CIRCADIAN RHYTHMS OF OVIPOSITION AND EMERGENCE OF THE ECTOPARASITOID *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMOLIDAE)

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Abstract

Catolaccus grandis (Burks) females oviposited 48.5-78.0% of their eggs between 0700 to 1300 h during a 24-h period. Moreover, 22.0-30.7% of their eggs were oviposited between 1300 to 1900 h. Only 3.9-20.8% of their eggs were oviposited between 1900 to 0700 h. The fecundity of parasitoid females held under the 14 h photophase regime (16.8 eggs per female per day) was significantly higher than fecundity of females held under the 10 h photophase (10.9 eggs per female per day). More female eggs were oviposited between 0700 to 1300 h (58.4-71.7%) in all photoperiods tested. During the 0700 to 1300 h period 30.0-50.0% of the adults emerged, and 32.2-70.0% of the adults emerged between 1900 to 0700 h compared with 0.0-17.8% emergence between 1300 to 1900 h.

Under constant light or dark conditions the pattern of circadian rhythms tended to disappear and the oviposition rates were constant throughout the day. The length of the photophase inversely affected developmental time of female and male parasitoids. The duration (days) of emergence by *C. grandis* was positively correlated with the length of the photophase, ranging from 2 d for the 14 h or 24 h photophase regimes to 5 d for the 10 h or 0 h photophase regimes.

Introduction

The boll weevil, *Anthonomus grandis grandis* (Boheman), is a key pest of cotton on most areas of the United States. The National Cotton Council estimates that the boll weevil costs the U.S. cotton industry $300 million annually (Suszkiw and De Quattro, 1994). One of the prevalent parasitoids attacking the boll weevil in its aboriginal home, Mexico and Central America, is the pteromalid *Catolaccus grandis* (Burks) (Chesnut and Cross, 1971). *C. grandis* is a selective ectoparasitoid; its larvae develop externally on third instar boll weevils (Johnson et al., 1973) and to a lesser extent on young pupae (Morales-Ramos and Cate, 1992).

The adult female may deposit one to five eggs in cavities of cotton squares and bolls containing boll weevil larvae. First instar parasitoids are cannibalistic; thus, only one *C. grandis* develops per host (Morales-Ramos and Cate, 1992). Boll weevil populations have been effectively suppressed following inoculative and augmentative releases of *C. grandis* (Morales-Ramos et al., 1994; Summy et al., 1992, 1994, 1995).

A laboratory rearing method to propagate *C. grandis* was developed by Cate (1987). Subsequent modifications as reported by Morales-Ramos et al. (1992) and Roberson and Harsh (1993) substantially expanded the capability for large-scale production of the parasitoid.

However, current rearing procedures for *C. grandis* are expensive and only about 1/3 of the weevil larvae exposed to *C. grandis* produce female parasitoids. Mass rearing of *C. grandis* requires the development of a mechanized, automated rearing system. This, in turn, requires substantial information on the biology, ecology, and genetics of *C. grandis* under field and laboratory conditions. Definition of the optimum parameters for growth, development, and reproduction of the parasitoid must be elucidated for mass rearing of *C. grandis* and designing equipment for mass production.

The activity of many insects is known to be governed by light (Tshernyshev, 1984). Humidity and temperatures are among the other factors of the physical environment that are known to influence the degree of activity of some insects. Nevertheless, light has been found to be the basic factor that sets the time of the activity phase in nearly every case (Edwards, 1964; Harker, 1961). Circadian rhythms of activity is a major ecological characteristic of insects (Beck, 1980; Neville, 1967; Pittendrigh, 1972; Roberts, 1965, 1974; Tshernyshev, 1984), and it is important to correctly select the optimal photoperiod for their maintenance. The time of peak activity is determined by physiological processes that trigger behavioral reactions in insects.

The objectives of this study were to determine the effects of photoperiod on (1) circadian rhythms of oviposition and emergence of *C. grandis* and (2) progeny sex ratio and reproductive potential of this parasitoid.

