Distribution, Phenology, and Notes on the Life History of Calyptra canadensis (Bethune) (Erebidae: Calpinae)

Authors: Snyder, Julia L., Powell, Gareth S., Behring, Robert S., Alford, Adam M., Mccarty, Megan E., et al.

Source: The Journal of the Lepidopterists' Society, 70(4) : 253-259

Published By: The Lepidopterists' Society

URL: https://doi.org/10.18473/lepi.70i4.a1

Journal of the Lepidopterists' Society
70(4), 2016, 253–259

DISTRIBUTION, PHENOLOGY, AND NOTES ON THE LIFE HISTORY OF *CALYPTRA CANADENSIS* (BETHUNE) (EREBIDAE: CALPINAE)

JULIA L. SNYDER, GARETH S. POWELL, ROBERT S. BEHRING, ADAM M. ALFORD, MEGAN E. MCCARTY AND JENNIFER M. ZASPEL

Department of Entomology, Purdue University, 901 West State Street, West Lafayette, IN 47907, USA
Corresponding author email: snyder65@purdue.edu

ABSTRACT. The genus *Calyptra* Ochsenheimer is known for its atypical behavior of exhibiting both obligate fruit piercing and facultative blood feeding as adults. The genus has been reported piercing a vast array of fruits including citrus, figs, grapes, and raspberries. One species, *Calyptra canadensis* (Bethune), more commonly known as the Meadow Rue Owlet moth, is the only member of the genus known to occur in the New World. The extent of this species' range, along with its adult host breadth, remains unknown. Museum specimens of *C. canadensis* from 20 institutions and private collections were examined and georeferenced to generate the most comprehensive distribution map for the species to date. Locality data was analyzed to explore the phenology of *C. canadensis*, recovering an adult activity period from May to October. Larval rearing experiments were also undertaken, documenting the presence of five larval instar stages and a development time ranging from 6 to 8 weeks. Overall this study expands what is currently known about the biology of *C. canadensis*, specifically its larval development, adult distribution, and activity period.

Additional key words: Meadow Rue Owlet, *Thalictrum*, development, rearing, collection records

Erebidae (Lepidoptera: Noctuoidea) is a diverse lineage composed of approximately 1,760 genera including around 24,600 species (van Nieukerken et al. 2011). Members of this family exhibit a vast array of feeding behaviors including lachryphagy (tear feeding), hematophagy (blood feeding), and frugivory (fruit eating) (Büttiker et al. 1996, Bänziger 2007, Zaspel et al. 2011). Within the subfamily Calpinae, obligate fruit piercing and facultative blood feeding behaviors have been documented. These feeding strategies have been observed in *Calyptra* Ochsenheimer; the vampire moth genus, both experimentally and in the laboratory (Bänziger 1982, 1986, 2007; Zaspel et al. 2007).

The genus *Calyptra* can be found on most continents, however only one species is known from the New World. This species, *Calyptra canadensis* (Bethune), commonly known as the Canadian Owlet or Meadow Rue Owlet moth, is distributed throughout northeastern and central North America, occurring in open habitats including fields, wet meadows, and woodland edges (Wagner 2005, Wagner et al. 2011). The larval host plant, Meadow Rue (*Thalictrum* spp. L.), is also known to occur in these types of habitats (Wagner 2005).

Like other members of the subfamily Calpinae (Kitching and Rawlins 1998), *C. canadensis* caterpillars, are heterochromatic, and exhibit a change in color as they progress from early to late instars. The early instars of *C. canadensis* tend to be "waxy yellow-green with or without dark subdorsal spots," present the length of the body. During later instars, the dorsal coloration changes to white; yellow and black maculations develop laterally, and the ventral surface darkens to a uniform black (Fig. 1c) (Wagner et al. 2011).

