

## DAILY ACTIVITY PATTERNS AND SEX PHEROMONE SPECIFICITY AS SEXUAL ISOLATING MECHANISMS IN TWO SPECIES OF *CHORISTONEURA* (LEPIDOPTERA: TORTRICIDAE)

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### Abstract

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The roles of adult activity patterns and sex pheromones were investigated in the sexual isolation of *Choristoneura fumiferana* and *C. pinus*, species which hybridize in cages but not in the field. Female *C. fumiferana* started calling (extruding the sex pheromone gland) earlier (50% of the females started calling by sunset) than female *C. pinus* (50% by 1½ hours after sunset). Male *C. fumiferana* were also attracted to traps by the females earlier, although the times of the peak catches were approximately the same in both species. Males, both in the laboratory and the field, responded only to the sex pheromone of their own species. It is therefore concluded that while *C. pinus* adults normally appear about 2 weeks later than those of *C. fumiferana*, which reduces the possibility of hybridization, when the adults do overlap, the ultimate barrier is the specificity of the sex pheromones.

### Introduction

The genus *Choristoneura* Lederer includes a complex of closely related coniferophagous species in North America (Freeman 1967). Two of these species, *Choristoneura fumiferana* (Clemens), the eastern spruce budworm, and *Choristoneura pinus pinus* Freeman, the jack-pine budworm, inhabit eastern Canada and are sympatric for part of their ranges (Freeman 1953; Stehr 1967). When confined in cages, the two species hybridize and produce fertile offspring, but in the field the opportunity for hybridization is largely obviated by ecological and temporal isolation; *C. pinus* adults appear about 2 weeks later than those of *C. fumiferana* (Smith 1953). However, even when these barriers break down, no hybrid matings occur (Smith 1954). Smith concluded that this was partly owing to a differential in mating times; the average times of entering into copulation were 16:51<sup>1</sup> for *C. fumiferana* and 18:54 for *C. pinus*. But at the time of these studies the importance of sex pheromones in the mating behaviour of the Lepidoptera was not appreciated. Female budworm attract males by extruding a gland located between the eighth and ninth abdominal segments which releases a sex pheromone (Sanders 1969). Hybridization between contemporaneous populations of *C. fumiferana* and *C. pinus* would be less likely to occur if the diurnal pattern of "calling" by the females and responding by the males were different in the two species, or alternatively if they had different sex pheromones, as has been recently demonstrated in other sibling species of Lepidoptera (Roelofs and Comeau 1969). This paper demonstrates that differences in the diurnal activity patterns of *C. fumiferana* and *C. pinus* are not sufficient to prevent the possibility of hybridization, but that the two species do have different sex pheromones.

### Methods and Results

#### *Female Activity*

Virgin female budworm between 2 and 4 days old were placed individually in clear plastic boxes. These were placed out of doors in the shade. There was no artificial illumination except for a weak flashlight which was used for observations after dark. The activity of each female was recorded at least once each hour. On each of seven nights between 16 and 25 July, 1968, observations were made in this

<sup>1</sup>All times quoted are Eastern Standard Time.

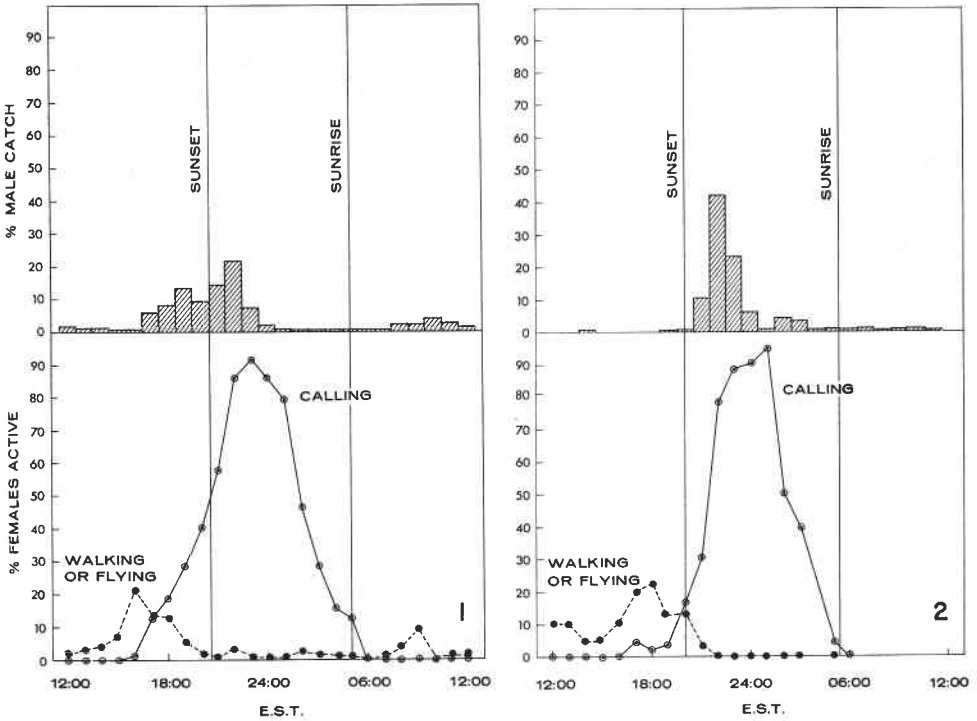


FIG. 1. Daily activity of adult *C. fumiferana*. Above: distribution of male catches in traps baited with virgin females. Below: female activity. Sunset and sunrise are 20:30 and 05:00 respectively, the approximate average for the period during which the observations were made (see text).

