Photoperiodic induction of adult reproductive diapause in the ladybird beetle *Cycloneda munda* (Coleoptera: Coccinellidae) *JOHN J. OBRYCKI*

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**Abstract.** The developmental response and the induction and duration of adult hibernal diapause in a North American population of *Cycloneda munda* (Say) (collected at 38°N, 84.5°W) was determined by rearing individuals at four photoperiods (L:D 16:8, 14:10, 12:12, and 10:14) at 22°C. Preimaginal development of *C. munda* individuals was slower at L:D 10:14 than at three longer photoperiods. No *C. munda* females reared at L:D 16:8 entered diapause, 5% of females at L:D 14:10 were in diapause, whereas shorter photoperiods (L:D 12:12 and 10:14) induced diapause in 84% and 100% of females. *Cycloneda munda* females demonstrated a long-day response to photoperiod, similar to that observed in several other species of predaceous Coccinellidae that diapause as adults.

**INTRODUCTION**

Interactions between native and non-native species of predaceous Coccinellidae are influenced by a number of factors, including predator-prey and pathogen-host interactions (Cottrell & Shapiro-Ilan, 2008; Colvin & Yeargan, 2014; Jackson et al., 2017), intraguild interactions (Bahlai et al., 2015), phenotypic plasticity (Hodek & Michaud, 2008), and the seasonal occurrence and phenology of these species (Li et al., 2021). In a 5-yr study of adult Coccinellidae in tree habitats in central Europe Honek et al. (2015) documented that the native species *Adalia decempunctata* was the most abundant species in spring, however the non-native *Harmonia axyridis* was consistently the most abundant species in late summer. Quantification of differences in seasonal occurrence when examining communities of Coccinellidae that include native and non-native species is an important consideration for an understanding of the interactions of these species (Honek et al., 2015).

Photoperiod influences the seasonal biology, particularly the induction of adult reproductive diapause, in many insect species, including predaceous Coccinellidae (Tauber et al., 1986; Hodek, 2012a, b; Tougeron, 2019). This area of research was of particular interest for Ivo Hodek, who elucidated and synthesized a large body of research on the effects of photoperiod and its interactions with temperature and food availability on the seasonal phenology of several species of ladybird beetles (e.g., Hodek, 1967, 2012a, b). One long-standing species of interest for Ivo Hodek was the seven spotted ladybird beetle, *Coccinella septempunctata*. Starting in the early 1960s and continuing into the 21st century, he published on the factors influencing the diapause and seasonal phenology of this widely distributed predatory species (e.g., Hodek & Cerkasov, 1960; Hodek, 1962; Hodek et al., 1977; Hodek & Michaud, 2008). In this research report, part of a special issue of the European Journal of Entomology to honor the contributions of Ivo Hodek, data are presented on the photoperiodic induction of reproductive diapause in one Nearctic ladybird species, *Cycloneda munda* (Say) (Coleoptera: Coccinellidae).

The native North American species *C. munda* is typically found in field crop agroecosystems (e.g., maize, small grains and alfalfa), grasslands, and roadside habitats (Wright & DeVries, 2000; Vandenberg, 2002; Nault & Kennedy, 2003; Pilcher et al., 2005; Hesler & Kieckhefer, 2008; Diepenbrock & Finke, 2013; Diepenbrock et al., 2016). This species is frequently not the most abundant species of Coccinellidae in these habitats, but is consistently observed as a component of the ladybird beetle fauna in these habitats (summarized in Honek, 2012). For example, in a two-yr. study (2005–2006) in soybean fields in the upper Midwestern USA, *C. munda* ranged from < 1% of adult coccinellids collected on sticky cards in Wisconsin to almost 5% of adults in Minnesota and Iowa (Gardiner et al., 2009).

The objectives of this study were to quantify the developmental response and the induction and duration of adult hibernal reproductive diapause in one North American population of *C. munda* in response to one abiotic factor, photoperiod.

**MATERIALS AND METHODS**

**Effect of photoperiod on preimaginal development and diapause induction**

Methods, that have previously been used to quantify diapause induction in three species of ladybird beetles in the genus

*This paper was contributed to a virtual special issue in memory of Ivo Hodek, a long-time editor of the European Journal of Entomology, who died on June 11, 2021, shortly after his ninetieth birthday.*
22°C ± 1.0°C. F1 offspring were individually reared in glass vials 4 to 6 females and placed in L
(Harris) (Hemiptera: Aphididae). Eggs were collected daily from the same larval photoperiod, provided water, a Wheast-honey mixture, and pea aphids. Pairs were fed daily and maintained at a photoperiod of L
mixture, and a daily supply of pea aphids, 1°C, and provided water, a Wheast (GreenMethods.com)-honey mixture, and pea aphids. Pairs were fed daily and maintained at each photoperiod for 110 days, when the experiment ended.