Although the general larval habits of *C. canadensis* have been previously summarized, the complete life history of the species remains largely uncharacterized. Thus, the objective of this study was to observe, document, and illustrate the life stages of *C. canadensis* and investigate the extent of its geographic range. An additional aim was to determine the degree to which flight period is linked with geographic location. This relationship has been recovered in Lepidoptera in previous studies using Satyrinae as a model (e.g., Brakefield 1987, Pollard 1991), similar methods were employed to test this potential trend. Within this study, summarization of development times for each larval instar, estimation of the number of instars required to reach pupation, and development of a specimen-level locality database of *C. canadensis* were completed. These data were used to generate a comprehensive distribution map for the species and to characterize adult phenology across its range. Here, for the first time, a detailed life history dataset for this species and a discussion of phenological patterns in the context of geographical distribution are provided.

MATERIALS AND METHODS

Rearing. Larvae of *C. canadensis* were collected from Marlborough, New Hampshire during the summer of 2015 and obtained as freshly emerged 1st and 2nd instars (Fig. 1a–b). Individual larvae were isolated in small plastic cups with damp filter paper and fed leaves of *Thalictrum dasycarpum* Fisch. and Avé-Lall. Larvae were placed in a controlled rearing room (24ºC) with a photoperiod of 16 hours. Due to a high desiccation rate within the first 24hrs, methods of rearing were modified: half of the persisting larvae remained in the rearing cups and were moved to a cooler location to prevent further desiccation. The other larvae were placed in a rearing cage on a potted *Thalictrum polygamum* Muhl. and left within the rearing room. These individuals were allowed to move freely about the plant and were not disturbed until their final instar. The change in host plant was solely due to availability of plant material.

Upon reaching the final instar, larvae were placed into a plastic rectangular container (2295 cm^3) with 5 cm of soil on the bottom and covered with a mesh lid. The soil was used in an attempt to provide the necessary resources for pupation based on the behaviors of related species and earlier observations of individuals surrounding themselves with dead leaf tissue, creating an outer casing of dry plant material. Future final instars were given ample plant material to facilitate this behavior and leaves that were not used in the pupation process were removed. Once pupation occurred, the pupae were moved to the plastic containers to give emerging moths adequate space to complete development.

Measurements and Images. To quantify larval instars, head capsules were measured periodically throughout the rearing process. Measurements were grouped based on similar widths and compared to an individual that was tracked across its complete development. Resulting head capsule widths were averaged within each group to define typical sizes for each larval instar of *C. canadensis* (Table 1). In some cases, high mortality of larvae during rearing resulted in inconsistent replicates of head capsule measurements.

High-resolution images of the larval head capsules were taken using a Leica DFC450 camera mounted onto a M165C stereomicroscope and measured using the Leica Application Suite version 4.2.0 (Leica Microsystems, USA). Larval head capsules were measured at the widest point from a dorsal perspective to investigate and document the changes in growth during development. Adult specimens were imaged with a Canon EOS Rebel T3i DSLR camera, Canon MP-E 65mm f/2.8 1-5X macro lens, controlled by Zerene Stacker automontage software.

TABLE 1. Larval head capsule measurements with estimated corresponding instar and Dyar's Law estimates calculated using an average ratio of 1.71.

Instar	Number of Individuals	Average Width (mm)	Dyar's Law
1	11	0.30	–
2	19	0.62	0.51
3	1	0.96	0.88
4	2	1.84	1.50
5	3	2.40	2.56

Adult Specimens Examined. A total of 264 adult specimens were examined, and 220 records were obtained from 20 collections; the Moth Photographers Group provided an additional 105 records. Institutions and private individuals that contributed locality data without the study of physical specimens by the authors are denoted with a "*" after the full collection name.

