Douglas (ed.), *Annual Review of Entomology*, Vol 64, vol. 64.
- Sanchez-Hernandez, C., M. G. Lopez, and J. P. Delano-Frier. 2006.** Reduced levels of volatile emissions in jasmonate-deficient spr₂ tomato mutants favour oviposition by insect herbivores. *Plant Cell and Environment* 29: 546-557.
- SAS 2010.** JMP user's guide, version 9.0 computer program, version By SAS, Cary NC.
- Stamp, N. E., and Y. Yang. 1996.** Response of insect herbivores to multiple allelochemicals under different thermal regimes. *Ecology* 77: 1088-1102.
- Trivedy, K., S. N. Kumar, N. Vinutha, and S. Qadri. 2011.** In vitro testing of common disinfectants used in sericulture to control the growth of fungi in rearing houses. *Research Journal of Microbiology* 6: 439-465.

- Turcotte, M. M., N. E. Turley, and M. T. Johnson. 2014.** The impact of domestication on resistance to two generalist herbivores across 29 independent domestication events. *New Phytol.* 204: 671-681.
- Wagner, D. L. 2005.** *Caterpillars of Eastern North America*, Princeton University Press, Princeton, NJ 08540.
- Waterbury, B., A. Potter, and L. K. Svancara. 2019.** Monarch butterfly distribution and breeding ecology in Idaho and Washington. *Frontiers in Ecology and Evolution* 7: 172.
- Williamson, J. G., and E. P. Miller. 2000.** Early fall defoliation of southern highbush blueberry inhibits flower bud initiation and retards flower bud development. *HortScience* 35: 505.
- Yang, Y., and N. E. Stamp. 1996.** Simultaneous effects of temperature and multiple allelochemicals on the performance of a Solanaceae specialist caterpillar (*Manduca sexta*). *Ecoscience* 3: 81-92.

Manuscript 2 was published in the Journal of Agricultural and Forest Entomology in 2019.

Reduced *Compsilura concinnata* parasitism of New England saturniid larvae

Alex K. Baranowski¹, Catherine Conroy¹, George H. Boettner², Joseph S. Elkinton^{2,3}, and
Evan L. Preisser¹

¹*Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881*

²*Department of Environmental Conservation, University of Massachusetts- Amherst,
Amherst, MA 01003*

³*Department of Organismal and Evolutionary Biology, University of Massachusetts-
Amherst, Amherst, MA 01003*

Introduction

Populations of silk moths (Lepidoptera: Saturniidae) in the northeastern United States have declined precipitously over the past half-century (Boettner et al. 2000, Wagner 2012, Goldstein et al. 2015). All saturniids have been affected, and the regal moth (*Citheronia regalis*) has been extirpated from the New England mainland, the imperial moth (*Eacles imperialis*) is currently so rare that it is on the Massachusetts Division of Fish and Wildlife Threatened species status, and *Hemileuca lucina* is a Species of Special Concern in Massachusetts, (Wagner 2012, Goldstein et al. 2015). Several anthropogenic causes for this decline have been hypothesized: these include habitat loss, human development (including electric lighting), and control efforts for gypsy moth (*Lymantria dispar*) and other forest pests (Fitzgerald 1995, Wagner 2012). The effects of human population densities and land usage on the populations of these moths has yet to be thoroughly investigated. The latter threat reflects the fact that since saturniids and gypsy moth larvae overlap in host usage, pesticide use on deciduous trees should affect both groups. However, most pesticide use was localized, and more common in practice in the 1940's and 1950's. One would expect silk moths should have recovered in the past 70 years. The increasing abundance of deer and birds in the wake of increased human development is another potential cause of decline, since deer browse plants containing moth eggs and larvae and birds prey heavily upon larvae and pupae (Wagner 2012). However, some of these silk moths produce acoustic and/or emetic signals, which may be defense strategies (Brown et al., 2007). Therefore, while each factor is

likely important, they cannot explain why saturniids outside of New England appear largely unaffected.

Researchers have suggested that the parasitoid *Compsilura concinnata* (Meigen 1824) (Diptera: Tachinidae; hereafter *Compsilura*) might help explain saturniid declines (Boettner et al. 2000, Kellogg et al. 2003, Wagner 2012, Goldstein et al. 2015). This multivoltine tachinid was first introduced to New England in 1906 (and repeatedly over the next 80 years) for control of *L. dispar* and 12 other pests (reviewed in Elkinton et al. 2006). *Compsilura* established quickly in North America, where it can attack and kill over 180 native insect species (Arnaud 1978). *Compsilura* was even used against one native saturniid moth, the range caterpillar, *Hemileuca oliviae* but with no known success (Boettner et al. 2000). Stamp and Bowers (1990) documented high *Compsilura*-induced mortality on the saturniid *Hemileuca lucina*, although a study of the closely related *H. maia* found lower parasitism rates (Selfridge et al. 2007). Between 1995-1998, Boettner et al. (2000) assessed the impact of *Compsilura* on the saturniids *Hyalophora cecropia* and *Callosamia promethea*. They found 60-80% of larvae were parasitized over a three-instar period, a rate high enough (if extrapolated over the larval period) to kill the entire experimental larval cohort (n=965). Their results suggested that *Compsilura* may have played a major role in saturniid decline and disappearance in the Northeast. In the Appalachian region of Virginia, saturniids remain common (Kellogg et al. 2003) despite the presence of *Compsilura*. *Actias luna* larvae deployed in the region were frequently (16-60% of cases) hyperparasitized by trigonalid wasps after *Compsilura* parasitism, a degree of top-down control that may dampen the suppression of moth populations

(Kellogg et al., 2003). This could also explain why *Compsilura* plays a minor role in gypsy moth mortality in Virginia. More recently, surveys failed to detect *Compsilura* on New England islands with high saturniid densities (Goldstein et al. 2015). While the fly's effect on saturniids is now accepted (Wagner and Van Driesche 2010), how *Compsilura* density responds to fluctuations of primary alternate host densities, such as from *L. dispar* abundance or scarcity (Hajek et al. 2015), is unknown.

