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CHANGES IN PHEROMONE TITER OF OBLIQUE-BANDED LEAFROLLER, *Choristoneura rosaceana*, VIRGIN FEMALES AS A FUNCTION OF TIME OF DAY, AGE, AND TEMPERATURE

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Abstract—Under a 16:8 hr light–dark photoperiod and 20°C constant temperature, the titers of (*Z*)-11-tetradecenyl acetate (*Z*11–14:Ac), (*E*)-11-tetradecenyl acetate (*E*11-14:Ac) and (*Z*)-11-tetradecenol (*Z*11–14:OH) produced by different-aged *Choristoneura rosaceana* virgin females varied significantly during the scotophase, with the maximum titer occurring before the onset of calling in day-0 and day-3 females, while in day-5 females the titer remained constant throughout the calling period. There was a significant decrease in the titer of all pheromone components with age, explaining the lesser attractiveness of day-5 females relative to day-0 and day-3 females observed in the field. Under a cold thermocycle simulating condition during the second flight period in the fall, the titers of all pheromone components did not vary with time of day. There was a significant decrease in the amount of *Z*11–14:Ac with age but no changes occurred in the minor components. Furthermore, for any given age tested, the amount of each component produced during the period of maximal calling activity remained relatively similar at the two temperature regimes. However, as with the expression of calling behavior, pheromone production was initiated earlier at cooler than at warmer temperatures. At both temperature regimes, female age and time of day influenced the ratio of each pheromone component. These results are discussed in relation to the hypothesis that by calling earlier, less attractive older females may increase their probability of mating.

Key Words—Pheromone titer, (*Z*)- and (*E*)-11-tetradecenyl acetate, (*Z*)-11-tetradecenol, age, time of day, constant and fluctuating temperature,

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Choristoneura rosaceana, oblique-banded leafroller, Lepidoptera, Tortricidae.

INTRODUCTION

It has been suggested that the advance in the onset time of calling as females age is an adaptation that permits older individuals in several species of Lepidoptera to increase their probability of attracting mates before younger females start calling (Swier et al., 1977; Turgeon and McNeil, 1982; Webster and Cardé, 1982). Similarly, the earlier onset of calling in response to decreasing ambient temperatures has been interpreted as an adaptation permitting females of all ages to attract mates before temperatures fall below a level that inhibits male flight activity (Cardé et al., 1975; Webster and Cardé, 1982; Turgeon and McNeil, 1983; Delisle and McNeil, 1987a, b).

Delisle (1992a) reported such age- and temperature-related changes in the onset time of calling of the oblique-banded leafroller (OBL), *Choristoneura rosaceana* (Harris), a bivoltine species in eastern Canada (Delisle, 1992b). However, whether or not these behavioral changes afford any reproductive advantage to older OBL females was further examined by comparing the relative attractiveness of different-aged (0-, 3-, and 5-day-old) females during both flight periods (Delisle, 1992a). Day-5 females attracted significantly fewer males than day-0 and day-3 individuals throughout the season, suggesting that older females may release less pheromone than younger ones. However, while the relative attractiveness of day-0 to day-3 females remained the same in both flights, day-5 females were comparatively more attractive in the fall than in the summer. Delisle (1992a) suggested that these results may support the hypothesis that by calling earlier, older females avoid competition with younger females and are particularly advantaged in the fall, when low temperatures limit male flight. However, another explanation for the lower degree of competitiveness of younger females in the fall is that temperature affected their pheromone production more than that of older individuals.

In order to verify if pheromone titer in OBL was influenced by age, and if there was a differential effect of both time and temperature on pheromone synthesis by different-aged females, we undertook a series of experiments to determine the periodicity of pheromone production of 0-, 3-, and 5-day-old females at 20°C constant temperature under a 16:8 hr light–dark photoperiod. The experiment was also repeated under a cold thermocycle that simulated temperature conditions prevailing during the fall flight.

METHODS AND MATERIALS

Moths. All females used in these experiments were obtained from an annually restocked laboratory colony, established using field-collected pupae at Quebec City, and maintained at 20 ± 0.5°C, 65± 1% relative humidity, under

a 16:8 hr light–dark photoperiod. Larvae were reared on a pinto bean artificial diet (Shorey and Hale, 1965). Upon pupation, individuals were sexed and only females retained. At emergence, females were kept individually in 150 cm^3 plastic vials with an 8% sucrose solution and held under standard colony conditions until needed.

Gland Extraction and Pheromone Quantification. The titers of the major component, (*Z*)-11-tetradecenyl acetate (*Z*11–14:Ac), as well as two minor components, (*E*)-11-tetradecenyl acetate (*E*11–14:Ac) and (*Z*)-11-tetradecenol (*Z*11–14:OH) (Hill and Roelofs, 1979) were determined. Quebec populations, like those of New York (Hill and Roelofs, 1979), do not produce (*Z*)-11-tetradecenal (*Z*11–14:Ald), a component isolated from OBL females in the Okanagan Valley, British Columbia (Vakenti et al., 1988). Prior to detailed quantification, the efficiency of pheromone extraction was determined by comparing the amount of *Z*11–14:Ac extracted from glands excised 2 hr after the onset of scotophase from day-1 females. Individual glands were soaked in 20 μl of hexane, containing 1.5 ng of dodecanoic acid methyl ester ($C_{13}H_{26}O_2$) as an internal standard, for 0.5, 1, 5, 10, 15, 20, 25, and 30 min. The titer of *Z*11–14:Ac did not vary significantly when extraction times exceeded 20 min. However, when glands were soaked >20 min, extraneous compounds were observed, so 20 min was chosen as the period of extraction.

To assess the effect of age, time of day, and temperature on the OBL pheromone titer, individual glands of 0-, 3-, and 5-day-old virgin females, held at 20°C constant temperature, were excised at −1, 0, 1, 2, and 3 hr, with 0 hr being the onset of the scotophase. As previously reported by Delisle (1992a), the mean onset time of calling (MOTC) of 0-, 3-, and 5-day-old females at 20°C constant temperature occurred 2.1, 0.9, and 0.7 hr after the onset of scotophase, respectively. The pheromone titer of similar-aged females was also quantified under a thermocycle (17°–9°C; $\bar{X}$ = 14.9°C) reflecting conditions during a cool summer or a fall night. Females were kept at 20°C constant temperature and at the appropriate age were transferred to the thermocycle 5 hr before lights-off on the day the pheromone titer was determined. At the time of transfer, the temperature was 17.5°C and decreased to 17.0, 16.5, 16.0, 14.5, and 13.0°C during the next 5 hr, respectively. Pheromone glands were excised at −2, −1, and 0 hr of the scotophase, coinciding with the period during which calling is initiated by the different-aged females under this thermocycle (Delisle, 1992a). The pheromone titer was not determined after the onset of scotophase as, based on the reduced male flight activity and the low incidence of matings observed at these temperatures in the field (Delisle, personal observations), it was unlikely that OBL females would attract mates at temperatures below 13°C, even though calling can be expressed under such conditions (Delisle, 1992a). As previous temperature conditions may affect some aspects of calling ecology (Baker and Cardé, 1979; Delisle and McNeil, 1987a), all females were precon-

ditioned under the same photoperiod and temperature prior to testing to allow direct comparisons of the effect of different ambient temperatures on pheromone production.

