



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

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Key words. Diapause induction, adult diapause, phenology, biological control

Abstract. The developmental response and the induction and duration of adult hibernial 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 decimpunctata* 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 hibernial 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.

Table 1. Egg, larval, pupal and pre-imaginal (egg to adult) developmental times (days) (Mean \pm 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 (*Acyrtosiphon pisum*) and *Ephesia kuehniella* eggs. Oneway 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
	16:8	14:10	12:12	10:14	
<i>Cycloneda munda</i>	N = 40	N = 44	N = 44	N = 39	
Egg	4.0 \pm 0.0a	4.0 \pm 0.0a	3.8 \pm 0.4b	4.0 \pm 0.0a	F = 10.29, P < 0.0001
Larval	11.3 \pm 0.8b	11.5 \pm 0.9b	11.5 \pm 0.7b	13.2 \pm 0.8a	F = 51.04, P < 0.0001
Pupal	5.0 \pm 0.2c	5.0 \pm 0.4c	5.3 \pm 0.6b	6.1 \pm 0.6a	F = 51.07, P < 0.0001
Pre-imaginal	20.2 \pm 0.7b	20.5 \pm 0.9b	20.6 \pm 0.9b	23.3 \pm 0.9a	F = 116.84, P < 0.0001

Hippodamia (Obrycki, 2018, 2020; Obrycki et al., 2018), were employed for *C. munda*. Adult *C. munda* were collected at the University of Kentucky Spindletop Research Farm in Fayette County, Kentucky, USA (38.12°N, 84.54°W).

Individual field collected females or mating pairs were placed in 0.24 L (8 oz.) paper containers (Choice®, webstaurantstore.com), maintained at a photoperiod of L:D 16:8 (light:dark), 22 \pm 1°C, and provided water, a Wheat (GreenMethods.com)-honey mixture, and a daily supply of pea aphids, *Acyrtosiphon pisum* (Harris) (Hemiptera: Aphididae). Eggs were collected daily from 4 to 6 females and placed in L:D 16:8, 14:10, 12:12, 10:14, at 22°C \pm 1.0°C. F1 offspring were individually reared in glass vials at each photoperiod on *A. pisum* and *Ephesia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Beneficial Insectary, Redding, CA).

Pairs of F1 adults were placed in 0.24 L paper containers at the same larval photoperiod, provided water, a Wheat-honey mixture, and pea aphids. Pairs were fed daily and maintained at each photoperiod for 110 days, when the experiment ended. This time period (110 days) is based upon previous field studies of hibernant adult reproductive diapause in predaceous Coccinellidae, in which diapause ends in most females within 3 to 4 months (Obrycki et al., 1983). The date of first oviposition was recorded for each female. If a female died, the date of her death was recorded. If a male died, a male from the same L:D condition was used as a replacement. Voucher specimens of adult *C. munda* are in the University of Kentucky, Department of Entomology Insect Museum. Data are deposited at UKnowledge: <https://doi.org/10.13023/t8ya-3j59>.

The length of the pre-oviposition period (days) was recorded to determine the proportion of females at a given photoperiod that was in diapause and to quantify the duration of diapause in females at the four photoperiods. The diapause or non-diapause condition of each female at the four photoperiods was based on twice the median pre-oviposition period (days) observed at L:D 16:8. This type of classification has been used to categorize fe-

males 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) for *C. 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. Relatively 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 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 \pm 1°C. Pre-oviposition period: Mean (days \pm SD); Min-Max days; Median (days); N = number of ovipositing females; N_F = total number of females set up at each photoperiod; N_F = 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	Daylength treatment (L:D)				Log-Rank ChiSq, df, P
	16:8	14:10	12:12	10:14	
<i>C. munda</i>					
Mean \pm SD	9.6 \pm 2.2	11.5 \pm 4.1	44.0 \pm 24.6	53.4 \pm 10.0	52.80,
Min-Max [N/N _F]	7–15 [16/19]	8–28 [19/20]	10–106 [18/20]	42–63 [9/11]	df = 3,
Median (days)	9	11	51.5	61	P < 0.0001
N _F	16	18	3	0	
% of females in diapause ¹	0%	5%	83%	100%	

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

(Table 2); at L:D 12:12, 84% of females were in diapause and all females reared and maintained at L:D 10:14 entered diapause.

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 hibernial 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 Kedrovaya 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 development (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 hibernial 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.

ACKNOWLEDGEMENTS. As a graduate student, Ivo Hodek's Biology of the Coccinellidae (1973) was one of the most important references for my thesis research. I first met Ivo in 1987 and always benefited from his kind words of advice and insights into the biology of Coccinellidae. This research was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch Program under accession number 1008480 and funds from the Bobby C. Pass Research Professorship. I thank H. Li and N. Mercer for maintaining the pea aphid colony

and N. Mercer for his assistance with this study. Data are deposited at UKnowledge: <https://doi.org/10.13023/t8ya-3j59>.

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Received February 1, 2022; revised and accepted March 11, 2022
Published online March 17, 2022