We report the results of surveys conducted in 2017 and 2018 that repeated work described in Boettner et al (2000). To investigate whether parasitism rate of saturniids by *Compsilura* have changed over the past 20 years, we deployed *C. promethea* and *H. cecropia* larvae in conditions replicating those of the previous study, then assayed parasitism rates. We found that parasitism was substantially lower on *H. cecropia* larvae than in the previous study and virtually nonexistent on *C. promethea* larvae. Our results, although only a two-year snapshot, suggest that the ecological importance of this parasitoid has changed.

Methods

Eggs of both *H. cecropia* and *C. promethea* were obtained by pairing unrelated captive individuals from New England (RI and CT) populations collected the prior year. Eggs were incubated in 240mL polypropylene cups (Pactiv, Lake Forest, Illinois). As per Boettner et al. (2000), the emerging *H. cecropia* and *C. promethea* larvae were reared on *Prunus serotina* and *Sassafras albidum*, respectively. Larvae were reared outdoors on live hosts within one-meter-long agribon (Berry Plastics, Evansville, Indiana) bags to

exclude predators and parasitoids. Pilot experiments found these bags effective at predator/parasitoid exclusion, and larvae reared outdoors within them experienced minimal mortality (A. Baranowski, *unpublished data*).

Once larvae had reached the 3rd-5th instar, they were deployed on naturally growing understory *P. serotina* and *S. albidum* saplings, respectively, along transects in Cadwell Memorial Forest (Pelham, Massachusetts). Both this site, and the location of the transects within it, were the same as reported in Boettner et al. (2000). As per the prior studies, three larvae (3rd-5th instar) were placed on each plant, which were spaced 1-3 m apart and marked with flagging tape; different transects were used for the two saturniid species. The 'stocking density' of three larvae/plant follows Boettner et al. (2000), who found no evidence for density-dependent parasitism. We also conducted our surveys at the same time of the year that the previous study did; Boettner et al. deployed *C. promethea* larvae on July 22-24 1995, while *H. cecropia* larvae were deployed on June 19-24 and July 2 1997.

The survey began on 2 July when we deployed 89 *C. promethea* larvae. We retrieved these larvae on 8 July; if all three larvae were not found, we searched the surrounding vegetation for five minutes. Following retrieval, we deployed an additional 104 *C. promethea* larvae. We retrieved these larvae on 15 July. That same day, we deployed 72 *H. cecropia* larvae; these were retrieved on 22 July. The final survey took place when we deployed 102 *C. promethea* larvae on 12 August (retrieved 19 August).

Retrieved larvae were reared individually in the lab in 473mL polystyrene cups (Pactiv, Lake Forest, Illinois) on fresh leaves from the appropriate host; wilted leaves were replaced with fresh foliage. Rearing cups were covered with cheesecloth held in place with a rubber band, and cups were placed on a bench over a moist section of absorbent liner to maintain humidity. Indoor rearing temperatures were 21-28° C during both years. Host sprigs were kept fresh by means of moist floral foam (Smithers-Oasis brand, Kent, OH). During 2018 rearing, cut host branches were briefly immersed in a 1% bleach solution and shaken dry before placement into rearing cups; this was done to reduce the spread of larval pathogens. Larvae were inspected and waste removed from cups daily, and their status (live/dead/pupated) recorded. Dead larvae were held individually for ten days to check for parasitoid emergence, then frozen for dissection to determine if they contained larval parasitoids. Cocoons were inspected for parasitoid emergence, then held outdoors in a mesh cage until moth emergence the following spring. Parasitoids were identified to species whenever possible.

In 2018, we repeated the above procedures for both moth species. We deployed *H. cecropia* and *C. promethea* larvae on 28 June, 5 July, 12 July, and 20 July; they were collected on 5 July, 12 July, 20 July, and 27 July, respectively. Numbers of *H. cecropia* larvae deployed per day were 63, 87, 71, and 16, respectively. Numbers of *C. promethea* larvae deployed per day were 89, 104, and 102 (we did not deploy this species on 20 July), respectively.

We analyzed data on the number of *Compsilura*-parasitized larvae in 2017 versus 2018, and in both years of our study versus the data reported in Boettner et al. (2000), using likelihood ratio χ^2 tests. All data were analyzed in JMP 9.0.0 (SAS 2010).

Results and Discussion

In 2017, we deployed 72 *H. cecropia* larvae and recovered 31 (Table 1); seven of the 31 (22%) survived to pupation. Six dead larvae had been parasitized by *Compsilura* (19.3%): these produced a total of ten *Compsilura* pupae. One prepupal larva was killed by the *H. cecropia* tachinid specialist *Lespesia samiae*; this single cecropia larva produced 22 adult parasitoids. None of the pupated *H. cecropia* had *Compsilura* emerge from them. In 2018, we deployed 198 larvae and recovered 32; only one survived to pupation. *Compsilura* killed one prepupal larva (3.1%) and produced a single parasitoid pupa; no other parasitoid species emerged. Parasitism rates were slightly higher in 2017 than in 2018 ($\chi^2_{1\text{df}} = 4.59$, $p=0.032$), but both years of our study differed greatly from the results reported in Boettner et al. (2000) ($\chi^2_{2\text{df}} = 98.5$, $p<0.001$).

In 2017, we deployed 295 *C. promethea* larvae and recovered 190 (64.4%) (Table 1); approximately 40% ($n=77$) of the retrieved larvae pupated. No *Compsilura* emerged from the dead larvae; a single *C. promethea* larvae was parasitized and killed by an unknown ichneumonid. Two *Compsilura* emerged in spring 2018 from *C. promethea* cocoons, having evidently overwintered inside them. This is the first record of how *Compsilura* overwinters we can find in the literature. No other cocoons contained either *Compsilura* pupae or adult flies. A subsequent examination revealed two cocoons

that each had one hole in its side, indicative of parasitoid escape. Since no other parasitoids emerged from these cocoons, we suspect the two *Compsilura* each emerged from a unique host larva. In 2018, we deployed 155 larvae and recovered 48. Thirty (55%) of the recovered larvae pupated; no parasitoids emerged from dead larvae. There was no difference in *Compsilura* parasitism rates between 2017 and 2018 ($\chi^2_{1\text{ df}} = 0.45$, $p=0.5$); again, however, there was a highly significant difference between our results and those of the previous study ($\chi^2_{2\text{ df}} = 223$, $p<0.001$). We dissected all larvae of both moth species that died prior to pupation to ascertain cause of death and count any immature larval parasitoids.