Materials and Methods

The *C. grandis* used in the study were derived from individuals imported from southern Mexico in 1987; the colony has been under continuous laboratory culture since then (Summy el al., 1992). The parasitoids were maintained at 26 ± 1°C, 65 ± 5% RH, and a photoperiod of 12:12 (L:D) h. Third instar boll weevils enclosed in Parafilm® capsules as described by Cate (1987) prior to exposure to the female wasps. The parasitoids were reared in transparent plexiglass cages measuring 26 cm height, 40 cm width, and 40 cm length (Morales-Ramos et al., 1992).

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Circadian rhythm of oviposition.

Groups of ten mated 10 day old female *C. grandis* were held at 5 different photoperiods: 10:14; 12:12; 14:10; 24:0; 0:24 (L:D) h. Photophase in all regimes was initiated at 0700. Females were individually placed into petri dishes (15 cm x 1.5 cm) with a circular screened (nylon) window, 5-cm diameter, on top. Each female was provided daily with water, honey, and 48 encapsulated boll weevil third instars. Six encapsulated boll weevil larvae were exposed to the parasitoids at 0700, 0900, 1100, 1300, 1500, 1700, 1900, and 2100 hour during the day. The group held at constant darkness received 3 sheets of 16 encapsulated boll weevils a day at 0700, 1300, and 1900 h. The number of eggs oviposited by each female during each period of exposure of boll weevils to the parasitoids was recorded. Parasitoids were then held at the conditions described above to complete development to the yellow abdomen pupal stage as defined by Morales-Ramos and Cate (1992). The number and weight of female pupae were recorded for each of the periods of exposure on the 5 photoperiodic conditions tested.

Influence of photoperiods on the legth of the preovipositional period was studied in 5 groups of 10 newly emergence females of *C. grandis*. Each group was held at one of 5 different photoperiods as described above. Females were individually placed into petri dishes. Each female was provided with 15 encapsulated boll weevil larvae every day. The encapsulated weevils were inspected daily until the females started to oviposit.

Circadian rhythm of emergence.

Third instar boll weevils (N=792) encapsulated in Parafilm® were exposed to a colony of *C. grandis* females (400, ten-day-old females per cage) for 6 h. The parasitized encapsulated weevils were divided into 5 groups (not less than 100 parasitized larvae per group). Then they were held at 5 different photoperiods as described above, at 26±1°C, 65±5% RH until the parasitoids completed development. The developmental time of males and females from egg to adult and the number of individuals emerging daily at 0700, 1300, and 1900 h were recorded for all photoperiods. The median (Me) of emergence distribution was calculated as described by Schefler (1980).

Analysis of variance (ANOVA) and Tukey's Studentized Range Test (SAS Institute, 1988) were used to analyze the data.

Results and Discussion

The circadian rhythm of oviposition showed a characteristic pattern in all photoperiods tested, but this pattern was expressed only slightly under constant light or dark. During the period between 0700 to 1300 h, females of *C. grandis* oviposited the highest proportion of eggs from the total oviposited during the day, 48.5 ± 2.9 to 78.0 ± 4.4% compared with 22.0 ± 4.4 to 30.7 ± 0.6% during the course of 1300 to 1900 h and 3.9 ± 0.6 to 20.8 ± 2.5% over the period of 1900 to 0700 h. Parasitoid females oviposited only during the photophase, but under constant dark and constant light conditions they oviposited throughout the 24 hours (Fig. 1).

C. grandis females held at the long photophase period (14:10 [L:D] h) showed significantly higher fecundity than females held at the short photophase period (10:14 [L:D] h), 16.8 ± 4.2 compared with 10.9 ± 2.0 eggs per female per day, respectively ($F = 4.9$; $df = 2, 12$; $P = 0.03$). Females held at constant light or dark regimes did not significantly differ in fecundity, 13.7 ± 1.7 compared with 10.2 ± 1.6 eggs per female per day ($F = 0.02$; $df = 1, 192$; $P = 0.9$). (Fig 2.) The length of photophase did not affect of the length of the preovipositional period of *C. grandis* females (from 3.4 ± 0.5 to 4.0 ± 0.7 days [$F = 0.6$; $df = 4, 20$; $P = 0.6$]) (Fig. 2).