For each pheromone gland extract, 5 μl were injected onto a fused silica capillary column, SP 2340 (30 m $\times$ 0.32 mm ID), in a 5890 HP gas chromatograph, equipped with a flame ionization detector (FID) and an HP-3393A integrator. The column temperature program was: 110°C for 30 sec, increased to 160°C at 6°C/min, held at 160°C for 15 sec, increased to 230°C at 15°C/min, and then held for 15 sec. The detector was at 300°C while the splitless injector was at 205°C. Hydrogen was used as the carrier gas, at a flow rate of 1 ml/min. The retention times of the internal standard and the three pheromone components, *E*11–14:Ac, *Z*11–14:Ac and *Z*11–14:OH were 2.57, 6.08, 7.15, and 8.36 min, respectively. For each age category, under both temperature regimes, a minimum of 20 pheromone glands were excised per time point.

Statistical Analyses. Each value of *Z*11–14:Ac and *E*11–14:Ac (expressed in nanograms) was raised to the power 0.4 while the naperian logarithm of each value of *Z*11–14:OH, ln (*Z*11–14:OH + 0.01) was used to normalize the data and stabilize the variance (homoscedasticity). Similarly, each value of the relative proportion of *Z*11–14:Ac, *E*11–14:Ac, and *Z*11–14:OH was transformed using: (1) ln (1.01 − proportion of *Z*11–14:Ac), (2) ln (proportion of *E*11–14:Ac + 0.01), and (3) ln (proportion of *Z*11–14:OH + 0.001), respectively. Transformed data were analyzed separately for each temperature regime, using an analysis of variance. In addition, orthogonal contrasts (Snedecor and Cochran, 1967) were performed to test for differences in the mean level of pheromone (least square means: adjusted means) as a function of age (three levels: 0, 3, and 5 days), time of day (five levels at 20°C: −1, 0, 1, 2, 3 hr and three levels under the cold thermocycle: −2, −1, 0 hr) and the interaction between the two factors. Additional contrasts were also carried out to assess the trend (linear, quadratic, cubic, or quartic) of each source of variation. Polynomial equations that best described the relationship between pheromone titer, female age, and time of day were determined and used to generate the response surfaces of each component as well as their relative proportion. In the calculation of these equations, the time values −1, 0, 1, 2, 3 and −2, −1, 0 were replaced by 1400, 1500, 1600, 1700, 1800, and 1300, 1400, 1500, respectively, which coincided with eastern standard time. Lights-off occurred at 1500 hr. All statistical analyses were performed using the GLM procedure of SAS/Stat (SAS Institute, 1990).

RESULTS

At 20°C, the titers of *Z*11–14:Ac and *E*11–14:Ac were generally low prior to lights-off but increased and reached a peak in the first or second hour of the scotophase (Table 1). These temporal variations were highly significant

TABLE 1. MEAN TITER (NANOGRAMS) (LEAST SQUARE MEAN OF BACK-TRANSFORMED VALUES) OF *Z*- AND *E*11–14:AC AND Z11–14:OH PRODUCED BY DIFFERENT-AGED (0-, 3-, AND 5-DAY-OLD) *Choristoneura rosaceana* VIRGIN FEMALES AT DIFFERENT HOURS OF 16:8 HR LIGHT–DARK PHOTOPERIOD AT 20°C CONSTANT TEMPERATURE OR COOL THERMOCYCLE (0 HR REPRESENTS ONSET OF SCOTOPHASE)[a]

	Z11–14:Ac at day				E11–14:Ac at day				Z11–14:OH at day			
Time	0	3	5	$\bar{X}$ (time)	0	3	5	$\bar{X}$ (time)	0	3	5	$\bar{X}$ (time)
20°C constant temperature												
−1	23.57	29.46	24.24	25.73	0.33	0.27	0.22	0.27	0.03	0.04	0.01	0.03
0	11.07	38.79	14.69	29.93	0.78	0.77	0.10	0.46	0.03	0.06	0.01	0.03
1	66.51	43.67	28.15	44.23	1.49	0.80	0.27	0.74	0.09	0.09	0.02	0.06
2	61.06	44.15	20.54	39.77	1.40	0.69	0.19	0.65	0.26	0.17	0.03	0.11
3	39.69	30.50	21.94	30.25	0.74	0.39	0.26	0.44	0.35	0.10	0.06	0.12
$\bar{X}$ (age)	44.65	37.01	21.63		0.88	0.55	0.20		0.09	0.08	0.02	
Cool thermocycle												
−2	37.68	43.91	19.63	32.60	0.67	1.15	0.85	0.88	0.07	0.11	0.08	0.09
−1	49.95	33.83	24.93	35.48	0.92	0.78	0.98	0.89	0.13	0.04	0.03	0.06
0	44.89	37.89	20.38	33.41	0.75	0.87	0.90	0.84	0.13	0.06	0.08	0.09
$\bar{X}$ (age)	43.91	38.56	21.63		0.78	0.93	0.91		0.11	0.06	0.06	

[a]The transformed LS mean values of the standard errors of Z11–14:Ac and E11–14:Ac were 0.09 and 0.25 at 20°C constant temperature and 0.08 and 0.24 under the cool thermocycle respectively, regardless of age and time. For the Z11–14:OH, the LS mean values varied with age and time between (0.37–0.40) at 20°C constant temperature and between (0.48–0.74) under the cool thermocycle.