Saturniid species	Data set	Larvae deployed	Days in field	Larvae retrieved (%)	Parasitoid-related rearing mortality	
					<i>C. concinnata</i>	Other parasitoids
<i>Hyalophora cecropia</i>	Boettner et al. 2000	300	5-7	134 (45%)	81%	2%
	This survey (2017)	72	7	31 (43%)	19%	3%
	This survey (2018)	198	6-7	32 (16%)	3%	0%
<i>Callosamia promethea</i>	Boettner et al. 2000	665	6-8	117 (18%)	68%	21%
	This survey (2017)	295	6-7	190 (65%)	1%	1%
	This survey (2018)	155	6-7	48 (31%)	0%	0%

Table 1: Larval retrieval and parasitoid-related mortality for *Hyalophora cecropia* and *Callosamia promethea* in 2017 and 2018 and as reported in Boettner et al (2000) for the same location and site in the late 1990s.

Despite lower rates of *Compsilura* parasitism, the *H. cecropia* larvae we recovered from the field experienced high rates of pathogen-related mortality in both 2017 (55%) and 2018 (94%). This also affected *C. promethea* larvae (58% and 45% in 2017 and 2018, respectively). Pathogen-killed larvae went from apparently healthy to dead, with their integument turning a distinctive black color, in a short (1-2 day) period. Pathogen presence was confirmed via dissection and microscopy (G.H. Boettner,

unpublished data); the dark fluid that we found oozing from the dead larvae is typical of viral epizootics in captive stocks of other saturniid moths (Skowron et al. 2015). While larvae occasionally died in the field sleeves, none exhibited the same symptoms as those dying post-retrieval. The >10-day lag between retrieval and the death of pathogen-killed larvae, in combination with the rapid decline and death of 'healthy' individuals, further suggests that the mortality resulted from a lab-associated pathogen, the stress of multiple moves (from field cages to survey trees to the lab over a 7-9 day period), or some combination of the two factors.

We found no larval parasitoids in the dissected larvae. While this might have resulted from the high rates of pathogen-induced mortality, the time from retrieval to death of pathogen-killed *H. cecropia* larvae was 10.8 ± 1.5 days, versus 6.2 ± 0.9 days for *Compsilura*-killed *H. cecropia* larvae. Because *Compsilura* develops quickly within its host, this difference in time suggests that any *Compsilura* larvae present should have grown to sizes detectable by dissection. The fact that *Compsilura* inserts its hatchlings into, and lurks within, the immune system-neglected peritrophic membrane of its host (Caron et al. 2008), also makes it unlikely that immune activity of sick larvae could reduce survival of this parasitoid.

While care must be taken when extrapolating from a two-year survey, the decline in *Compsilura* parasitism suggests that important changes in the ecological impact of this tachinid may be occurring. There are many possible explanations for our results; we discuss below several of the more compelling hypotheses for the apparent decline.

One possible explanation for our results involves climatic differences: extreme temperatures can alter or disrupt host-parasitoid interactions (Hance et al. 2007), and changes in precipitation can impact caterpillar abundance (e.g., Karban et al. 2017). An analysis of daily weather data from the periods when caterpillars were deployed in 1995 (22-31 July) and 1997 (19-24 June and 2-9 July) revealed that temperatures averaged $22.4^{\circ} \pm 3.1^{\circ}$ (SE) C and weekly rainfall averaged 2.4 ± 1.13 cm (NOAA 2018). During our work in 2017 and 2018, temperatures averaged $23.0^{\circ} \pm 1.5^{\circ}$ (SE) C and weekly rainfall averaged 4.0 ± 1.1 cm (NOAA 2018). Although rainfall values are higher in our study, our means include one extremely wet week (28 June - 4 July 2018) in which 8.1 cm of rain fell; excluding this week reduces our weekly rainfall means to 3.25 ± 0.86 cm.

The reduction in parasitism may also have resulted from increased predation on *Compsilura* itself. Kellogg et al. (2003) found that ~50% of *Compsilura* pupae collected in Virginia were hyperparasitized by trigonalid wasps and hypothesized that this might explain the continued local abundance of silk moths. We assessed hyperparasitism by rearing the 14 *Compsilura* pupae we collected: 13 emerged as adults and one died without producing any hyperparasitoids. In addition, no hyperparasitoids emerged from dead saturniid larvae.

A third possibility is that *Compsilura*-saturniid interactions are influenced by the interplay between *L. dispar* and its fungal pathogen *Entomophaga maimaiga*. *Compsilura* was introduced to control *L. dispar*, and Culver (1919) found lower *C. promethea* densities at tachinid release sites. *Compsilura* parasitizes early season *L. dispar* caterpillars (Gould et al. 1990), and the ensuing generations of parasitoids attack

both early and late season saturniids (Wagner and Van Driesche 2010). Starting in the late-1980s, the establishment of *E. maimaiga* in the northeastern U.S. reduced both the frequency and magnitude of *L. dispar* outbreaks (Hajek 2007, Hajek et al. 2015). This pathogen also kills *L. dispar* larvae so quickly that co-infecting parasitoids like *Compsilura* are unable to complete their development (Hajek and van Nouhuys 2016). *Compsilura* also tends to attack *L. dispar* during early instars, while *Entomophaga* kills later instar larvae. If early-season hosts determine mid and late season *Compsilura* densities, the impact of *E. maimaiga* on *L. dispar* could affect the ability of *Compsilura* to suppress saturniids, however, there is little evidence to confirm this.