The parasitoid females oviposited more fertilized eggs (females) during the time interval of 0700 to 1300 h in all photoperiods tested, from 58.4 to 71.7% compared with 18.4 - 56.3% female progeny during the time period between 1300 to 1900 h. The percentage of female progeny was not significantly different at any particular period of time during the day when they were held 24 hours in constant light or dark conditions (Table 1).

Pupal weight is an important predictor of progeny quality of *C. grandis* ; it is positively correlated with pupal survival, fecundity, reproductive potential, and intrinsic rate of increase (Greenberg et al., 1994). The authors provisionally divided the female pupae into 5 weight classes, as follows: 4 mg or less, 4.1-5.0 mg, 5.1-6.0 mg, 6.1-7.0 mg and >7 mg.In these experiments eggs oviposited during the period of time between 0700 to 1300 h produced females with a pupal weight ranging from 6.2 to 6.9 mg in all photoperiods tested. The pupal weight of females developing from eggs oviposited during the time period between 1300 to 1900-2100 h was 6.0 mg or less. Eggs oviposited under constant light or dark conditions produced females with a pupal weight ranging from 5.1 to 6.0 mg.

The rhythm of emergence of parasitoid progeny was affected by the photoperiod at which they were held during their development. When parasitoids developed in a long photophase day (14 h light), the average of emergence was 53.2 ± 4.4% during 0700 - 1300 h; 13.7 ± 3.9% - during the interval between 1300 to 1900 h; and 33.1 ± 2.1% during the interval between 1900 to 0700 h. The average of emergence was 45.1 ± 4.5, 15.3 ± 3.2, and 39.6 ± 2.0%, respectively, during the same periods of time when parasitoids developed in a photoperiod of 12 : 12 (L : D) h. When parasitoids developed in a short-photophase day (10 h light), the average of emergence was 45.9 ± 4.2, 13.8 ± 2.1, and 40.3 ± 3.6%, respectively. The circadian rhythm of emergence disappeared when *C. grandis* developed under constant light or dark conditions. When the

parasitoid developed under constant light regime, 32.4 ± 5.9% individuals emerged during 0700 to 1300 h, 39.1 ± 3.8% from 1300 to 1900, and 28.5 ± 4.8% from 1900 to 0700h. When parasitoids developed under constant dark regime, emergence of individuals was 29.1 ± 6.2, 34.2 ± 5.2, and 36.7 ± 1.1%, respectively, during the same intervals (Table 2).

The length of photophase inversely affected developmental time of female and male parasitoids. When *C. grandis* developed at photoperiods 14:10 and 24:0 (L:D) h, the females completed development in 11.9 and 11.4 d and males in 10.4 and 10.2 d, respectively. Developmental times were 14.9 and 15.1 d for females and 13.9 and 14.6 d for males at photoperiods 10:14 and 0:24 (L:D) h, respectively. These observations are consistent with the report by Morales-Ramos et al. (in press) that photoperiod significantly affects developmental time in *C. grandis*.

The length of distribution of emergence of *C. grandis* in days was positively correlated with the length of photophase and ranged from 2 d (14:10 or 24:0 [L:D] h) to 5 d (10:14 or 0:24 h). Long-light day resulted in a compact distribution of emergence. The median of emergence was 46.5 and 45.5% on the 1st day and 53.5 and 54.0% on the 2nd day when *C. grandis* developed at 14 and 24 h photophase, respectively. Short photophase resulted in a looser distribution of emergence. Medians were 28.5 and 26.0% on the 1st day and 40.0 and 42.0% on the 2nd day at 10 and 0 h photophase, repsectively (Fig. 3).