TABLE 2. MEAN SQUARE (MS) VALUES AND LEVEL OF SIGNIFICANCE (P) ASSOCIATED WITH EACH ORTHOGONAL CONTRAST USED TO TEST FOR DIFFERENCES IN TITER OF Z- AND E11-14:Ac AND Z11-14:OH PRODUCED BY *Choristoneura rosaceana* VIRGIN FEMALES UNDER 16:8 HR LIGHT-DARK PHOTOPERIOD AT 20°C CONSTANT TEMPERATURE OR UNDER COOL THERMOCYCLE, IN RESPONSE TO AGE (A), TIME DURING PHOTOPERIOD (T), AND INTERACTION BETWEEN THE TWO (AT)

		Z11-14:Ac		E11-14:Ac		Z11-14:OH	
Source of variation	*df*	MS	*P*	MS	*P*	MS	*P*
20°C constant temperature							
Time	4	14.447	0.0001	1.431	0.0006	32.565	0.0001
Linear (T)	1	9.653	0.0419	1.287	0.0342	121.950	0.0001
Quadratic (T^2)	1	37.473	0.0001	4.129	0.0002	0.007	0.9616
Cubic (T^3)	1	5.036	0.1412	0.085	0.5850	7.500	0.1090
Quartic (T^4)	1	5.625	0.1200	0.224	0.3754	0.542	0.6658
Age	2	61.283	0.0001	8.030	0.0001	63.208	0.0001
Linear (A)	1	107.880	0.0001	15.000	0.0001	91.517	0.0001
Quadratic (A^2)	1	14.686	0.0122	1.073	0.0530	36.119	0.0005
Age * time	8	5.726	0.0124	0.594	0.0359	3.781	0.2416
AT	1	9.763	0.0407	0.621	0.1409	9.887	0.0659
AT^2	1	26.807	0.0007	2.594	0.0027	0.296	0.7497
AT^3	1	0.081	0.8519	0.014	0.8264	4.123	0.2342
AT^4	1	1.580	0.4095	0.081	0.5955	0.549	0.6637
A^2T	1	1.178	0.4765	0.161	0.4530	7.894	0.1001
A^2T^2	1	0.677	0.5892	0.587	0.1521	5.137	0.1843
A^2T^3	1	0.489	0.6463	0.338	0.2770	0.242	0.7729
A^2T^4	1	5.234	0.1336	0.358	0.2634	2.775	0.3289
Error IV	510	2.319		0.285		2.901	
Cool thermocycle							
Time	2	0.417	0.7848	0.012	0.9383	1.754	0.5929
Linear (T)	1	0.051	0.8640	0.011	0.8084	0.001	0.9851
Quadratic (T^2)	1	0.785	0.4999	0.013	0.7945	3.496	0.3086
Age	2	32.567	0.0001	0.119	0.5397	2.993	0.4112
Linear (A)	1	53.471	0.0001	0.176	0.3395	5.467	0.2036
Quadratic (A^2)	1	11.731	0.0095	0.061	0.5726	0.979	0.5893
Age * time	4	2.099	0.3024	0.152	0.5316	2.611	0.5391
AT	1	0.740	0.5125	0.007	0.8489	1.043	0.5773
AT^2	1	0.203	0.7313	0.052	0.6025	5.294	0.2109
A^2T	1	1.850	0.3003	0.211	0.2960	2.914	0.3524
A^2T^2	1	5.557	0.0734	0.336	0.1874	0.381	0.7363
Error IV	269	1.720		0.192		3.335	

for both acetate components (Table 2). Furthermore, the titers of both acetates varied with female age, particularly during the period of maximal pheromone production (Table 1), with younger females producing significantly more *Z*11–14:Ac and *E*11–14:Ac than older ones (Table 2). However, there was a significant interaction between age and time for both components (Table 2), indicating that the temporal pattern described above was not typical of all OBL females. The titers of both acetates produced by day-5 females remained relatively constant throughout the 5-hr period compared with those produced by day-0 and day-3 females (Table 1), which could explain the significant interaction.

Temporal changes in the titer of *Z*11–14:OH were also noted (Table 1), with the production increasing linearly as the night progressed (Table 2). Furthermore, as observed with respect to the two acetate components, female age had a significant effect on the titer of *Z*11–14:OH (Table 2), with younger females producing more alcohol than older ones. The best model that described the relationship between titer in nanograms ($\hat{Y}$), time of day (T), and female age (A) for each acetate, as well as the alcohol component, was expressed by the following polynomial equations

$$\hat{Y}_{Z11\text{-}14:Ac} = (-84.0304 + 10.9383\,T - 0.3349\,T^2 + 17.5113\,A - 0.0595\,A^2 - 2.1499\,AT + 0.0657\,AT^2)^{2.5}$$

$$\hat{Y}_{E11\text{-}14:Ac} = (-26.9178 + 3.4754\,T - 0.1075\,T^2 + 5.1883\,A - 0.0161\,A^2 - 0.6542\,AT + 0.0204\,AT^2)^{2.5}$$

$$\hat{Y}_{Z11\text{-}14:OH} = \exp(-9.7615 + 0.4623\,T + 0.3461\,A - 0.127\,A^2) - 0.01$$

and served to generate the response surfaces shown in Figure 1A–C, respectively.

Given these differences, one would expect the relative proportion of each pheromone component to vary with time and age. As seen in Tables 3 and 4, there was a significant linear decline in the proportion of present *Z*11–14:Ac with time during the scotophase, and older females produced relatively more *Z*11–14:Ac (~0.99) than younger individuals (~0.96) at any given hour. There was no significant interaction between the two factors. The proportion of *E*11–14:Ac remained constant during the 5-hr period but, as expected, it decreased significantly from 0.02 to 0.003 with age. In contrast, the relative proportion of *Z*11–14:OH increased linearly with time and younger females produced more *Z*11–14:OH (~0.003) than older ones (~0.001). However, there was significant interaction between age and time with respect to the proportion of *Z*11–14:OH in the pheromone blend (Table 4). The best relationship between age and time on the relative proportion of each OBL pheromone component is described by the following polynomial equations,