AMNH American Museum of Natural History (New York, NY)
ARC Albert J. Cook Arthropod Research Collection (East Lansing, MI)
CNC Canadian National Collection (Ottawa, ON, CAN)*
CUIC Cornell University Insect Collection (Ithaca, NY)*
FMNH Field Museum of Natural History (Chicago, IL)
FLMNH Florida Museum of Natural History (Gainesville, FL)
SEMC Snow Entomological Museum Collection (Lawrence, KS)*
LACM Los Angeles County Museum (Los Angeles, CA)
LAFC Les Ferge Private Collection (Middleton, WI)*
MCZ Museum of Comparative Zoology (Cambridge, MA)
MEM Mississippi Entomological Museum (Starkville, MS)*
NHM The Natural History Museum (London, UK)
NCSU North Carolina State University (Raleigh, NC)*
OSUC Charles A. Triplehorn Insect Collection (Columbus, OH)*
PERC Purdue Entomological Research Collection (West Lafayette, IN)
UCBC University of Connecticut Biodiversity Research Collections (Storrs, CT)*
UMSP University of Minnesota Collection (St. Paul, MN)
UNSM University of Nebraska State Museum (Lincoln, NE)*
USNMNH U.S. National Museum of Natural History (Washington, D.C.)
WIRC Wisconsin Insect Research Collection (Madison, WI)

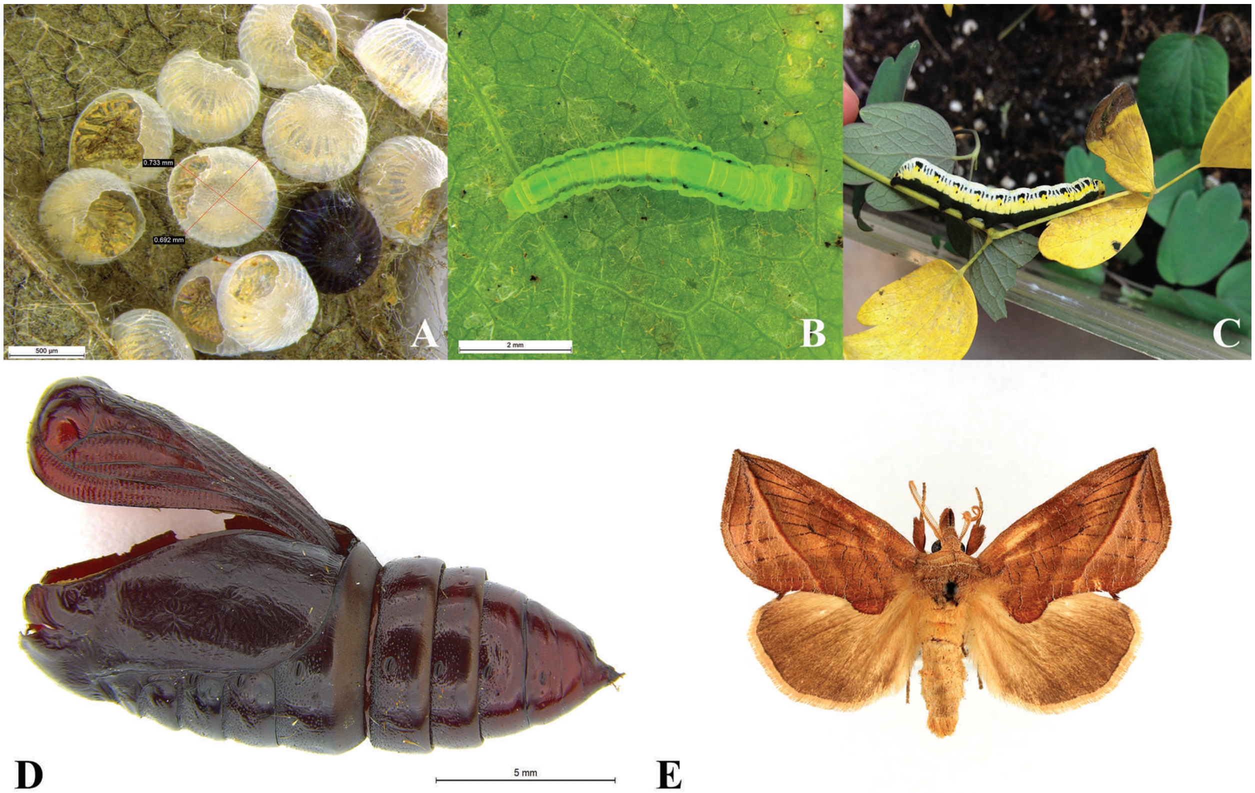

Fig. 1. *Calyptra canadensis* (Bethune) life stages **A**. eggs, **B**. 2nd instar larva, **C**. 5th instar larva, **D**. pupal case, **E**. adult moth.

