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CALLING BEHAVIOR OF TWO DIFFERENT FIELD POPULATIONS OF *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE): EFFECT OF AGE AND PHOTOPERIOD

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Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae), is an oligophagous leafminer of Rutaceae, especially of *Citrus* spp. (Jacas et al. 1997), occurring in all citrus-growing areas worldwide. Pheromone traps have been used successfully in Japan (Ando et al. 1985, Ujiye 1990) but unsuccessfully in China (Tongyuan et al. 1989), Italy (Ortu 1996), Spain and Florida (pers. obs.), and Turkey (Uygun, N., Univ. Çukurova, Adana, Turkey, pers. comm.). Negative results could be attributed to the use of a non-specific attractant, and to a poor understanding of the behavior of the pest. The objective of this study was to determine patterns of calling of two populations of *P. citrella* when exposed to three different photoperiods as a first step toward determination of a specific sex pheromone.

A first set of experiments was carried out during June-July 1998 at the Institut Valencià d'Investigacions Agràries (IVIA) Spain (0.4°W long., 39.6°N lat., 33 m alt). A second set was performed at the Tropical Research and Education Center (TREC) Florida (80.2°W long., 25.3°N lat., 1 m alt.), during October-November 1998. Pupae of *P. citrella* were gathered from citrus orchards. Female pupae (Garrido & Jacas 1996) were placed in 12 cm-diameter petri dishes containing 2% agar and filter paper and held in a cabinet at 25 ± 1°C, under a photoperiod matching local one at that moment. Emerging adults were transferred daily to analogous petri dishes (max. 6 moths per dish) and fed droplets of honey plus pollen. These were placed in a cabinet at 25 ± 1°C, and exposed to one of the three photoperiods: L12:D12, L16:D8, and L8:D16. At 60 min intervals during scotophase, and using a portable red light, moth activity (quiescence, locomotion, or calling) was observed in the cabinet and recorded. Calling females opened and vibrated their wings and rhythmically protruded and retracted the ovipositor. Observations were classified per scotophase hour and sorted by photoperiod, age, and geographical/seasonal origin. Age was expressed as the scotophase number (1 to 7), starting with the first complete scotophase after emergence. The onset of calling was determined as the time (hours after lights off) halfway between the first time calling was observed and the previous observation. Differences in percentages of female calling were subjected both to G-test (Sokal & Rohlf 1969) and two-way ANOVA for unbalanced designs (SAS Institute 1985). Onset calling times were also analyzed using a two-way ANOVA. About 10,000 observations of moth activity were recorded.

Table 1 shows the influence of age and photoperiod on the percentage of calling females. Calling patterns for moths subjected to the same photoperiod regime at the two locations were significantly different ($G = 24.71$; df = 6; $P < 0.005$). Nevertheless, the age pattern exhibited by females exposed to L16:D8 at IVIA did not significantly differ from that exhibited by those exposed to L8:D16 at TREC ($G = 7.59$; d.f. = 6; $P = 0.2245$). Photoperiod ($F = 15.56$; df = 2, 38; $P < 0.0001$) and age ($F = 2.67$; df = 6, 38; $P = 0.0352$) affected the proportion of females calling, whereas the geographical/seasonal origin did not ($F = 0.70$; df = 1, 38; $P = 0.4102$). Calling activity of females exposed to L12:D12 photoperiod was the highest, especially for age 1 females. Moths aged 4 to 7 called more than those aged 2 and 3, with 1-day old females being the less active ones. Females exposed to a photoperiod opposite to the one occurring when they were collected in the field barely responded during the whole experimental period.

Mean onset calling times (MOCT) are shown in Figure 1. Both geographical/seasonal origin ($F = 781.26$; df = 1, 374; $P < 0.0001$) and photoperiod ($F = 31.06$; df = 2, 374; $P < 0.0001$) affected the MOCT, but age did not ($F = 1.35$; df = 6, 374; $P = 0.2337$). Moths studied during the first set of assays called significantly earlier than those tested during the second set, and, similarly, the longer the photophase experienced by a moth, the sooner it called. The MOCT for females collected at IVIA was 8.62 ± 1.29 h (N = 130; mean ± SE) when exposed to L12:D12 and 8.11 ± 0.93 h (N = 90) when exposed to L16:D8, whereas for those collected at TREC, it was 11.67 ± 0.58 h (N = 71) when exposed to L8:D16, and 10.65 ± 0.61 h (N = 84) when exposed to L12:D12. Calling occurred mostly toward the end of the scotophase. Non-calling females (8L:16D for the experiments at IVIA and 16L:8D at TREC) were usually very active toward the end of the scotophase.

Phyllocnistis citrella has been considered a crepuscular and dawn-active moth. Our results

TABLE 1. INFLUENCE OF AGE (NUMBER OF SCOTOPHASES EXPERIENCED BY THE INSECTS: 1-7) ON THE PERCENTAGE OF CALLING VIRGIN FEMALES OF *P. CITRELLA* MEASURED AT 1-H INTERVALS AFTER LIGHTS OFF (1-16) UNDER 3 DIFFERENT PHOTOPERIODS (16L:8D; 12L:12D; 8L:16D). THE FIRST SET OF ASSAYS WAS CARRIED OUT AT IVIA AND THE SECOND ONE WAS AT TREC. INITIAL NUMBER OF FEMALES WAS 30.