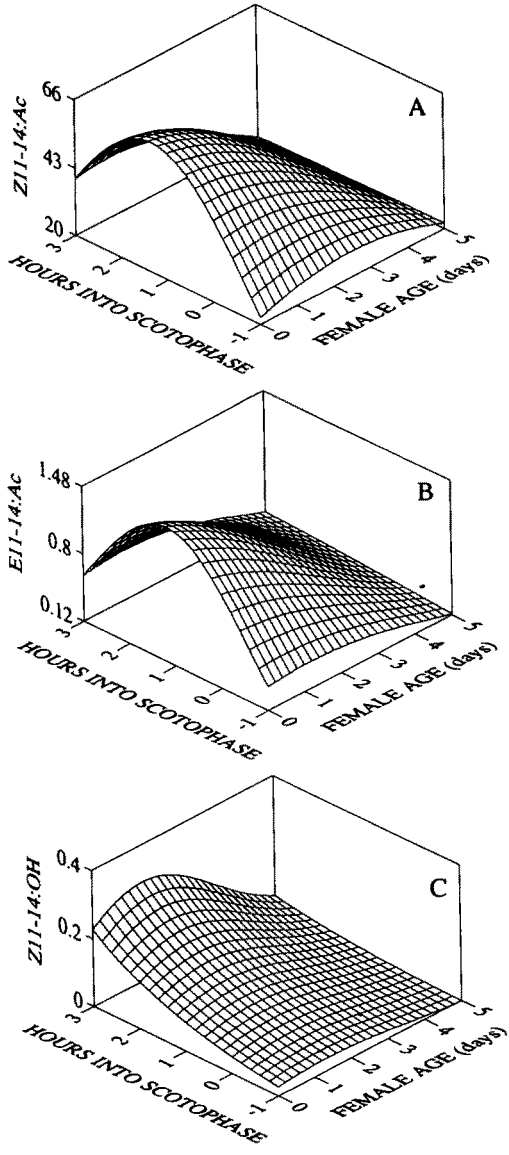

FIG. 1. Predicted values (ng) of Z11-14:Ac (A), *E*11-14:Ac (B), and Z11-14:OH (C) produced by different-aged *Choristoneura rosaceana* virgin females at different hours of the 16:8 hr light-dark photoperiod at 20°C constant temperature. 0 hr represents the onset of the scotophase.

TABLE 3. MEAN PROPORTIONS (LEAST SQUARE MEAN OF BACK-TRANSFORMED VALUES) OF *Z* AND *E*11-14:Ac AND *Z*11-14:OH PRODUCED BY DIFFERENT-AGED (0-, 3-, AND 5-DAY-OLD) *Choristoneura rosaceana* VIRGIN FEMALES AT DIFFERENT HOURS OF 16:8 HR LIGHT-DARK PHOTOPERIOD AT EITHER 20°C CONSTANT TEMPERATURE OR COOL THERMOCYCLE (0 HR REPRESENTS ONSET OF SCOTOPHASE)[a]

	*Z*11-14:Ac at day				*E*11-14:Ac at day				*Z*11-14:OH at day			
Time	0	3	5	$\bar{X}$ (time)	0	3	5	$\bar{X}$ (time)	0	3	5	$\bar{X}$ (time)
20°C constant temperature												
−1	0.971	0.983	0.994	0.985	0.016	0.007	0.003	0.007	0.002	0.002	0.000	0.001
0	0.974	0.979	0.995	0.984	0.017	0.013	0.003	0.009	0.001	0.002	0.000	0.001
1	0.971	0.980	0.992	0.982	0.023	0.010	0.004	0.100	0.002	0.003	0.001	0.002
2	0.957	0.978	0.992	0.979	0.028	0.011	0.003	0.100	0.006	0.004	0.001	0.003
3	0.963	0.983	0.983	0.978	0.019	0.007	0.006	0.100	0.010	0.003	0.002	0.005
$\bar{X}$ (age)	0.968	0.981	0.991		0.020	0.009	0.003		0.003	0.003	0.001	
Cool thermocycle												
−2	0.977	0.956	0.969	0.968	0.019	0.038	0.023	0.026	0.002	0.003	0.003	0.002
−1	0.972	0.955	0.951	0.960	0.024	0.038	0.044	0.034	0.003	0.002	0.001	0.002
0	0.957	0.966	0.939	0.955	0.029	0.030	0.050	0.035	0.005	0.002	0.004	0.003
$\bar{X}$ (age)	0.969	0.959	0.954		0.024	0.035	0.037		0.003	0.002	0.002	

[a]The transformed LS mean values of the standard errors of the proportions of *Z*11-14:Ac, *E*11-14:Ac and *Z*11-14:OH varied with age and time between 0.15–0.16, 0.29–0.31 and 0.25–0.27 at 20°C constant and 0.14–0.16, 0.16–0.21, and 0.30–0.41 under the cool thermocycle, respectively.

TABLE 4. MEAN SQUARE (MS) VALUES AND LEVEL OF SIGNIFICANCE (P) ASSOCIATED WITH EACH ORTHOGONAL CONTRAST USED TO TEST FOR DIFFERENCES IN PROPORTIONS OF Z AND E11–14:Ac AND Z11–14:OH PRODUCED BY *Choristoneura rosaceana* VIRGIN FEMALES UNDER 16:8 HR LIGHT–DARK PHOTOPERIOD AT 20°C CONSTANT TEMPERATURE OR UNDER COOL THERMOCYCLE IN RESPONSE TO AGE (A), TIME DURING PHOTOPERIOD (T) AND INTERACTION BETWEEN THE TWO (AT)

		Z11–14:Ac		E11–14:Ac		Z11–14:OH	
Source of variation	*df*	MS	*P*	MS	*P*	MS	*P*
20°C constant temperature							
Time	4	0.747	0.1702	0.910	0.7244	14.154	0.0001
Linear (T)	1	2.834	0.0139	2.418	0.2429	50.088	0.0001
Quadratic (T^2)	1	0.008	0.8924	1.060	0.4391	2.247	0.1945
Cubic (T^3)	1	0.127	0.6006	0.014	0.9301	3.906	0.0875
Quartic (T^4)	1	0.019	0.8398	0.030	0.8965	0.878	0.4169
Age	2	16.512	0.0001	59.868	0.0001	21.043	0.0001
Linear (A)	1	32.456	0.0001	117.017	0.0001	32.322	0.0001
Quadratic (A^2)	1	0.665	0.2313	3.115	0.1853	10.147	0.0061
Age * time	8	0.399	0.5487	1.488	0.5853	1.748	0.2353
AT	1	0.073	0.6914	0.163	0.7618	2.843	0.1447
AT^2	1	0.157	0.5607	2.286	0.2562	1.784	0.2475
AT^3	1	0.528	0.2862	1.933	0.2963	2.058	0.2143
AT^4	1	0.417	0.3430	0.850	0.4884	0.388	0.5892
A^2T	1	0.742	0.2062	1.542	0.3509	5.369	0.0454
A^2T^2	1	1.200	0.1083	2.600	0.2263	1.562	0.2792
A^2T^3	1	0.027	0.8105	0.122	0.7925	0.011	0.9293
A^2T^4	1	0.192	0.5196	2.628	0.2235	0.115	0.7684
Error IV	293	0.462		1.766		1.329	
Cool thermocycle							
Time	2	0.592	0.0950	0.950	0.0968	1.095	0.4880
Linear (T)	1	1.109	0.0360	1.492	0.0555	0.549	0.5487
Quadratic (T^2)	1	0.037	0.6976	0.298	0.3878	1.776	0.2818
Age	2	0.754	0.0509	1.642	0.0190	0.661	0.6477
Linear (A)	1	1.507	0.0150	3.152	0.0059	1.086	0.3993
Quadratic (A^2)	1	0.028	0.7349	0.322	0.3700	0.366	0.6244
Age * time	4	0.518	0.0852	0.770	0.1102	1.400	0.4539
AT	1	0.000	0.9968	0.105	0.6084	0.766	0.4789
AT^2	1	0.139	0.4534	0.221	0.4576	1.043	0.4089
A^2T	1	1.975	0.0056	2.690	0.0107	2.093	0.2428
A^2T^2	1	0.046	0.6661	0.008	0.8860	1.311	0.3546
Error IV	100	0.245		0.397		1.515	