Pairs of F1 adults were placed in 0.24 L paper containers at the four photoperiods. The diapause or non-diapause condition of each female at the four photoperiods was based on the length of the pre-oviposition period (days) as a measure of induction and duration of adult diapause and percentage of female reproductive diapause in predatory Hemipterans (Ruberson et al., 2000, 2001) and for three species of ladybird beetles in the genus Hippodamia (Obrycki, 2018, 2020; Obrycki et al., 2018). Pairs were fed daily and maintained at each photoperiod on the same larval photoperiod, provided water, a Wheast-honey mixture, and pea aphids. This type of classification has been used to categorize females into diapause and non-diapause groups in previous studies of adult reproductive diapause in predatory Hemipterans (Ruberson et al., 2000, 2001) and for three species of ladybird beetles in the genus Hippodamia (Obrycki, 2018, 2020; Obrycki et al., 2018).

### Statistical analyses

Egg, larval, pupal, and pre-imaginal (egg to adult) developmental times (days) (Mean ± SD) for Cycloneda munda were compared among the four photoperiods using one-way ANOVA (JMP Pro 15.2.0; https://www.jmp.com/en_us/software/predictive-analytics-software.html). Pre-imaginal developmental times at each photoperiod were compared using Tukey-Kramer HSD (P < 0.05) (JMP Pro 15.2.0). Days from female eclosion to first oviposition (pre-oviposition period) at each photoperiod was compared using a non-parametric log-rank analysis (JMP Pro 15.2.0). Females that died or did not oviposit within 110 days, the duration of the experiment, were censored, because the pre-oviposition period for these individuals was not measured. Relative few females died during the experiment: three at L:D 16:8 and two at 10:14 and one at each of the other two photoperiods. The censored females (died or did not oviposit) were included in the non-parametric log-rank analysis of the pre-oviposition period.

### RESULTS

Photoperiod influenced egg, larval, pupal and total pre-imaginal development of C. munda (Table 1). The observed variation in the duration of egg development at L:D 12:12 may be due to experimental error related to checking for first instar eclosion once each day. Pre-imaginal development was slower at L:D 10:14 compared to development at the three longer photoperiods (Table 1). The number of days to initiate oviposition by C. munda females at each photoperiod varied significantly (Table 2). The median pre-oviposition periods for females ranged from 9 days at L:D 16:8 to 61 days at L:D 10:14 (Table 2). The percentage of C. munda females in diapause increased at shorter photoperiods.

### Table 1. Egg, larval, pupal and pre-imaginal (egg to adult) developmental times (days) (Mean ± SD) for Cycloneda munda at four constant photoperiod conditions (L:D 16:8, 14:10, 12:12, 10:14) at 22°C. Population collected in Fayette County, Kentucky, USA. F1 laboratory reared individuals on pea aphids (Acyrthosiphon pisum) and Euphoria kuehniella eggs. One-way ANOVA for effect of photoperiod on developmental times. Tukey-Kramer HSD (P < 0.05) used to determine significant differences in development times at each photoperiod (indicated by different letters within rows).

| Species developmental period | Daylength treatment (L:D) | ONEWAY ANOVA df = 3, 163 |
|------------------------------|--------------------------|--------------------------|
| Cycloneda munda | 16:8 | 14:10 | 12:12 | 10:14 |
| Egg | N = 40 | N = 44 | N = 44 | N = 39 |
| 4.0 ± 0.0a | 4.0 ± 0.0a | 3.8 ± 0.4b | 4.0 ± 0.0a |
| Larval | 11.3 ± 0.6b | 11.5 ± 0.9b | 11.5 ± 0.7b | 13.2 ± 0.8a |
| Pupal | 5.0 ± 0.2c | 5.0 ± 0.4c | 5.3 ± 0.6b | 6.1 ± 0.6a |
| Pre-imaginal | 20.2 ± 0.7b | 20.5 ± 0.9b | 20.6 ± 0.9b | 23.3 ± 0.9a |