Distribution Mapping. To develop a comprehensive distribution map for *C. canadensis*, locality data were transcribed and georeferenced for 474 specimens from 20 institutions, private collections, and the Moth Photographers Group. Databased records were georeferenced in Google Maps with the Lat-Long Crosshairs (Canadensys) plug-in, resulting in GPS points in decimal degrees following the methods of Wieczorek et al. (2012). The georeferenced coordinates were then mapped using QGIS v2.12.1 Lyon (Quantum GIS Development Team 2009).

Phenology. To estimate the adult activity period of *C. canadensis* museum records were aggregated representing almost 150 years of collection efforts. Specimen data were first summarized by month, and then adult frequencies were compiled. The original collection dates were transformed from the various formats on specimen labels to numeric ordinal date. This is a date format where each day of the year is numbered sequentially 1–365 (or 1–366 on leap years). The georeferenced dataset was then used to compare adult collection date with latitude of collection locality. A simple linear regression was performed in RStudio v.0.99.879 (RStudio Team 2016) to demonstrate the relationship between latitude as a predictor of adult activity period. Prior to the linear regression, ordinal dates were binned by two degrees latitude and a standard deviation (SD) was calculated for each bin. Calculating the SD of ordinal date allowed for the summarization of total variation of adult activity within its representative bin. In this manner, a larger SD corresponds to a longer adult active period than that of a smaller SD. All graphs were created with the package ggplot2 (Wickham 2009) in RStudio v.0.99.879 (RStudio Team 2016).

Results and Discussion

Larval Morphology. Throughout the rearing process, morphological changes were observed and recorded. Eggs were spherical with longitudinal ridges radiating from the micropylar region (Fig. 1a). First instar larvae were uniformly bright green and developed dark, lateral spots in the second instar (Fig. 1b). Two dark bands running the length of the body were present in the second instar but were secondarily lost in the third; lateral spots were more prominent during the third instar. The head capsule transformed from a very light, almost translucent green, to an opaque darker