	Site 1: IVIA																				
	16L:8D							12L:12D							8L:16D						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1																					
2																					
3																					
4																					
5		2																			
6								6	2		22										
7		3						9	15	7		10									
8	4		9	11	10	11	13	16	36	10		10									
9	6	30	42	54	69	63	66	21	33	33	49	52	25								
10								47	44	84	68	75	100	60							
11								21	48	48	52	75	75	60							
12								6	5	26	11	43	19	33							
13																					
14																					
15																					
16																8	10				

	Site 2: TREC																				
	16L:8D							12L:12D							8L:16D						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1																					
2																					
3																					
4																					
5																					
6																					
7																					
8		1		8			13														
9		11	2	3	6									14							
10		1	2						13	11		29									
11								18	53	56	53	60	50	57	4	31	17	20		40	
12								45	86	67	100	57	75	71	9	43	53	33	67	60	33
13															9	40	59	56	67	70	17
14																24	36	42	67	50	50
15																29	13	5	33		17
16																17					

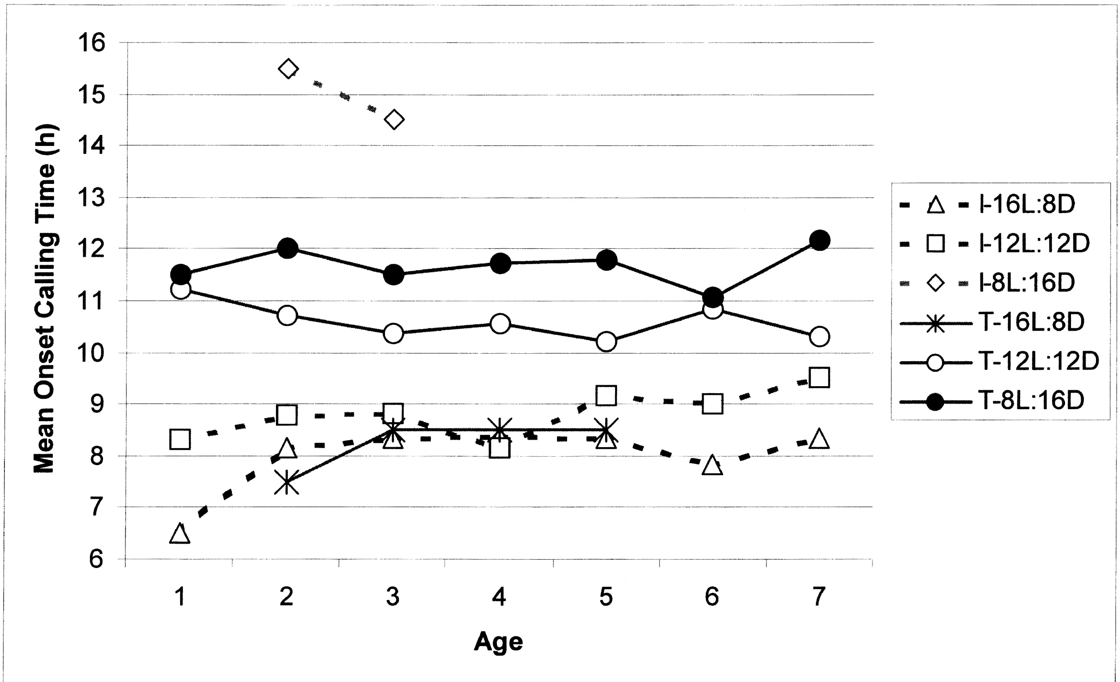

Fig. 1. Influence of age (1-7) on the onset of calling (in h since start of scotophase) of virgin females of *P. citrella* under three different photoperiods (16l:8d; 12l:12d; 8l:16d). The first set of assays was carried out at ivia (i-) and the second one was at TREC (T-). initial number of females was 30.

show a marked dawn sexual activity. Virgin females modified their calling patterns in response to photoperiod with some limitations. Females could not adapt to the photoperiods completely opposite to the ones they had experienced in the field. Furthermore, for a given location, the MOCT under different photoperiods extraordinarily matched current natural scotophases (9 h at IVIA and 14.5 h at TREC). Therefore, it is presumed that differences observed at the two locations were due to seasonal changes rather than to geographical differences between populations. The ability of virgin females of many Lepidoptera to modify the periodicity of their calling in response to different environmental changes, including day length (Haynes & Birch 1984), has been interpreted as an adaptation to reduce the impact of climatic fluctuations on mating success (Delisle & McNeil 1987).

Onset calling time was not affected by moth age, although age increased the proportion of females calling (1 < 2 = 3 < 4 = 5 = 6 = 7 days). At a given temperature, virgin females of numerous crepuscular and nocturnal species of Lepidoptera advance the onset time of calling on successive nights (Delisle 1992). This has been interpreted as an adaptation increasing the probability of older females to attract a mate before younger moths initiate their calling (Swier et al. 1977).

Age and photoperiod are two of the many factors affecting the occurrence of calling behavior of female insects (Howse et al. 1998). Further laboratory experimentation and field testing should take our results into account.

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Summary

The effect of age and photoperiod on the calling behavior of *Phyllocnistis citrella* was studied. *P. citrella* is sexually active at dawn. Activation is presumably caused by the cumulative time elapsed since sunset. Onset calling time was not affected by the age of moths, but the proportion of females calling increased with age.

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