### Table 2. Pre-oviposition period (days) as a measure of induction and duration of adult diapause and percentage of female Cycloneda munda in diapause, Fayette County, KY, USA. Constant photoperiods: L:D 16:8, 14:10, 12:12, and 10:14 at 22°C ±1°C. Pre-oviposition period: Mean (days ± SD); Min-Max days; Median (days); N = number of ovipositing females; Nt = total number of females set up at each photoperiod; Ns = number of females ovipositing within 2X median pre-oviposition period at L:D 16:8. Non-parametric log-rank analysis within row examines response to the 4 photoperiods.

| Species | C. munda | Daylength treatment (L:D) | Log-Rank ChiSq df, P |
|---------|----------|--------------------------|----------------------|
| Mean ± SD | 9.6 ± 2.2 | 11.5 ± 4.1 | 44.0 ± 24.6 | 53.4 ± 10.0 | 52.80, df = 3 |
| Min–Max [N/Ns] | 7–15 [16/19] | 8–28 [19/20] | 10–106 [18/20] | 42–63 [9/11] | df = 3, P < 0.0001 |
| Median (days) | 9 | 11 | 51.5 | 61 | 0 |
| Ns | 16 | 18 | 3 | 0 | 100% |
| % of females in diapause | 0% | 5% | 83% | 100% | 1 |

1 The criteria for reproductive diapause in a female was 2X the median pre-oviposition period (days) observed at L:D 16:8.
DISCUSSION

Pre-imaginal developmental times in this *C. munda* population from central Kentucky, USA, were similar at L:D 16:8, 14:10, and 12:12. However, pre-imaginal developmental times in this population were longer at L:D 10:14 (Table 1). Considerable inter- and intra-specific variation in the pre-imaginal developmental response to photoperiod has been documented in ladybird beetles that overwinter in adult hibernal diapause. Faster development at shorter photoperiods has been considered an adaptation to increase the proportion of females that would successfully enter diapause and overwinter (Reznik & Vaghina, 2011). For example, pre-imaginal development of a *Harmonia axyridis* population from the Kenrova Pad Nature Reserve (Khasanskiy region, Primorskiy territory of Russia) was faster at shorter photoperiods, however, variation in this developmental response was reported in other *H. axyridis* populations (Reznik & Vaghina, 2011; Reznik et al., 2015). Several ladybird beetle species show no pattern in the relationship between photoperiod and pre-imaginal developmental times (McMullen, 1967; Storch & Vaundell, 1972; Obrycki, 2020). The slower pre-imaginal development of this *C. munda* population at L:D 10:14 requires additional study to determine the biological significance of this observation.

The photoperiodic induction of adult hibernal reproductive diapause has been documented in numerous species of predatory ladybird beetles that are characterized as long-day species, e.g., *Adalia bipunctata*, *Coccinella septempunctata*, *Harmonia axyridis* and *Hippodamia convergens* (Hodek & Cerkasov, 1960; Obrycki et al., 1983, 2018, reviewed by Hodek, 2012a; Reznik & Vaghina, 2011). In late summer and early fall, individuals likely respond to interactions among photoperiod, temperature and prey abundance leading to the possible production of an additional generation or an alternative pathway leading to diapause (Reznik & Vaghina, 2013). Subsequently, diapausing adult Coccinellidae move to overwintering habitats, which may involve long distant movements to remote sites (e.g. *Hippodamia convergens*) or more localized movements to field borders (e.g. *Coleomegilla maculata*) followed by movement from these sites in spring to initiate reproduction as prey becomes available (Hagen, 1962; Roach & Thomas, 1991; Hodek et al., 1993).

The response of this population of *C. munda* to photoperiods is characteristic of long-day species, in which females under long daylengths avert diapause and short daylengths induce diapause (Tauber et al., 1986). Additional studies are needed to determine levels of intraspecific variation in North American populations of *C. munda* in responses to temperature and photoperiod, two factors influencing its seasonal phenology (e.g. Obrycki et al., 2015). Considerable phenotypic variation in responses to these abiotic factors has been documented for *C. septempunctata* and *H. axyridis* (Hodek & Michaud, 2008; Reznik et al., 2015; Belyakova et al., 2021); two invasive non-native ladybird species interacting with *C. munda* in North America.