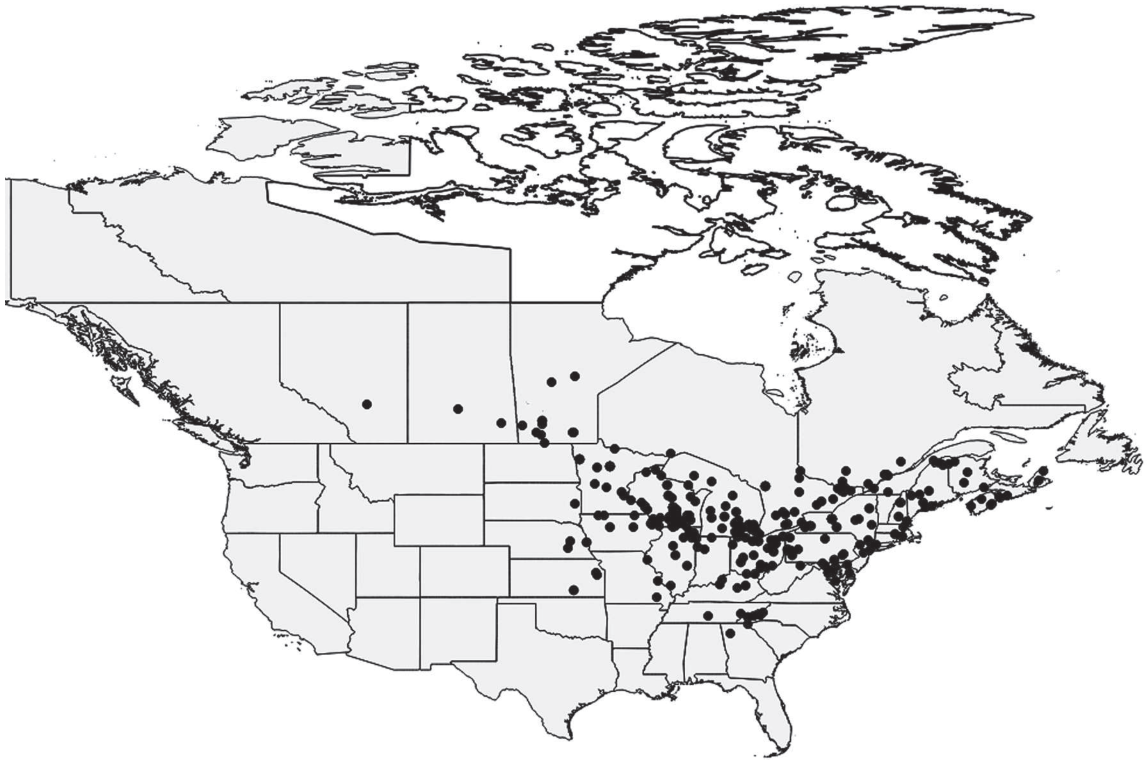

FIG. 2. Complete distribution map for *Calyptra canadensis* (Bethune), with larval host plant (*Thalictrum* spp.) range shaded in dark grey.

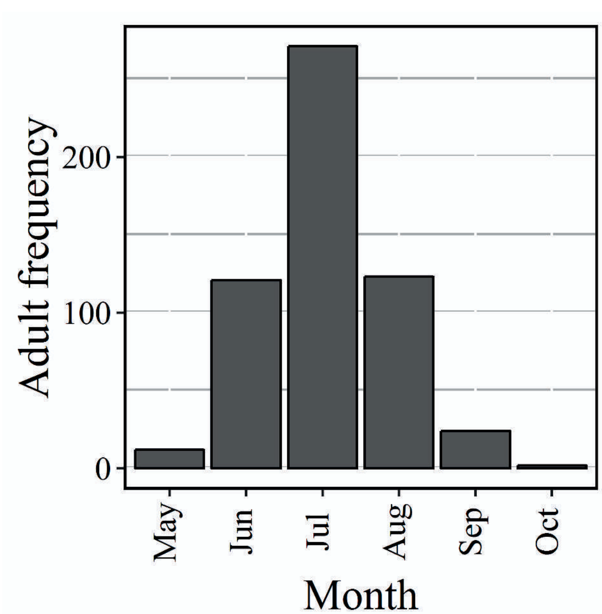

FIG. 3. *Calyptra canadensis* (Bethune) adult museum records grouped by month collected.

green, and then finally yellowing with the addition of some black maculations. During the 4th and 5th instars, the body coloration became more complex. The ventral surface darkened significantly and a clear division between that and the dorsal surface was apparent. Lateral spots remained clear on a generally uniform yellow background. The dorsum was green in coloration and developed intricate black and yellow markings that persisted the full length of the larva (Fig. 1c).

The analysis of larval instars provided evidence for a total of five instar stages for *C. canadensis*. Head capsule width increased by an average ratio of 1.71 for each successive instar. The reported head capsule widths are in general agreement with predicted widths using Dyar's method (Dyar 1890). Deviations from the predicted head capsule widths in the last two instars are likely due to a small sample size and it is unlikely an instar was missed. Development times varied under the rearing conditions, yet most individuals on the plant reached pupation at approximately 4–5 weeks. The individuals kept in rearing cups had an average development time of 5–6 weeks. Pupation in all individuals spanned an average of 2 weeks before emerging as adult moths.