In summary, we consistently found lower rates of saturniid parasitism by *Compsilura* than previously reported (Boettner et al. 2000) from the same location. If *Compsilura* is not presently the primary driver and sustainer of saturniid decline, then the reasons for it, particularly in the Northeast region, remain indeterminate. In addition to surveying for parasitoid presence or absence, future studies should explore interactions between *Compsilura* and other lepidopteran hosts. Specifically, it seems important to investigate the phenology of both *L. dispar* and its natural enemies for possible overlap in pathogen and parasitoid activity. The factors responsible for wild mortality in other life stages of these declining moths, biotic and abiotic, are also relatively unexplored. While much remains to be done, our results highlight the importance of longitudinal survey work capable of detecting cryptic but important changes in community structure.

References Cited

- Arnaud, P. H., Jr. 1978.** A host-parasite catalog of North American Tachinidae (Diptera). 1319, U.S. Science and Education Administration, Washington D.C.
- Boettner, G. H., J. S. Elkinton, and C. J. Boettner. 2000.** Effects of a biological control introduction on three nontarget native species of Saturniid moths. *Conservation Biology* 14:1798-1806.
- Caron, V., A. F. Janmaat, J. D. Ericsson, and J. H. Myers. 2008.** Avoidance of the host immune response by a generalist parasitoid, *Compsilura concinnata* Meigen. *Ecological Entomology* 33:517-522.
- Culver, J. J. 1919.** A study of *Compsilura concinnata*, an imported tachinid parasite of the gypsy moth and brown-tail moth USDA Bulletin 766:1-27.
- Elkinton, J., D. Parry, and G. Boettner. 2006.** Implicating an introduced generalist parasitoid in the invasive browntail moth's enigmatic demise. *Ecology* 87:2664-2672.
- Fitzgerald, T. D. 1995.** The Tent Caterpillars. Cornell University Press, Ithaca, NY.
- Goldstein, P. Z., S. Morita, and G. Capshaw. 2015.** Stasis and flux among Saturniidae and Spingidae (Lepidoptera) on Massachusetts' offshore islands and the possible role of *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) as an agent of mainland New England moth declines. *Proceedings of the Entomological Society of Washington* 117:347-366.
- Gould, J. R., J. S. Elkinton, and W. E. Wallner. 1990.** Density-dependent suppression of experimentally created gypsy moth, *Lymantria dispar* (Lepidoptera:

Lymantriidae), populations by natural enemies. *Journal of Animal Ecology* 59:213-233.

Hajek, A. E. 2007. Introduction of the entomopathogenic fungus *Entomophaga maimaiga* into North America. Pages 53-62 in C. Vincent, M. Goettel, and G. Lazarovits, editors. *Biological Control: a Global Perspective*. CABI Publishing, Wallingford.

Hajek, A. E., P. C. Tobin, and K. J. Haynes. 2015. Replacement of a dominant viral pathogen by a fungal pathogen does not alter the collapse of a regional forest insect outbreak. *Oecologia* 177:785-797.

Hajek, A. E., and S. van Nouhuys. 2016. Fatal diseases and parasitoids: from competition to facilitation in a shared host. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 283.

Hance, T., J. van Baaren, P. Vernon, and G. Boivin. 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. *Annual Review of Entomology* 52:107-126.

Karban, R., P. Grof-Tisza, and M. Holyoak. 2017. Wet years have more caterpillars: interacting roles of plant litter and predation by ants. *Ecology* 98:2370-2378.

Kellogg, S. K., L. S. Fink, and L. P. Brower. 2003. Parasitism of native luna moths, *Actias luna* (L.) (Lepidoptera: Saturniidae) by the introduced *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) in central Virginia, and their hyperparasitism by trigonalid wasps (Hymenoptera : Trigonalidae). *Environmental Entomology* 32:1019-1027.

- NOAA. 2018.** Climate Data Online Search. Pages <https://www.ncdc.noaa.gov/cdo-web/search>. National Climatic Data Center.
- SAS. 2010.** JMP user's guide, version 9.0. SAS Institute Inc., Cary NC.
- Selfridge, J. A., D. Parry, and G. H. Boettner. 2007.** Parasitism of barrens buck moth *Hemilueca maia* Drury in early and late successional pine barrens habitats. *Journal of the Lepidopterists' Society* 61:213-221.
- Skowron, M. A., B. Guzow-Krzeminska, S. Baranska, P. Jedrak, and G. Wegrzyn. 2015.** A rapidly progressing, deadly disease of *Actias selene* (Indian moon moth) larvae associated with a mixed bacterial and baculoviral infection. *Journal of Biosciences* 40:487-495.
- Stamp, N. E., and M. D. Bowers. 1990.** Parasitism of New England buckmoth caterpillars (*Hemileuca lucina*: Saturniidae) by tachinid flies. *Journal of the Lepidopterists' Society* 44:199-200.
- Wagner, D. L. 2012.** Moth decline in the northeastern United States. *News of the Lepidopterists' Society* 54:52-56.
- Wagner, D. L., and R. G. Van Driesche. 2010.** Threats posed to rare or endangered insects by invasions of nonnative species. *Annual Review of Entomology* 55:547-568.

Manuscript 3 was published in *Frontiers in Ecology and Evolution* in 2018.

Predator Cues Increase Silkmoth Mortality

Alex K. Baranowski¹, Evan L. Preisser¹

¹ *Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881*

Introduction

Predation risk can affect various aspects of prey behavior and physiology (Lima and Dill, 1990; Werner and Peacor, 2003; Adamo, 2012; Sheriff and Thaler, 2014). These changes are generally seen as adaptive since they reduce the likelihood of capture and/or consumption. Because they require prey to alter their investment in activities such as foraging, however, these changes can also negatively affect growth, physical condition, fecundity, and, in the most extreme cases, survival (Zanette et al., 2011; Siepielski et al., 2014; Duong and Mccauley, 2016). Exposure to predator cues decrease tadpole survival (Hetttyey et al., 2015), for instance, and have a similar effect on dragonflies (McCauley et al., 2011) and grasshoppers (Schmitz et al., 1997).

Lepidopteran larvae are important terrestrial herbivores attacked by a wide variety of predators and parasitoids. Because they are slow-moving and lack a hard exoskeleton, they often rely on camouflage to avoid detection (Lichter-Marck et al., 2015). Foraging-related movement greatly increases caterpillar predation risk (Bernays, 1997), and camouflaged species may be especially likely to forego feeding in response to risk (Ruxton et al., 2004). Even individuals that resume feeding may suffer from the combined impact of reduced energy intake and the physiological costs of stress responses (Sheriff and Thaler, 2014).