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REFERENCES

Bahlai C.A., Colunga-Garcia M., Gage S.H. & Landis D.A. 2015: The role of exotic ladybird beetles in the decline of native ladybeetle populations: evidence from long-term monitoring. — * Biol. Invas. 17*: 1005–1024.

Belyakova N.A., Ovchinnikov A.N., Bezman-Moseyko O.S. & Reznik S.Y.A. 2021: Comparative study of the phenotypic structure and photoperiodic responses of female Asian ladybirds *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) from Moscow, Belgorod, and Sochi. — *Entomol. Rev.* **101**: 733–742.

Colvin S.M. & Yeargan K.V. 2014: Predator fauna associated with oleander aphids on four milkweed species and the effect of those host plants on the development and fecundity of *Cy cloeneda munda* and *Harmonia axyridis*. — *J. Kans. Entomol. Soc.* **87**: 280–298.

Cottrell T.E. & Shapiro-Ilani D.J. 2008: Susceptibility of endemic and exotic North American ladybirds (Coleoptera: Coccinellidae) to endemic fungal entomopathogens. — *Eur. J. Entomol.* **105**: 455–460.

Depenbrock L.M. & Finke D.L. 2013: Refuge for native lady beetles (Coccinellidae) in perennial grassland habitats. — *Insect Conserv. Divers.* 6: 671–679.

Depenbrock L.M., Fothergill K., Tindall K.V., Lowey J.E., Smyth R.R. & Finke D.L. 2016: The influence of exotic lady beetle (Coleoptera: Coccinellidae) establishment on the species composition of the native lady beetle community in Missouri. — *Environ. Entomol.* **45**: 855–864.

Gardiner M.M., Landis D.A., Gratton C., Schmidt N., O’Neal M., Mueller E., Chacon J., Hempl G.E. & DiFonzo C.D. 2009: Landscape composition influences patterns of native and exotic lady beetle abundance. — *Diversity Distr.* **15**: 554–564.

Hagen K.S. 1962: Biology and ecology of predaeous Coccinellidae. — *Annu. Rev. Entomol.* 7: 289–326.

Heesler L.S. & Kueckhoper R.W. 2008: Status of exotic and previously common native Coccinellid (Coleoptera) in South Dakota landscapes. — *J. Kansas Entomol. Soc.* **81**: 29–49.

Hodek I. 1962: Experimental influencing of the imaginal diapause in *Coccinella septempunctata* L. (Col., Coccinellidae), 2nd part. — *Cas. Cs. Spol. Entomol.* 59: 297–313.

Hodek I. 1967: Bionomics and ecology of predaeous Coccinellidae. — *Annu. Rev. Entomol.* **12**: 79–104.

Hodek I. 2012a: Adult diapause in Coleoptera. — *Psyche* 2012: 249081, 10 pp.

Hodek I. 2012b: Diapause / Dormancy. In Hodek I., van Emden H.F. & Honek A. (eds): *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae).* Wiley-Blackwell, New York, pp 275–342.

Hodek I. & Cerkasov J. 1960: Prevention and artificial induction of the imaginal diapause in *Coccinella 7-punctata* L. — *Nature* **187**: 345.

Hodek I. & Michaud J.P. 2008: Why is *Coccinella septempunctata* so successful? (A point-of-view). — *Eur. J. Entomol.* **105**: 1–12.

Hodek I., Iperti G. & Rolley F. 2015: Two invasive non-native ladybird species interacting with *C. munda* in North America. — *Diversity Distr.* **10**: 297–313.

Hodek I., Iperti G. & Poddova M. 1993: Long-distance flights in Coccinellidae (a review). — *Eur. J. Entomol.* **90**: 403–414.
HONEK A. 2012: Distribution and habitats. In Hodek I., van Emden H.F. & Honk a. (eds): Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). Wiley-Blackwell, New York, pp. 110–140.

HONEK A., MARTINKOVA Z. & DIXON A.F.G. 2015: Detecting seasonal variation in composition of adult Coccinellidae communities. — Ecol. Entomol. 40: 543–552.

JACKSON K.A., MCCORD J.S. & WHITE J.A. 2017: A window of opportunity: Subdominant predators can use suboptimal prey. — Ecol. Evol. 7: 5269–5275.

LI H., LI B., LOVEG L., KRING T.J. & OBRYCKI J.J. 2021: Interactions among native and non-native predatory Coccinellidae influence biological control and biodiversity. — Ann. Entomol. Soc. Am. 114: 119–126.