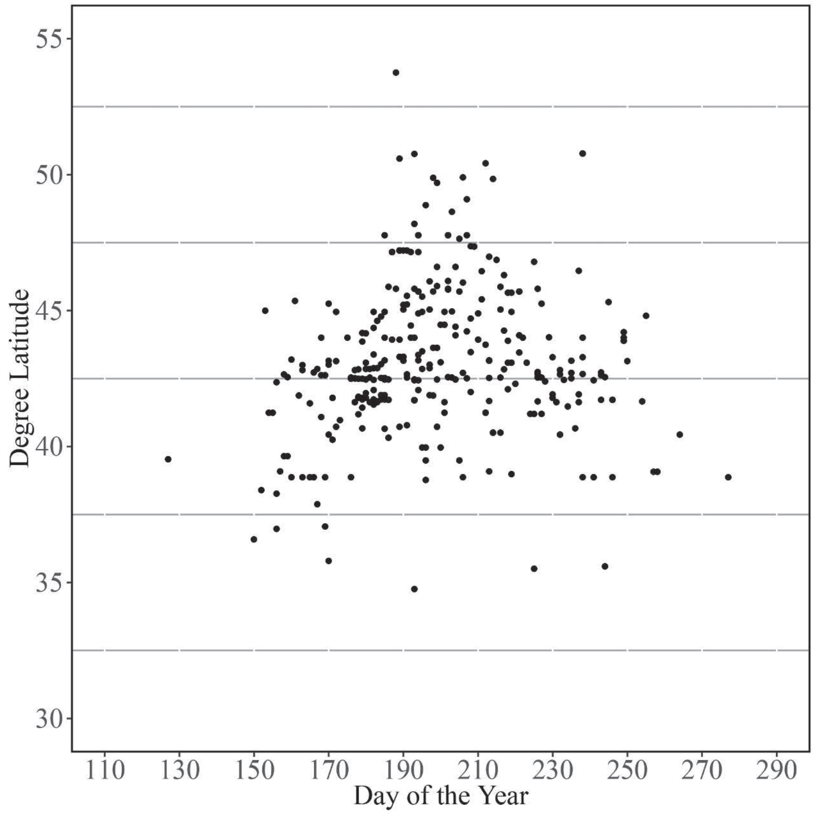

FIG. 4. Collection data for *Calyptra canadensis* (Bethune) graphed across latitude gradient of locality. Date of specimen collection transformed to ordinal numbering scheme to visualize breadth of adult activity period.

Distribution. Locality data collected as part of the study resulted in a comprehensive range map for *C. canadensis* (Fig. 2). Black points represent adult specimen records and the range for the host plant, *Thalictrum* spp., is illustrated with grey shading. The breadth of the species' range begins in the Canadian province of Alberta and extends east to the province of Nova Scotia persisting as far south as Georgia and continues west across the Midwest to Kansas. For the northern extent of *C. canadensis*' range, the distribution and habitat data largely agree with Wagner (2005) and Wagner et al. (2011). Specifically, records documenting collection events in bogs, fens, prairies, and other fragile habitats were observed across southern Canada and the northern Midwest, while specimens from the east coast and across the southern extreme of the range were more commonly collected in woodland habitats.

From the resulting dataset, complete overlap between *C. canadensis* and its host plant was observed, although the range for *Thalictrum* spp. is much broader. This discrepancy could be driven, in part by the vast diversity of the genus *Thalictrum* or unknown host limits for *C. canadensis*. The expansive distribution of the larval host, but relatively restricted range of the adult moth, suggests other factors could be influencing or driving distribution in this species. The adult feeding behaviors and host preferences of *C. canadensis* are also vastly uncharacterized, but it is likely an obligate fruit piercer like most other species within the genus. Recent laboratory experiments resulted in the first video observations of adult *C. canadensis* piercing fruit for 15 minutes (Zaspel et al. unpublished). Thus, the availability of adult fruit hosts could be another factor shaping the range of *C. canadensis*.

Phenology. Museum records were used to estimate the adult phenology of *C. canadensis* throughout its distribution. The results from this study confirm the adult activity period for *C. canadensis* is from May to October (Fig. 3). The majority of adult specimen records (49%) were recorded from July; 22% of *C. canadensis* records were recorded from both June and August, respectively. These three months account for 515 out of the 553 total records that were included. The adult activity period was then visualized using a scatterplot (Fig. 4). Our dataset shows a wider range of collection dates for *C. canadensis* associated with lower latitude with the range narrowing as latitude increases. Above the 47th parallel, the flight period of *C. canadensis* was limited to approximately 60 days; below the 40th parallel the flight period range roughly doubled. A linear regression comparing the variation of dates (y) collected across the latitude gradient (x) showed a significant trend (y = -4.65x + 46.09; $F_{1,6}$=55.46; p-value <0.001; R^2=0.8861) of decreasing variation, and therefore a reduction in adult activity period with increasing latitude. This could mean that individuals of *C. canadensis* are reproductively active for longer periods of time the further south in the range they are found. In general, our findings support the hypothesis that *C. canadensis*' activity period tends to be longer in warmer temperatures, and gives valuable information on the times of the year that it could be collected.