The luna moth *Actias luna* (Lepidoptera: Saturniidae; *Actias* hereafter) is a silkmoth native to eastern North America (Wagner, 2005). The solitary larvae of this species require 3–6 weeks to mature, during which time camouflage provides their

primary protection against predators and parasitoids (Tuskes et al., 1996). [Sourakov (2018); p. 488] characterized *Actias* defenses against predation as “. . . relying mostly on cryptic colouration and being motionless when not feeding,” although they possess spines that may reduce their vulnerability to vertebrate predators (Sourakov, 2018). Vespid wasps (Hymenoptera: Vespidae) are generalist predators that hunt caterpillars (Stamp and Bowers, 1988; Lichtenberg and Lichtenberg, 2003); we have repeatedly observed yellowjacket wasps (*Vespula maculifrons*; wasps hereafter) attacking and killing *Actias* larvae in the field (A. Baranowski, personal observation).

We conducted two separate experiments measuring the survivorship of *Actias* caterpillars in the presence of wasps rendered unable to either sting or bite. We determined whether these responses were predator-specific by also exposing caterpillars to (1) wasp-sized scavenging flies that had been similarly treated; and (2) a no-insect control. We hypothesized that survivorship would be lowest in the wasp treatment, and higher in both the fly and no-insect treatments.

Methods

2016 Experiment

In July 2016, a newly-emerged captive female *Actias luna* was mated to a wild male at East Farm (Kingston RI), an agricultural research facility managed by the University of Rhode Island. Eggs were incubated in a 473 mL polypropylene deli cup (Pactiv brand, Lake Forest, IL) and began hatching 10 days later. Hatchlings were offered hickory (*Carya glabra*) foliage, with waste removed and new foliage added as needed.

Larvae were reared communally within the cup until the 2nd instar. To prevent overcrowding, larvae were then transferred to a 6L polypropylene bin (Sterilite brand, Townsend, MA) until they averaged 1.5 cm in length, when they were transferred to a 12L bin. Twenty-six days after hatching, 54 larvae were individually weighed (mean 0.673 ± 0.045 [SE] g) and transferred into individual 6L bins containing hickory foliage kept hydrated using water-filled floral tubes. Once all larvae had been transferred, each bin was randomly assigned to one of three 18-bin treatment groups: a predator that had been rendered non-lethal, a similarly-treated harmless detritivore, and no-insect control.

Bins in the non-lethal predator (“wasp”) treatment each contained a single adult *V. maculifrons* collected from either flowers or overripe fruit; prior to the experiment, we had repeatedly observed wasps attacking and dismembering free-living *Actias* larvae. Captured wasps were first anesthetized by brief chilling in a freezer; when the adults were motionless, we applied one drop of UV-bonded plastic (Bondic brand, Aurora, ON) to both the mandible and stinger. Once each drop was applied, we immediately hardened it via exposure to a UV light. This procedure rendered each wasp “non-lethal,” alive and mobile but unable to either sting or bite potential prey; their non-lethal nature was confirmed via our repeated handling of test specimens with no stings or bites. We explored whether our addition of the glue affected wasp behavior via a pilot experiment in which we visually assessed the behavior of non-lethal and lethal wasps added singly to plastic bins. When resting, the non-lethal wasps spent more time grooming their mandibles than the lethal wasps; there were no other noticeable

differences in time of flight or exploratory behavior. The wasp in each bin was checked daily and replaced with a new wasp when it died.

Bins in the harmless detritivore (“fly”) treatment each contained a single adult scavenging fly (families Caliphoridae and Sarcophagidae), of similar size as the wasps, collected from trash or reared from eggs. As with the wasps, we added UV-bonded plastic to the mouthparts and terminal abdominal segment of each fly. Each fly was handled the same way we handled the wasps. Flies were checked daily and replaced with a new one as needed.

Bins in the no-insect (“control”) treatment each received a single section of bamboo toothpick of wasp length, with one dot of UV-bonded plastic dots added to the end. Each bamboo toothpick was replaced every 2 days to simulate the level of disturbance received by the other two treatments.

After the experiment started, each bin was checked daily; food was replaced, waste removed, and treatments renewed as necessary. Larvae were weighed weekly and at either pupation or death; time (days) to either event was recorded for each larva. Each treatment was replicated 18 times for a total of 54 larvae.

2017 Experiment

To ensure that our results were robust, we repeated the experiment in 2017. The two experiments were identical except for the following differences. We collected eggs from three pairings (=broods) of different captive *Actias* females with wild males; eggs and larvae from the three different broods were held in separate containers. Brood

one larvae hatched on July 13–18, brood two larvae hatched on August 3, and brood three larvae hatched on August 8. Larvae were reared on *Juglans nigra* throughout the experiment because of greater vegetation availability, and cut foliage was kept hydrated using microcentrifuge tubes filled with agar water (3 g/L agar:water). This latter procedure kept foliage fresh while preventing floral tube leakage. Larvae reaching their 3rd instar were transferred in groups of 25 to individual 6 l bins to prevent overcrowding. Data collection for experiment #2 was the same as for experiment #1, with the exception that the brood identity of each larva was recorded. A total of 86 larvae were used in the experiment, with each treatment replicated 28–29 times. Of these larvae, 21 larvae were from brood one (n = 7/treatment), 40 from brood two (n = 13–14), and 25 from brood three (n = 7–9).

Statistical Analysis

For experiment #1, we analyzed treatment-level differences in larval outcome [died, pupated] by fitting a GLM with a binomial distribution and logit link (maximum likelihood estimation method). We used GLM with a normal distribution and identity link to assess differences in percentage weight gain at, and time to, death or pupation, analyzed separately for each outcome. All p-values were obtained using likelihood-ratio χ^2 tests.