$$\hat{Y}_{(\text{ratio of } Z11\text{-}14:\text{Ac})} = 1.01 - \exp(-4.2492 + 0.0699\,T - 0.1621\,A)$$

$$\hat{Y}_{(\text{ratio of } E11\text{-}14:\text{Ac})} = \exp(-3.7933 - 0.3078\,A) - 0.001$$

$$\hat{Y}_{(\text{ratio of } Z11\text{-}14:\text{OH})} = \exp(-11.0921 + 0.3484\,T + 2.7968\,A - 0.6105\,A^2 - 0.1648\,AT + 0.0340\,A^2T) - 0.001$$

and were used to produce the response surfaces shown in Figure 2A–C, respectively.

Under cold thermoperiodic conditions, females of the same age produced a similar titer of *Z*11–14:Ac over the 3-hr period (Table 1) but, overall, older individuals produced significantly less than younger ones (Table 2). This was similar to the trend observed at 20°C constant temperature. No significant interaction was found between age and time (Table 2), and the best model

$$\hat{Y}_{Z11\text{-}14:\text{Ac}} = (4.5498 + 0.1387\,A - 0.0729\,A^2)^{2.5}$$

was used to simulate the response surface (Figure 3). It is worth noting that, for any given age tested, the titers of *Z*11–14:Ac were quite comparable under both temperature regimes (Table 1, Figures 1A and 3).

The production of *E*11–14:Ac, as well as *Z*11–14:OH, under the cold thermocycle did not vary significantly with either age or time during the scotophase (Tables 1 and 2). The mean titer of *E*11–14:Ac was ~1 ng compared with 0.1 ng for the alcohol component.

There was considerable variation in the relative proportion of both acetate components produced by the different-aged OBL females over time due to significant interactions between factors (Tables 3 and 4) under the cool thermocycle. While there was no clear pattern for *Z*11–14:Ac, older females produced relatively more *E*11–14:Ac than younger ones. The best relationship between time and age on the ratios of both acetate components was described using the following polynomial equations

$$\hat{Y}_{(\text{ratio of } Z11\text{-}14:\text{Ac})} = 1.01 - \exp(-6.3025 + 0.2221\,T + 4.0339\,A - 0.8198\,A^2 - 0.2835\,AT + 0.0586\,A^2T)$$

$$\hat{Y}_{(\text{ratio of } E11\text{-}14:\text{Ac})} = \exp(-5.1545 + 0.1040\,T + 4.5671\,A - 0.9229\,A^2 - 0.3189\,AT + 0.0658\,A^2T) - 0.001$$

which served to generate the response surfaces of Figure 4.

However, contrary to the situation observed at 20°C, the ratio of *Z*11–14:OH did not vary with female age or time of day (Tables 3 and 4).

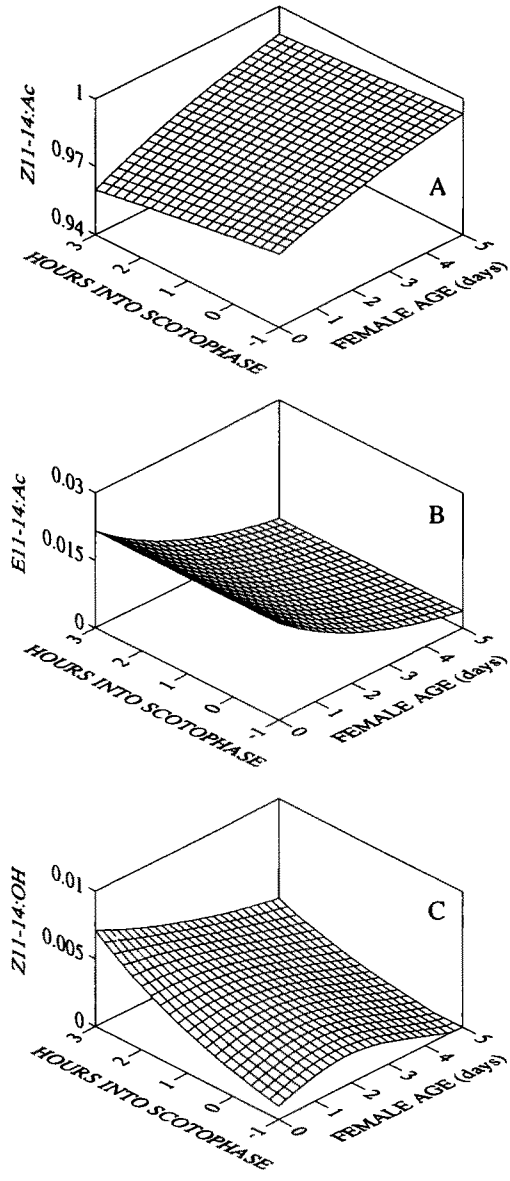

FIG. 2. Predicted values of the relative proportions of Z11-14:Ac (A), E11-14:Ac (B), and Z11-14:OH (C) of different-aged *Choristoneura rosaceana* virgin females at different hours of the 16:8 hr light-dark photoperiod at 20°C constant temperature. 0 hr represents the onset of the scotophase.