This study provides a comprehensive characterization of the life history and distribution of the Meadow Rue Owlet Moth, *C. canadensis*. This species completes development through five larval instar stages and takes approximately eight weeks to reach adulthood. An analysis of distribution and activity period for *C. canadensis* was documented across a latitude gradient, providing necessary temporal data that could be used in targeted collection of this species. A thorough understanding of *C. canadensis*' life history, distribution, and phenology will ensure the establishment of laboratory colonies for future physiological and behavioral experiments. Prior to this work, controlled studies on a vampire moth species were not possible. This contribution will allow researchers to unravel the molecular and environmental underpinnings of the enigmatic adult feeding behaviors in vampire moths and their fruit-piercing relatives.

Acknowledgements

The authors would like to thank Samuel Jaffe for obtaining eggs and larval material. The following individuals and institutions are graciously acknowledged for searching museum holdings to provide data vital to this study: Robert Blinn (NCSU), James Boone (FMNH), Brian Brown (LACM), Richard Brown (MEM), Anthony Cognato (MSUC), Eric Day (Virginia Tech Insect Collection, VTEC), Andy Deans (Frost Entomological Museum, PSUC), Jason Dombroskie (CUIC), Zachary Falin (SEMC), Les Ferge (LAFC), Patricia Gentili-Poole (USNMNH), Paul Goldstein (USNMNH), David Grimaldi (AMNH), Rachel Hawkins (MCZ), James Hayden (FLMNH), Laura Hlusko (CUIC), Richard Hoebeke (University of Georgia Collection of Arthropods, UGCA), Christi Jaeger (MPG), Kyle Johnson (WIRC), Owen Lonsdale (CNC), Crystal Maier (FMNH), Paul Marek (VTEC), John Morse (Clemson University Arthropod Collection, CUAC), Luciana Musetti (OSUC), Eugenio Nearns (PERC), Jane O'Donnell (UCBC), Gary Parsons (MSUC), Philip Perkins (MCZ), Brett Ratcliffe (UNSM), Hojun Song (Texas A&M University Insect Collection, TAMUIC), Terence Schiefer (MEM), Christian Schmidt (CNC), Derek Sikes (University of Alaska Museum, UAM), Kristen Simpson (The Enns Entomology Museum, UMC), Jennifer Thomas (SEMC), Robin Thomson (UMSP), and Alberto Zilli (NHM). Also we would like to thank Ian Kaplan and Michael Garvey for providing space and equipment necessary for rearing. We appreciate the assistance from Eugenio Nearns with imaging, Brigette Zacharczenko with the acquisition of specimens, and Jeffrey Holland with data analysis. The authors would like to acknowledge the Prairie Biotic Research Inc. (PBR), Agriculture Research at Purdue (ARP), and Molecular Agriculture Summer Institutes (MASI) for partially funding this study.

Literature Cited

BÄNZIGER, H. 1982. Fruit-piercing moths (Lep., Noctuidae) in Thailand: a general survey and some new perspectives. Mitt. Schweiz. Entomol. Ges. 55: 213–240.

———. 1986. Skin-piercing blood-sucking moths IV: biological studies on adults of 4 *Calyptra* species and 2 subspecies (Lep., Noctuidae). Mitt. Schweiz. Entomol. Ges. 59: 111–138.

———. 2007. Skin-piercing blood-sucking moths VI: fruit-piercing habits in *Calyptra* (Noctuidae) and notes on the feeding strategies of zoophilous and frugivorous adult Lepidoptera. Mitt. Schweiz. Entomol. Ges. 80: 271–288.