The circadian rhythms of oviposition and emergence, and photo- and gravity-tropisms of *Trichogramma* spp. have been reported in Afonina et al., 1985, 1986; Azad Sing Dakhiya et al., 1991; and Greenberg, 1991. The highest oviposition of *Trichogramma evanescens* Westw. occurred during the afternoon and lasted until the evening. The oviposition stopped during the night and again increased on the next day. The emergence of the parasitoid started at 0600 h. The maximum emergence of *T. evanescens* occurred from 0800 to 1000 h. Abrupt reduction of emergence after 1400 h was reported. The emergence stopped in the interval from 2200 to 0600 h. Hawkes (1972) in the cabbage fly (*Erioischia brassicae* Bouche = *Delia brassicae* Bouche) and Campan, 1968 (in Tshernyshev 1984), in the flower flies (*Syrphidae* spp.), observed that the maximum oviposition occurred at the end of day. Sharp drops in duration of daylight also stimulated deposition of eggs by Pyralidae at dusk (Tshernyshev 1984).

Drosophila spp. is the classic example of the influence of photophase on synchronization of emergence (Pittendrigh 1967). Butterflies emerged during the morning (Tshernyshev 1984), but most of the moths emerged during the evening (Banerjee & Decker 1966).

It is possible that the rhythms of parasitization and emergence of *C. grandis* are endogenous because they were maintained during constant conditions of light and temperature and could not be induced by direct influence of any non-control factors. Moreover, the endogenic rhythm is more typical for the insects of tropical or subtropical origin (Tshernyshev 1984). *C. grandis* is a tropical species (Cross and Mitchell, 1969). Investigation of the circadian rhythms of oviposition and emergence of parasitoid provides knowledge for impoving the mass rearing processes for *C. grandis*.

Summary

C. grandis females oviposited the highest number of eggs between 0700 - 1300 h. The fecundity of females held in long day photoperiods (14 or 24 h of light) was significantly higher than fecundity of females held in short day photoperiods (10 or 0 h of light). Photoperiod did not significantly affect the preovipositional period. Parasitoids oviposited more females eggs during 0700 to 1300 h than at any other time during the day. Most of *C. grandis* emerged during 0700 -1300 and 1900 -700 h. Circadian rhythms were especially evident in the fluctuating light-dark regimes. The length of the photophase affected the developmental time and the length of the distribution of emergence of *C. grandis* in days.

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Table 1. Influence of different photoperiods on the sex of *Catolaccus grandis* progeny.

Time of oviposition, hours	Percentage female progeny in photoperiods (L:D),h		
	14:10[a]	12:12[a]	10:14[a]
0700-0900	71.7a	64.0a	58.4ab
0900-1100	69.6a	70.0a	65.7b
1100-1300	64.0a	67.7a	69.8b
1300-1500	55.6b	31.0b	41.5c
1500-1700	56.3b	25.7b	48.2ac
1700-1900	50.0b	18.4b	42.7c
1900-2100	29.0c	0.0	0.0
2100-0700	0.0	0.0	0.0

Table 1 (continued)

Time of oviposition, hours	Percentage female progeny in photoperiods (L;D), h	
	24:0[a]	0:24[a]
07-1300	57.5a	60.8a
1300-1900	50.4a	69.6a
1900-0700	44.9a	59.1a

[a]Means followed by the same letter within column are not significantly different (P>0.05, Tukey's studentized range HSD test [SAS Institute1988])

Table 2. Influence of different photoperiods on the circadian rhythm of emergence and developmental time of *C. grandis*.