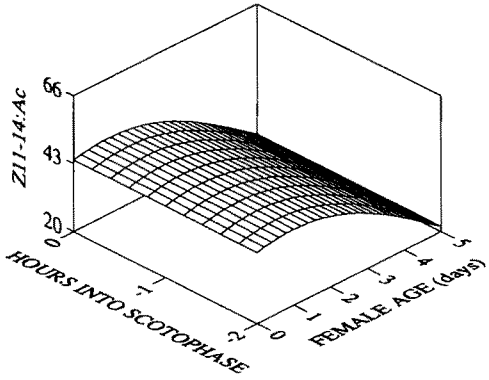

FIG. 3. Predicted values (ng) of Z11-14:Ac produced by different-aged *Choristoneura rosaceana* virgin females at different hours of the 16:8 hr light–dark photoperiod under a cool thermocycle. 0 hr represents the onset of the scotophase.

DISCUSSION

The present study demonstrates that younger OBL females produced more pheromone than older ones, both at warm and cold temperatures, thereby supporting the hypothesis that the greater attractiveness of day-0 and day-3 females compared with that of day-5 individuals during both flight periods (Delisle, 1992a) was probably associated with their higher pheromone content, if the amounts released are proportional to the amounts produced. This interpretation is further supported by the fact that the capture of OBL males in traps baited with the three-component pheromone blend (Z11-14:Ac, E11-14:Ac, Z11-14:OH) increased significantly with concentration, irrespective of the flight period (Delisle, 1992b). The decline in pheromone titer as females age has been linked with senescence associated with increased oviposition in *Helicoverpa zea* (Giebultowicz et al., 1990) and *Lymantria dispar* (Teal and Tumlinson, cited in Giebultowicz et al., 1990). However, in this study, senescence could not account for the decline observed in pheromone titer with age, as all ovipositing females, irrespective of age, were excluded from the experiment.

The relative composition of the OBL pheromone blend varied both in time and with age, although variation in the ratios of Z- and E11-14:Ac isomers was relatively small (less than 10%). These results fit within the generalization made by Ono et al. (1990) that species using geometrical isomers, such as *Argyrotaenia velutinana* (Miller and Roelofs, 1980) and *Pectinophora gossypiella* (Collins and Cardé, 1985), exhibit a narrow range of ratios compared with species such as *Phthorimaea operculella* (Ono et al., 1990), *Agrotis segetum* (Löfstedt et al., 1985), and *Ephestia cautella* (Barrer et al., 1987) that

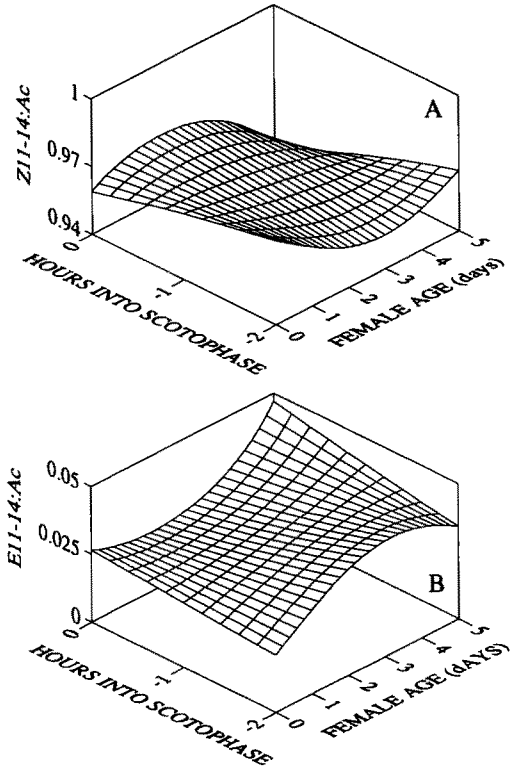

FIG. 4. Predicted values of the relative proportions of $Z11$–14 : Ac (A) and $E11$–14 : Ac (B) of different-aged *Choristoneura rosaceana* virgin females at different hours of the 16 : 8 hr light–dark photoperiod under a cool thermocycle. 0 hr represents the onset of the scotophase.

produce blends with very different compounds. The variation observed in the pheromone blend produced by OBL females fell within the range of variation that gave maximum male response in the field (Hill and Roelofs, 1979; Vakenti et al., 1988; Delisle, 1992b). This is not surprising as, in the field, it has been shown that males generally respond to a wider range of isomeric ratios (Flint et al., 1977; Baker et al., 1988) than those produced by females, and this may be accentuated by seasonal changes in temperature (Linn and Roelofs, 1988). However, the presence or absence of a pheromone component generally has a stronger influence on male attraction (Linn and Roelofs, 1983) than variation in blend ratios. In the case of OBL, it is clear that males responded preferentially to blends containing three pheromone components rather than the acetates only

(Delisle, 1992b). In this context, the lower amounts of Z11–14:OH in the pheromone blend of older females at 20°C, combined with the lower total pheromone titer, could reasonably explain their lower ability to attract males during the summer flight. However, under a cool thermocycle there were no differences in the amount or proportion of Z11–14:OH and thus does not support the idea that seasonal changes in the relative attractiveness of older females (Delisle, 1992a) could be attributed to the effects of age and temperature on pheromone production. Therefore, we believe our data support the original hypothesis that the relative increase in attractiveness of older females over younger ones observed in the fall (Delisle, 1992a) is due to the significant advance in the onset time of calling with age (Delisle, 1992a): by doing so, older females not only avoid competition with the more attractive younger females but also would be less affected by the limiting effect of low temperatures on male flight activity. On the other hand, when the mating periodicity of different-aged OBL females was examined under field conditions (Delisle, unpublished data), the onset of calling of older females coincided with peak foraging activity of a presently unidentified spider species that preyed on tethered females (Delisle, personal observation). This suggests there may be a cost associated with calling early and could explain why younger females do so later than older individuals, irrespective of temperature conditions (Delisle, 1992a).

This model probably holds true for other Lepidoptera, such as the omnivorous leafroller, *Platynota stultana*. All *P. stultana* virgin females call on the day following emergence and the onset time of calling occurs earlier on successive nights (Webster and Cardé, 1982). Furthermore, the progressive decline in the pheromone titer with age (Webster and Cardé, 1982) would explain the lower attractiveness of older females (day-5 and -6) compared with younger ones (day-2) under field conditions (AliNiazee and Stafford, 1971). However, whether the earlier onset of calling of less attractive older females enhances their degree of competitiveness, particularly in response to cool nights, remains to be tested. This seems likely as under cooler conditions calling is initiated earlier, especially in older females (Webster and Cardé, 1982). On the other hand, this scenario does not necessarily hold for all species where it has been shown that pheromone titer varies with age (Table 5). For example, in *Plusia chalcites*, even though older females initiate calling earlier than younger ones, pheromone production also increases with age (Snir et al., 1986).