BRAKEFIELD, P. M. 1987. Geographical variability in, and temperature effects on, the phenology of *Maniola jurtina* and *Pyronia tithonus* (Lepidoptera, Satyrinae) in England and Wales. Ecol. Entomol., 12.2, 139-148.

BÜTTIKER, W., H. W. KRENN, & J. PUTTERILL. 1996. The proboscis of eye- frequenting and piercing Lepidoptera. Zoomorphology. 116: 77–83.

DYAR, H. G. 1890. The number of molts of lepidopterous larvae. Psyche 5:420–422.

KITCHING, I. J. & J. E. RAWLINS. 1998. "The Noctuoidea." Lepidoptera, Moths and Butterflies. Ed. Niels P. Kristensen. Vol. 1. Berlin: W. De Gruyter, 355–402.

VAN NIEUKERKEN, E. J., L. KAILA, I. J. KITCHINGI, N. P. KRISTENSEN, D. C. LEES, J. MINET, C. MITTER, M. MUTANEN, J. C. REGIER, T. J. SIMONSEN, N. WAHLBERG, S. YEN, R. ZAHIRI, D. ADAMSKI, J. BAIXERAS, D. BARTSCH, B. Å. BENGTSSON, J. W. BROWN, S. R. BUCHELI, D. R. DAVIS, J. DE PRINS, W. DE PRINS, M. E. EPSTEIN, P. GENTILI-POOLE, C. GIELIS, P. HÄTTENSCHWILER, A. HAUSMANN, J. D. HOLLOWAY, A. KALLIES, O. KARSHOLT, A. Y. KAWAHARA, S. (J.C.) KOSTER, M. V. KOZLOV, J. D. LAFONTAINE, G. LAMAS, J. LANDRY, S. LEE, M. NUSS, K. PARK, C. PENZ, J. ROTA, A. SCHINTLMEISTER, B. C. SCHMIDT, J. SOHN, M. A. SOLIS, G. M. TARMANN, A. D. WARREN, S. WELLER, R. V. YAKOVLEV, V. V. ZOLOTUHIN, & A. ZWICK. 2011. Order Lepidoptera Linnaeus, 1758. *In* Zhang, Z.-Q. (Ed.) Animal biodiversity: an outline of higher level classification and survey of taxonomic richness. Zootaxa. 3148: 212–221.

POLLARD, E. 1991. Changes in the flight period of the hedge brown butterfly *Pyronia tithonus* during range expansion. J. Anim. Ecol., 737-748.

QUANTUM GIS DEVELOPMENT TEAM. 2009. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. Available at: http://qgis.osgeo.org (Accessed January 2015).

RSTUDIO TEAM. 2016. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/ (Accessed February 2016).

WAGNER, D. L. 2005. Caterpillars of eastern North America: a guide to identification and natural history. Princeton University Press, Princeton, NJ., p. 370.

WAGNER, D. L., D. F. SCHWEITZER, J. BOLLING SULLIVAN & R. C. REARDON 2011. Owlet caterpillars of eastern North America, Princeton University Press, Princeton, NJ, p 80.

WICKHAM, H. 2009. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

WIECZOREK, J., D. BLOOM, H. CONSTABLE, J, FANG, M. KOO, C. SPENCER, & K. YAMAMOTO. 2012. Georeferencing quick reference guide. Available at: http://manisnet.org/docs/Georeferencing QuickReferenceGuide.pdf (Accessed January 2015).

ZASPEL, J. M., V. S. KONONENKO, & P. Z. GOLDSTEIN. 2007. Another blood feeder? Experimental feeding of a fruit-piercing moth species on human blood in the Primorye Territory of far eastern Russia (Lepidoptera: Noctuidae: Calpinae). J. Insect Behav. 20: 437–451.

ZASPEL, J. M., S. J. WELLER, & M. A. BRANHAM. 2011. A comparative survey of proboscis morphology and associated structures in fruit-piercing, tear-feeding and blood-sucking moths in the subfamily Calpinae (Lepidoptera: Noctuidae). Zoomorphology. 130: 203–225.

Submitted for publication 26 April 2016; revised and accepted 19 July 2016.