For experiment #2, we assessed the individual effects of treatment, brood identity, and their interaction on larval outcome by fitting a GLMM with a binomial error distribution and logit link function. Because the Hessian matrix suggested quasi-

complete separation, we reran the model using biasadjusted estimates (Firth adjusted maximum likelihood). Brood identity was used as a random effect in the model, and p-values were obtained by performing likelihood-ratio χ^2 tests. A linear mixed effects modeling approach was also used to analyze weight at and time to pupation (for surviving larvae), or weight at and time to death. These variables were analyzed separately for each outcome; treatment was coded as a fixed effect and brood identity as a random effect. Chi-square and p-values were obtained as above. All analyses were conducted using JMP 9.0.0 (SAS Institute, Cary NC).

Results

2016 Experiment

More wasp-cue larvae died than in the other two treatments. Only 17% of wasp-exposed larvae pupated, vs. 50% of larvae in the fly-cue and control treatments [Figure 1A; χ^2 (2df) = 6.04, $p = 0.049$]. Of the larvae that pupated, mass at pupation and time to pupation did not differ among the three treatments (both $p > 0.20$; Table 1).

Although the treatments did not affect pupated larvae, there was a marginal between-treatment difference in the time to death of larvae dying prior to pupation (Figure 1B). Control larvae that died prior to pupation lived seven and 4 days longer than larvae in the fly- and wasp-cue treatments, respectively [χ^2 (2df) = 5.15, $p = 0.076$]. Larvae that died prior to pupation also gained similar amounts of weight prior to their death [Figure 1C; χ^2 (2df) = 4.30, $p = 0.116$].

2017 Experiment

Although more larvae in all three treatments survived to pupation (39 and 61% survival in 2016 and 2017, respectively), only the wasp-cue treatment differed from the control [Figure 1D; χ^2 (2df) = 13.30, $p = 0.001$]. As in 2016, there were no treatment-level differences in time to, or weight gain at, pupation (both $p > 0.3$; Table 1).

There were substantial treatment-level differences between larvae that died before pupation. Larval longevity was greatest in the control treatment and lower in the fly- and wasp-cue treatments [Figure 1E; χ^2 (2df) = 6.18, $p = 0.045$]; a similar pattern was seen in percentage weight gain prior to death [Figure 1F; χ^2 (2df) = 6.36, $p = 0.042$].

The three broods differed overall and in their treatment response (Supplementary Information). Overall survival was highest for brood three [88%; “brood”: χ^2 (2df) = 15.3, $p < 0.001$], while brood one responded most strongly to the treatments [“treatment*brood”: χ^2 (4df) = 12.9, $p = 0.012$]. Brood three also took longer to pupate [χ^2 (2df) = 14.8, $p < 0.001$] and gained more weight prior to pupation [χ^2 (2df) = 9.2, $p = 0.010$]; this likely reflects the greater number of brood three larvae surviving to pupation.

While we did not take any data on predator or prey behavior, the wasps and flies appeared to behave similarly in both experiments. For the first several hours following their individual addition to an *Actias*-containing plastic bin, both types of insects spent most of their time flying between perches where they sat while attempting to groom their mouthparts. Wasps appeared more agitated than flies during the grooming period and would often buzz their wings while grooming; this behavior was never observed

with flies. After this first period, both wasps and flies were predominantly found walking on the walls of the plastic bin with occasional short (5–8 cm) flights between walls. Neither type of insect appeared interested in the *Actias* larva and were only rarely observed in physical contact with it.

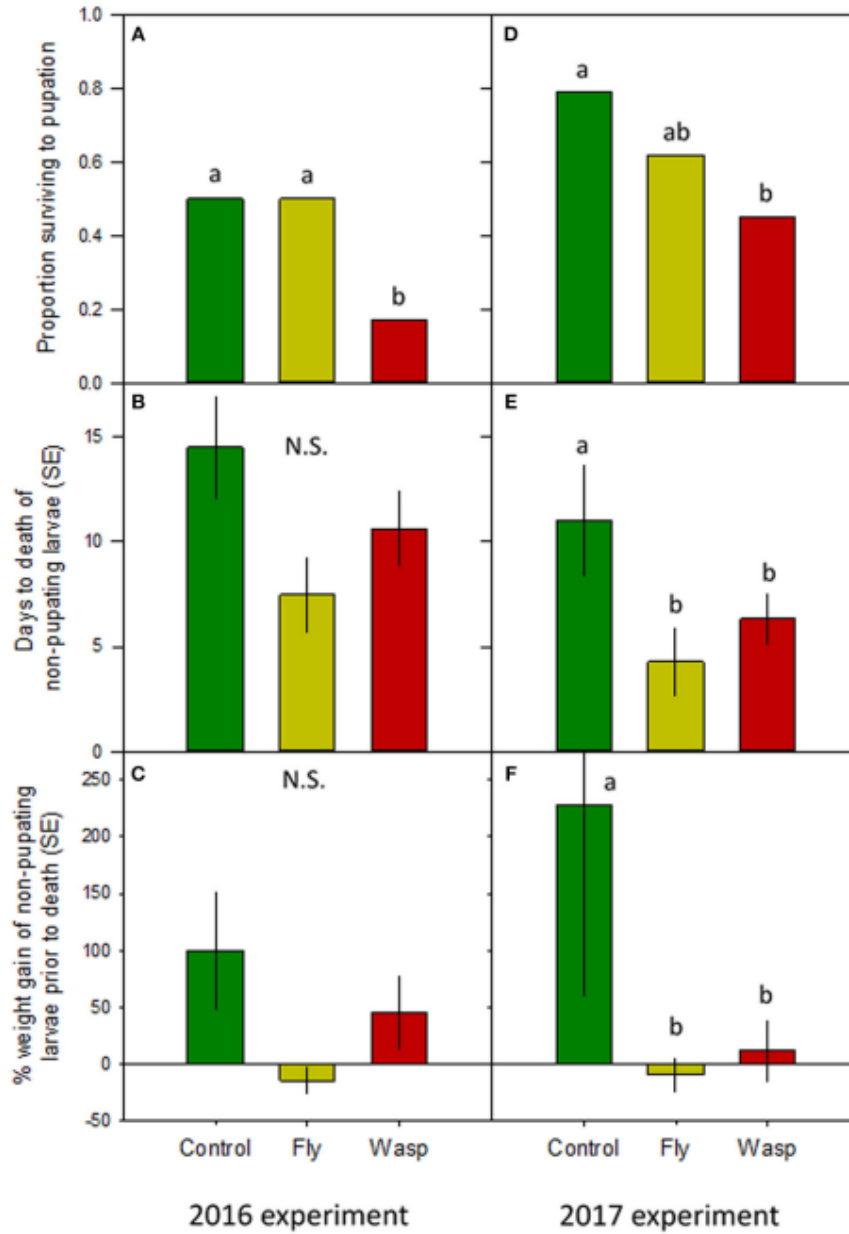


Figure 1: Impact of varying risk cues on *Actias luna* larval development. Left-hand panels: 2016 experiment (n = 18/treatment); right-hand panels: 2017 experiment (n = 28–29/treatment). (A,D): Proportion of *A. luna* larvae surviving to pupation.