The literature available on the diel patterns of pheromone production and calling in Lepidoptera (Table 6) indicates that most species (15 species of 25) show a close synchrony between the timing of calling and maximal pheromone production. Two different asynchronous patterns have been reported in the others: in six species, the peak of pheromone production occurs before the peak of calling, while in two noctuids, *Pseudaletia unipuncta* (Delisle and McNeil, 1987a) and *P. chalcites*, (Snir et al., 1986) the peak of pheromone production

TABLE 5. LEPIDOPTERAN SPECIES IN WHICH PHEROMONE TITER, DETERMINED BY GC ANALYSIS OR MALE RESPONSE, VARIED WITH FEMALE AGE[a]

Family	Age(s) of maximal production	Species	Reference
Arctiidae	4	*Holomelina lamae*	Schal et al. (1987)
Gelechiidae	1	*Phthorimaea operculella*	Ono et al. (1990)
Geometridae	3–4[b]	*Rheumaptera hastata*	Werner (1977)
Lymantriidae	4	*Lymantria dispar*	Giebultowicz et al. (1992),
	2–3		Tang et al. (1992)
	6–8[b]	*Orgyia pseudotsugata*	Swaby et al. (1987)
Noctuidae	7–20[b]	*Euxoa ochrogaster*	Struble and Jacobson (1970)
	5–8	*Heliothis peltigera*	Dunkelblum and Kehat (1992)
	3	*H. virescens*	Mbata and Ramaswamy (1990)
	2–3	*Helicoverpa zea*	Raina et al. (1986)
	7–9[b]	*Mamestra configurata*	Struble et al. (1975)
	4	*Plusia chalcites*	Snir et al. (1986)
	2	*Spodoptera littoralis*	Dunkelblum et al. (1987),
	2		Martinez and Camps (1988)
	2[b]	*S. frugiperda*	Sekul and Cox (1967)
	6	*Trichoplusia ni*	Bjostad et al. 1980
Pyralidae	3[b]	*Anagasta kühniella*	Traynier (1970)
	1–5	*Diaphania nitidalis*	Valles et al. 1992
	1–4	*Plodia interpunctella*	Sower and Fish (1975)
	2	*Sesamia nanogrioides*	Babilis and Mazomenos (1992)
Tortricidae	4–5	*Argyrotaenia velutinana*	Miller and Roelofs (1977)
	3–4	*Choristoneura fumiferana*	Sanders and Lucuick (1972),
	1–3		Morse et al. (1982),
	1–4		Grant et al. (1982)
	5[b]	*Epiphyas postvittana*	Lawrence and Bartell (1972)
	2	*Platynota stultana*	Webster and Cardé (1982)

[a]The age at which the maximal pheromone production occurred is also given.
[b]In these species, the age of maximal production was determined using male response.

occurs after the peak of calling. In a third noctuid, *H. zea*, the relationship is far from clear as conflicting data have been presented in two papers (Raina et al., 1986, 1991).

If one assumes that calling and pheromone biosynthesis are both circadian, as demonstrated experimentally for *P. unipuncta* (Delisle and McNeil, 1987a), then species that showed synchrony probably used the same *zeitgeber* for the two processes. However, in most species that show asynchrony (Table 6), calling is expressed relatively early in the scotophase, while the accumulation of either pheromone or the precursors occurs in the photophase. Thus, one could

TABLE 6. RELATIONSHIP BETWEEN TIMING OF CALLING AND PHEROMONE PRODUCTION DETERMINED BY GLAND EXTRACT (G) OR AIRBORNE COLLECTION (A) IN LEPIDOPTERA

Family	Pheromone quantification	Species	Period of calling	Reference
Peak of pheromone coincides with peak of calling				
Lymantriidae	A	*Lymantria dispar*	early scotophase	Charlton and Cardé (1982),
	G			Tang et al. (1992),
	G			Giebultowicz et al. (1992)
Noctuidae	G	*Heliothis peltigera*	late scotophase	Dunkelblum and Kehat (1992)
	G, A	*H. subflexa*	middle scotophase	Heath et al. (1991)
	G	*H. virescens*	middle-late scotophase	Heath et al. (1991),
	G, A[a]			Mistrot Pope et al. (1982),
	G			Mbata and Ramaswamy (1990, 1991)
	A	*Mamestra brassicae*	late scotophase	Bestmann et al. (1988),
				Subchev (1983)
	A	*Spodoptera sunea*	late scotophase	Bestmann et al. (1988)
	G	*S. frugiperda*	throughout scotophase	Ramaswamy et al. (1988)
Pterophoridae	A[a]	*Platyptilia carduidactyla*	middle scotophase	Haynes et al. (1983)
Pyralidae	G, A	*Diaphania nitidalis*	middle scotophase	Valles et al. (1992)
	G	*Ephestia cautella*	throughout scotophase	Coffelt et al. (1978),
	A			Barrer et al. (1987)
	G	*Plodia interpunctella*	early scotophase	Coffelt et al. (1978),
	A			Sower and Fish (1975)
	G	*Sesamia nanogrioides*	late scotophase	Babilis and Mazomenos (1992)
Tortricidae	A	*Cacoecimorpha pronubana*	throughout photophase	Witzgall and Frérot (1989)
	A	*Choristoneura funiferana*	early scotophase	Ramaswamy and Cardé (1984),
	G, A			Morse et al. (1982)
	A	*Chryptophlebia leucotreta*	late photophase	Bestmann et al. (1988)

TABLE 6. CONTINUED

Family	Pheromone quantification	Species	Period of calling	Reference
Peak of pheromone occurs before peak of calling				
Arctiidae	A, G	*Holomelina lamae*	late photophase	Schal et al. (1987)
Gelechiidae	G	*Phthorimaea operculella*	early scotophase	Ono et al. (1990)
Noctuidae	G	*Spodoptera littoralis*	early scotophase	Dunkelblum et al. (1987),
	G			Martinez and Camps (1988)
	G,A[a]	*Trichoplusia ni*	early scotophase	Hunt and Haynes (1990)
Pyralidae	G	*Conogethes punctiferalis*	middle scotophase	Konno (1986)
Tortricidae	G	*Adoxophyes* sp.	middle scotophase	Kou (1992)
	G	*Platynota stultana*	early scotophase	Webster and Cardé (1982)
Peak of pheromone occurs after peak of calling				
Noctuidae	G	*Plusia chalcites*	early scotophase	Snir et al. (1986)
	G	*Pseudaletia unipuncta*	late scotophase	Delisle and McNeil (1987a)
Conflicting results				
Noctuidae	G	*Helicoverpa zea*	early/middle scotophase	Raina et al. (1986, 1991)

[a]The release rate was determined from forcibly extruded glands.

hypothesize that the lack of synchrony is due to different cues being used for the two processes: lights-on for pheromone biosynthesis and lights-off for calling.