L : D, h	Percentage of emergence in time during 24 h			Developmental time, d	
	0700-1300	1300-1900	1900-0700	Male	Female
14:10	53.0	13.7	33.1	10.4	11.9
12:12	45.1	15.3	39.7	11.2	13.9
10:14	45.9	13.8	40.3	13.9	14.9
24:0	32.4	39.1	28.6	10.2	11.4
0:24	29.1	34.2	36.7	14.6	15.1

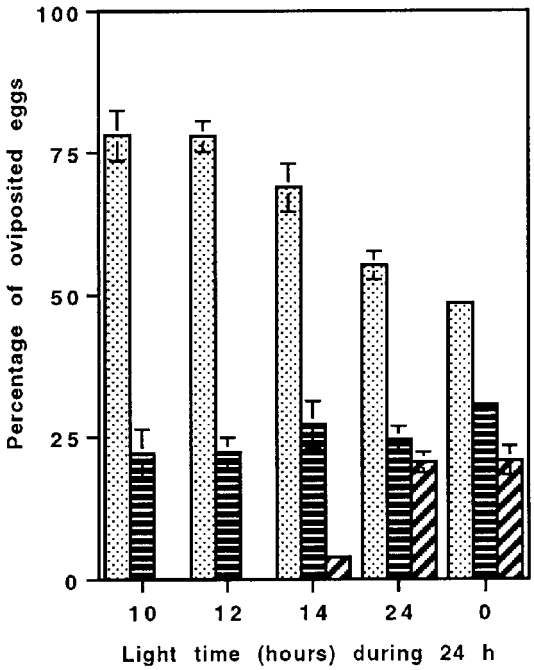

Fig. 1. Circadian rhythm of parasitization by *Catolaccus grandis* females

during 700 to 1300

during 1300 to 1900

during 1900 to 700

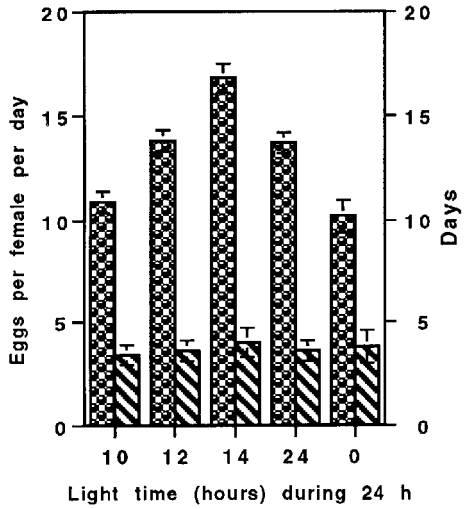

Fig. 2. Influence of different photoperiods on fecundity and length of the preovipositional period

fecundity, eggs per female per day

preovipositional period

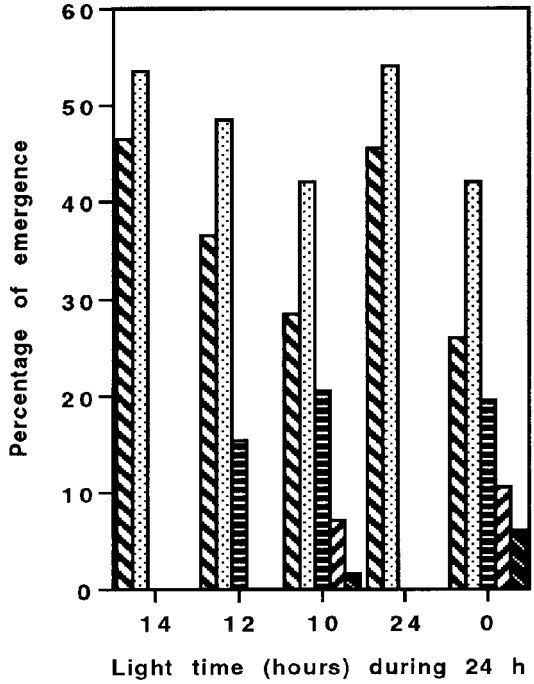

Fig. 3. Effect of different photoperiods on emergence *Catolaccus grandis* (Me)

on the first day

on the second day

on the third day

on the fourth day

on the fifth day