(B,E): Mean \pm SE days from the start of the experiment to death of non-pupating larvae. (C,F): Percentage \pm SE weight gain from hatching to death of non-pupating larvae. Green bars: no risk cues; yellow bars: risk cues from harmless scavenging fly; red bars: risk cues from *Vespula* sp. predatory wasp. Lower-case letters indicate treatments similar at $\alpha = 0.05$ (post-hoc Tukey HSD); N.S. = no significant differences among treatments.

Experiment	Treatment	Pupal weight (g)	Days to pupation
2016	Control	2.5 + 0.12	20.7 + 0.94
	Non-lethal fly	2.4 + 0.12	19.3 + 0.94
	Non-lethal wasp	2.7 + 0.21	21.0 + 1.63
2017	Control	2.5 + 0.14	18.5 + 0.61
	Non-lethal fly	3.0 + 0.15	20.2 + 0.67
	Non-lethal wasp	2.8 + 0.21	22.1 + 0.85

There were no significant ($\alpha = 0.05$) treatment-level differences in either variable in either experiment.

Table 1: Weight at pupation (g) \pm SE and days to pupation \pm SE for larvae surviving to pupation in the 2016 (top) and 2017 (bottom) experiments.

Discussion

Our two experiments, conducted in different years with different populations, found that predation risk decreases *Actias* survival by ~55% (66 and 43% in 2016 and 2017, respectively) relative to control treatments, while exposure to a similarly-sized and -treated detritivorous fly did not. This appears to be the first direct evidence that risk alone can increase prey mortality in lepidopterans.

The impact of predator cues may reflect the heavy reliance of *Actias* larvae on camouflage for predator defense. While their spines and strong grip on twigs and branches may deter vertebrate predators (Sourakov, 2018), *Vespula* sp. wasps

dismember larger caterpillars in situ (Lichtenberg and Lichtenberg, 2003). Feeding by caterpillars greatly increases their vulnerability to wasp predation (Bernays, 1997), and *Actias* that perceive risk “freeze” in place (A. Baranowski, personal observation). Confining larvae and wasps together (the drawbacks of which are discussed below) decreases or stops feeding, as indicated by the minimal weight gain of larvae dying in the wasp treatment (Figure 1F). Exposure to foraging honeybees similarly reduces feeding, and thus plant damage, by *Spodoptera exigua* caterpillars (Tautz and Rostás, 2008). The fact that honeybees pose no threat to *S. exigua* suggests that hymenopteran buzzing, especially in combination with volatile and visual cues, may be a general risk cue for caterpillars (Tautz and Markl, 1978).

The risk-induced increase in *Actias* mortality is consistent with findings from aquatic predator-prey systems in which predator cues reduced the survivorship of both tadpoles (Hetttyey et al., 2015) and larval dragonflies (McCauley et al., 2011). In a terrestrial system, Stamp and Bowers (1991) used data on weight gain of buckmoth (*Hemileuca lucina*) caterpillars in the presence and absence of wasps to infer the risk-induced increase in caterpillar mortality. They estimated that exposure to wasps reduced survival by 20.3% via reductions in food intake that slowed growth and increased the larval period. Our study builds on theirs by providing the first directly-measured evidence that risk increases mortality in a terrestrial predator-prey system. More generally, the strong responses of multiple lepidopteran species to risk cues (Tautz and Markl, 1978; Stamp, 1997; Johnson et al., 2007) suggests that similar results may occur in a range of systems and play an important but relatively unappreciated role

in plant-herbivore-natural enemy interactions in natural and managed ecosystems.

Low mortality in the fly treatment (Figures 1A,D) can be interpreted as suggesting that *Actias* differentiate between predators and other similarly-sized but harmless flying insects. This interpretation agrees with work in both aquatic and terrestrial systems (e.g., Bass and Gerlai, 2008; Zanette et al., 2011) showing that prey can distinguish between cues from similarly-sized dangerous and harmless species. It is not, however, consistent with our data on larvae that died prior to pupation. If *Actias* perceived flies as less risky, the time to death (Figures 1B,E) and weight gain prior to death (Figures 1C,F) of fly-exposed larvae should be either similar to, or slightly less than, the control treatment. Instead, both metrics were identical to those seen in the wasp treatment: fewer larvae died in the fly treatment, but those that did appeared to respond as strongly to flies as their counterparts did to wasps. This may suggest that individual *Actias* have different “risk thresholds” that determine their reaction to cues (i.e., the shy-bold continuum; Sih et al., 2012). Larvae with high risk thresholds would err on the side of boldness and continue to forage even when a predator might be present. Conversely, larvae with lower thresholds would cease feeding even when exposed to low-risk cues such as the buzzing of a fly. Such risk thresholds would also explain the similar size and larval period of successfully-pupating larvae; individuals that did not perceive their environment as risky should have similar times to and size at pupation. If true, then our work may point more toward *Actias* larvae distinguishing between different risk levels rather than discriminating between predatory wasps and similarly-sized but harmless flies.

The brood-level differences we observed suggest the potential for ecologically-relevant intraspecific variation in risk responses (Bolnick et al., 2011). Since the broods emerged at different times, we cannot rule out the possibility that our results are explained by phenology rather than genetic differences. Our increasing awareness of the impact of maternal stress on offspring phenotypes (Sheriff et al., 2018), however, argues strongly for additional research into this topic.