However, care must be taken when making generalizations based on available literature due to differences in the experimental approaches used to determine the pattern of pheromone production. For example, while the onset time of calling differs with age in most species examined (McNeil, 1991), the pheromone titer of different-aged females was usually measured at one specific hour of the scotophase and/or photophase within the calling window. Similarly, the diel changes in the pheromone titer are generally examined at a specific chronological age and only at constant temperatures. A comparison of the pattern of calling and pheromone production by different-aged OBL females at 20°C and under the cool thermocycle (Figure 5) demonstrates that these approaches may lead to somewhat biased estimates and thus to potentially erroneous conclusions. If the pheromone titers were determined at the time of peak calling activity at 20°C, one would conclude that day-0 and day-3 females produce similar amounts of pheromone (~45 ng), with day-5 females producing significantly less (~20 ng) than younger females. However, if the pheromone titers were determined at the mean onset time of calling at 20°C, then the conclusion would be that day-0 females (61 ng) produce significantly more pheromone than day-3 females (43.7 ng), with day-5 females still producing less than 30 ng. In

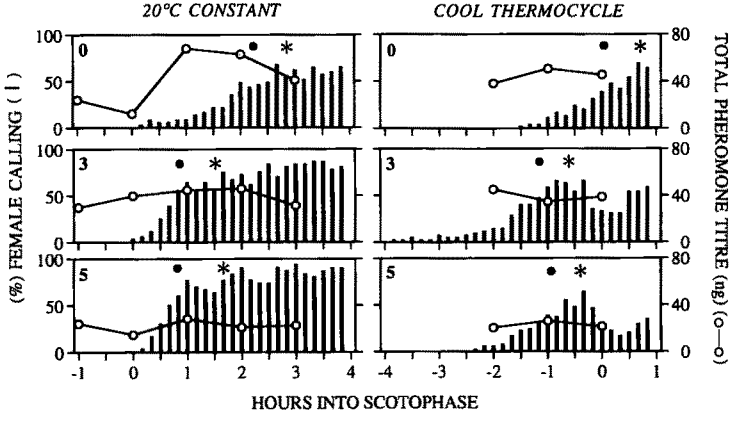

FIG. 5. The calling pattern (bars), and the mean total pheromone titer (circles) produced by 0-, 3-, and 5-day-old *Choristoneura rosaceana* virgin females at different hours of the 16:8 hr light–dark photoperiod at either 20°C constant temperature or a cool thermocycle. 0 hr represents the onset of the scotophase. The mean onset time of calling (solid circle) as well as the peak of call (*) are indicated for each age category and temperature regime. The proportion of females calling were obtained from Delisle (1992a).

contrast, under the cool thermocycle, estimates of the amount of pheromone produced by either 0- or 3-day-old females would be similar whether analyses were carried out at the mean onset time of calling or peak period of calling. As before, estimates of day-5 females would be less than those of younger ones at both times.

Furthermore, the age at which the patterns of calling and pheromone production is estimated could influence the conclusions drawn. At 20°C, the pheromone titer of day-0 OBL females reached its peak 1 hr prior to the onset of calling and dropped as soon as calling began, suggesting that the events are asynchronous, with the peak of pheromone preceding the peak of calling. In contrast, the pheromone patterns of day-3 and day-5 females showed that the peak of both calling and pheromone occurred at the same time, therefore leading to the conclusion that the two events are synchronous. The temperature regime at which the analyses are carried out could also affect the results, for as seen under the cold thermocycle, pheromone titers did not fluctuate considerably with age, regardless of whether analyses were made at the onset time of calling or at the peak calling time.

Using chronological rather than calling age (Turgeon and McNeil, 1982) is an additional factor that may result in errors when evaluating age and diel pheromone titers of species where females do not all initiate calling on the night following emergence. For example, in species such as the true armyworm (Turgeon and McNeil, 1982; Delisle and McNeil, 1986), the cotton bollworm (Kou and Chow, 1987), or the oriental armyworm (Han and Gatehouse, 1991), a population of 5-day-old females will have some individuals that have not yet started calling, while others are on their first, second, or third night of calling. Thus, any diel and/or age patterns obtained could be confounded if, as seen in this study of the OBL, there are changes in titer on successive days of calling. To make the point, let us assume that the OBL, rather than initiating calling on the night following emergence, did so at different ages. If we sampled a population of 5-day-old individuals that had equal numbers of females in their first, fourth and sixth night of calling (the first scotophase being day 0) then, using the data from Figure 5, we would obtain the pattern seen in Figure 6. While this would be an accurate population pattern, it would not give a clear diel pattern of females of a specific calling age. In fact, under both temperature regimes, the periodicity of calling, as well as pheromone production of the population (Figure 6), would be very close to the pattern expressed by females in their fourth night of calling (3-day-old) (Figure 5), but not for those in their first (0-day-old) or sixth night of calling (5-day-old) (Figure 5).

We would, therefore, recommend that future studies examining the synchrony of pheromone biosynthesis and calling take into account the importance of determining the temporal pattern of calling and pheromone biosynthesis as a function of calling age rather than chronological age, thus avoiding possibly

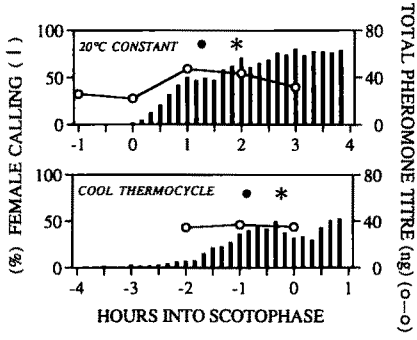

FIG. 6. The calling pattern (bars), and the mean total pheromone titer (circles) of a 5-day-old population of *Choristoneura rosaceana* virgin females that contained females in their first, fourth, and sixth night of calling under a 16:8 hr light–dark photoperiod at either 20°C constant temperature or a cool thermocycle. 0 hr represents the onset of the scotophase. The mean onset time of calling (solid circle) as well as the peak of calling (*) are indicated for each population under both temperature regimes. The proportion of females calling were obtained from Delisle (1992a).

incorrect estimates. This, we believe, is essential for valid interspecies comparisons, as well as for experiments examining the external cues governing the diel periodicity of calling and pheromone synthesis.

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