MCMULLEN R.D. 1967: The effects of photoperiod, temperature, and food supply on rate of development and diapause in Coccinella novemnotata. — Can. Entomol. 99: 578–586.

NAULT B.A. & KENNEDY G.C. 2003: Establishment of multicolored Asian lady beetle in Eastern North Carolina: Seasonal abundance and crop exploitation within an agricultural landscape. — BioControl 48: 363–378.

OBRYCKI J.J. 2020: Comparative studies of reproductive diapause in North American populations of three Hippodamia species (Coleoptera: Coccinellidae). — Environ. Entomol. 49: 1164–1170.

OBRYCKI J.J. 2018: Reproductive diapause in North American populations of the introduced lady beetle Hippodamia variegata (Coleoptera: Coccinellidae). — Environ. Entomol. 47: 1337–1343.

OBRYCKI J.J., TAUBER M.J., TAUBER C.A. & GOLANDS B. 1983: Environmental control of the life cycle of Adalia bipunctata (Coleoptera, Coccinellidae). — Environ. Entomol. 12: 416–421.

OBRYCKI J.J., TAUBER M.J. & TAUBER C.A. 2015: Thermal requirements for development of predatory insects: Contributions of Alois Honék, including data on three Nearctic species. — Acta Soc. Zool. Bohem. 79: 105–111.

OBRYCKI J.J., MCCORD J.S., MERCER N.H. & WHITE J.A. 2018: Photoperiodic induction of adult diapause in North American populations of the lady beetle Hippodamia convergens (Coleoptera: Coccinellidae). — Environ. Entomol. 47: 1596–1600.

PILCHER C.D., RICE M.E. & OBRYCKI J.J. 2005: Impact of transgenic Bacillus thuringiensis corn and crop phenology on five nontarget arthropods. — Environ. Entomol. 34: 1302–1316.

REZNIK S.YA. & VAGHINA N.P. 2011: Photoperiodic control of development and reproduction in Harmonia axyridis (Coleoptera: Coccinellidae). — Eur. J. Entomol. 108: 385–390.

REZNIK S.YA. & VAGHINA N.P. 2013: Effects of photoperiod and diet on diapause tendency, maturation and fecundity in Harmonia axyridis (Coleoptera: Coccinellidae). — J. Appl. Entomol. 137: 452–461.

REZNIK S.YA., OCHINIKOV A.N., DOLGOVSKY M.YU. BELYAKOVA N.A. 2015: Interspecific variation in photoperiodic effect on the rate of preimaginal development in Harmonia axyridis (Pallas) (Coleoptera, Coccinellidae). — Entomol. Rev. 95: 15–27.

ROCH S.H. & THOMAS W.M. 1991: Overwintering and spring emergence of three coccinellid species in the coastal plain of South Carolina. — Environ. Entomol. 20: 540–544.

RUBERSON J.R., SHEN Y.J. & KRING T.J. 2000: Photoperiodic sensitivity and diapause in the predator Orius insidiosus (Heteroptera: Anthocoridae). — Ann. Entomol. Soc. Am. 93: 1123–1130.

RUBERSON J.R., YEARGAN K.V. & NEWTON B.L. 2001: Variation in diapause responses between geographic populations of the predator Geocoris punctipes (Heteroptera: Geocoridae). — Ann. Entomol. Soc. Am. 94: 116–122.

STORCH R.H. & VAUNDELL W.L. 1972: The effect of photoperiod on diapause induction and inhibition in Hippodamia tredecimpunctata (Coleoptera: Coccinellidae). — Can. Entomol. 104: 285–288.

TAUBER M.J., TAUBER C.A. & MASAKI S. 1986: Seasonal Adaptations of Insects. Oxford University Press, New York, 411 pp.

TOUGERON K. 2019: Diapause research in insects: historical review and recent work perspectives. — Entomol. Exp. Appl. 167: 27–36.

VANDENBERG N.J. 2002: The New World genus Cycloneda Crotch (Coleoptera: Coccinellidae: Coccinellini): historical review, new diagnosis, new generic and specific synonyms, and an improved key to North American species. — Proc. Entomol. Soc. Wash. 104: 221–236.

WRIGHT R.J. & DE VRIES T.A. 2000: Species composition and relative abundance of Coccinellidae (Coleoptera) in south central Nebraska field crops. — J. Kansas Entomol. Soc. 73: 103–111.

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