The fact that risk alone is sufficient to reduce caterpillar survival suggests several important areas of future research. Perhaps the most important is the nature of the predator cue; while other researchers have found “buzzing” important in caterpillar risk assessment (Tautz and Markl, 1978; Tautz and Rostás, 2008), chemical and visual cues may well complement and enhance auditory inputs. In retrospect, it would have been useful to include a “no-contact” treatment in which the non-lethal wasps were prevented from having the opportunity to physically touch the caterpillar; this would have allowed us to determine whether direct contact between the predator and its prey contributed to the observed effect. It is also important to consider how continuously confining caterpillars and cues together in an enclosed space might have affected our results. Although many studies have used continual risk exposure to understand its effects (reviewed in Ferrari et al., 2009), the variability of risk in natural settings should affect caterpillar responses. Finally, our work highlights the need for future studies linking risk-mediated changes in foraging time (Johnson et al., 2007) and assimilation efficiency (Thaler et al., 2012) to individual survival.

References Cited

- Adamo, S. A. (2012).** The effects of the stress response on immune function in invertebrates: an evolutionary perspective on an ancient connection. *Horm. Behav.* 62, 324–330.
- Bass, S. L. S., and Gerlai, R. (2008).** Zebrafish (*Danio rerio*) responds differentially to stimulus fish: The effects of sympatric and allopatric predators and harmless fish. *Behav. Brain Res.* 186, 107–117.
- Bernays, E. A. (1997).** Feeding by lepidopteran larvae is dangerous. *Ecol. Entomol.* 22, 121–123.
- Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., et al. (2011).** Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* 26, 183–192.
- Duong, T. M., and Mccauley, S. J. (2016).** Predation risk increases immune response in a larval dragonfly (*Leucorrhinia intacta*). *Ecology* 97, 1605–1610.
- Ferrari, M. C. O., Sih, A., and Chivers, D. P. (2009).** The paradox of risk allocation: a review and prospectus. *Anim. Behav.* 78, 579–585.
- Hettyey, A., Tóth, Z., Thonhauser, K. E., Frommen, J. G., Penn, D. J., and Van Buskirk, J. (2015).** The relative importance of prey-borne and predator-borne chemical cues for inducible antipredator responses in tadpoles. *Oecologia* 179, 699–710.
- Johnson, M.-L., Armitage, S., Scholz, B. C. G., Merritt, D. J., Cribb, B. W., and Zalucki, M. P. (2007).** Predator presence moves *Helicoverpa armigera* larvae to distraction. *J. Insect Behav.* 20, 1–18.

- Lichtenberg, J. S., and Lichtenberg, D. A. (2003).** Predation of caterpillars on understory saplings in an Ozark forest. *Southeastern Nat.* 2, 423–432.
- Lichter-Marck, I. H., Wylde, M., Aaron, E., Oliver, J. C., and Singer, M. S. (2015).** The struggle for safety: effectiveness of caterpillar defenses against bird predation. *Oikos* 124, 525–533.
- Lima, S., and Dill, L. (1990).** Behavioral decisions made under the risk of predation: A review and prospectus. *Can. J. Zool.* 68, 619–640. doi: 10.1139/z90-092
- McCauley, S. J., Rowe, L., and Fortin, M.-J. (2011).** The deadly effects of “nonlethal” predators. *Ecology* 92, 2043–2048.
- Ruxton, G., Sherratt, T., and Speed, M. (2004).** *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry.* Oxford: Oxford University Press.
- Schmitz, O., Beckerman, A., and O’Brien, K. (1997).** Behaviorally-mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* 78, 1388–1399.
- Sheriff, M. J., Dantzer, B., Love, O. P., and Orrock, J. L. (2018).** Error management theory and the adaptive significance of transgenerational maternal-stress effects on offspring phenotype. *Ecol. Evol.* 2018, 1–10.
- Sheriff, M. J., and Thaler, J. S. (2014).** Ecophysiological effects of predation risk; an integration across disciplines. *Oecologia* 176, 607–611.
- Siepielski, A. M., Wang, J., and Prince, G. (2014).** Non-consumptive predator-driven mortality causes natural selection on prey. *Evolution* 68, 696–704.

- Sih, A., Cote, J., Evans, M., Fogarty, S., and Pruitt, J. (2012).** Ecological implications of behavioural syndromes. *Ecol. Lett.* 15, 278–289.
- Sourakov, A. (2018).** Size, spines and crochets: defences of luna moth caterpillars against predation by brown anoles. *J. Nat. Hist.* 52, 483–490.
- Stamp, N. E. (1997).** Behavior of harassed caterpillars and consequences for host plants. *Oikos* 79, 147–154.
- Stamp, N. E., and Bowers, M. D. (1988).** Direct and indirect effects of predatory wasps (*Polistes* sp.: Vespidae) on gregarious caterpillars (*Hemileuca lucina*: Saturniidae). *Oecologia* 75, 619–624.
- Stamp, N. E., and Bowers, M. D. (1991).** Indirect effect on survivorship of caterpillars due to presence of invertebrate predators. *Oecologia* 88, 325–330.
- Tautz, J., and Markl, H. (1978).** Caterpillars detect flying wasps by hairs sensitive to airborne vibration. *Behav. Ecol. Sociobiol.*
- Tautz, J., and Rostás, M. (2008).** Honeybee buzz attenuates plant damage by caterpillars. *Curr. Biol.* 18, R1125–R1126.
- Thaler, J., Mcart, S., and Kaplan, I. (2012).** Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12075–12080.
- Tuskes, P. M., Tuttle, J. P., and Collins, M. M. (1996).** *The Wild Silk Moths of North America: A Natural History of the Saturniidae of the United States and Canada.* Ithaca NY: Cornell University Press.

Wagner, D. L. (2005). Caterpillars of Eastern North America. Princeton NJ: Princeton University Press.

Werner, E., and Peacor, S. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84, 1083–1100.

Zanette, L. Y., White, A. F., Allen, M. C., and Clinchy, M. (2011). Perceived predation risk reduces the number of offspring songbirds produce per year. *Science* 334, 1398–1401.