FIG. 2. Daily activity of adult *C. pinus*. Above: distribution of male catches in traps baited with virgin females. Below: female activity. Sunset and sunrise are 20:00 and 05:30 respectively, giving a photoperiod 1 hour shorter than in Fig. 1.

way on 20 female *C. fumiferana* at Black Sturgeon Lake, northwestern Ontario (lat. 49°20' N., long. 88°54' W.). The females were replaced after they were 4 days old, or if they behaved abnormally. A further 23 females of *C. fumiferana* were observed for 48 hours, 8 and 9 July, 1969, at Sault Ste. Marie, Ont. (46°31' N., 84°20' W.). Twenty female *C. pinus* were observed for 48 hours, 6 and 7 August, 1969, at Thessalon, Ont. (46°15' N., 83°33' W.).

Figures 1 and 2 show that the females of both species have distinct rhythms of calling. Comparisons between the two figures, however, are slightly confounded by the difference in times of sunset and sunrise during the observations on the two species. The approximate average times of sunset and sunrise during the two sets of observations on *C. fumiferana* were 20:30 and 05:00, respectively (Fig. 1), and the comparable times during the observations on *C. pinus* were 20:00 and 05:30 (Fig. 2). The first female *C. fumiferana* started calling about 4½ hours before sunset (Fig. 1), confirming previous laboratory observations (Sanders 1969). By sunset 50% of the females were calling and the peak number was reached 2½ hours later. By dawn virtually all calling had ceased. *C. pinus* females started calling ½ to 1 hour before sunset, reaching a peak soon after midnight. As in *C. fumiferana* virtually all calling ceased by dawn.

Table I. Catches of male *C. fumiferana* and *C. pinus* in traps baited with virgin females showing location, dates of trapping, and average catches per trap

Study area	Dates of trapping	Lure	No. of nights	No. of traps	No. of males caught:		Av. no. males/trap-night
					<i>C. fumiferana</i>	<i>C. pinus</i>	
Black Sturgeon L.*	18-27 Jul. '67	<i>C. fumiferana</i> ♀	9	12	190	0	1.75
Black Sturgeon L.*	16-25 Jul. '68	" "	5	12	63	0	1.05
Burchell Lake*	23-25 Jul. '68	" "	3	12	391	0	10.9
Parkinson Twp.*	14-16 Jul. '69	" "	3	36	1165	0	10.8
Chapleau*	25-27 Jul. '69	" "	3	18	516	0	9.56
"	"	<i>C. pinus</i> ♀	3	4	5	0	0.42
"	"	Control	3	6	4	0	0.22
Thessalon†	6-8 Aug. '69	<i>C. pinus</i> ♀	3	20	0	258	4.30
		<i>C. fumiferana</i> ♀	3	4	0	2	0.17
		Control	3	6	0	3	0.17

\*In white spruce - balsam fir stands.

†In jack pine stand.

### Male Response in Field

Traps baited with virgin female moths were placed out to determine at what time the males were attracted to the females and whether males were attracted by females of the opposite species. The traps consisted of boards, 1 cm thick, 30 cm square, smeared with tanglefoot. Each board was attached to a wooden arm 1.5 m long. A square hole in the centre of each board contained a screen cage housing a virgin female budworm. The boards were attached to trees about 1.5 m above ground level. They were checked hourly, at which time the males were counted and removed. The numbers of traps and the number of nights the traps were operated are shown in Table I. Four of the study areas were in white spruce-balsam fir forests, three of which, Burchell Lake (48°35' N., 90°35' W.), Parkinson Twp. (46°25' N., 83°15' W.), and Chapleau (47°55' N., 83°15' W.) were areas of known high *C. fumiferana* population density, while the fourth (Black Sturgeon Lake) was an area of endemic *C. fumiferana* population density. One study area was in a jack pine stand (Thessalon) where *C. pinus* populations were declining from outbreaks of the previous 2 years.

In three of the *C. fumiferana* study areas, Black Sturgeon Lake, Burchell Lake, and Parkinson Twp., all the traps were baited with *C. fumiferana* females. In the fourth area, Chapleau, four traps baited with female *C. pinus* and six unbaited controls were set up along with the traps baited with female *C. fumiferana*. Similarly, in the *C. pinus* study area (Thessalon) four traps baited with female *C. fumiferana* and six unbaited controls were set up along with the traps baited with female *C. pinus*.

As with the observations on female activity, the trapping results are slightly confounded by time differences in the periods of trapping which affect the length of the photoperiod (Figs. 1, 2). The distribution of male catches showed pronounced daily rhythmicity in both species. In *C. fumiferana* some males were caught at all times during the 24 hour period (Fig. 1). The peak catch was 1½ hours after sunset, with a minor peak 1½ to 2½ hours before sunset and another in mid-morning. The minor peaks varied in intensity and probably depended upon climatic conditions. For instance, at Parkinson Twp., the morning catches were almost as high as the evening peak, and the only difference noted between this and

the other areas was the unusually high humidity (maximum daily temperature 24° to 25° C, minimum relative humidity 60 to 70%) during the period of observation. *C. pinus* males showed a more restricted period of activity (Fig. 2). No appreciable numbers were caught before sunset. Peak catches occurred 2 hours after sunset and thereafter catches tailed off rapidly, with a small increase shortly after midnight.

The numbers of males caught in the traps baited with females of the alien species show very clearly that attractance is species specific. Both at Chapleau and at Thessalon the number of males caught by the alien females did not differ significantly from the numbers caught at the unbaited controls (Table I).

#### *Male Response in the Laboratory*

A crude wash containing sex pheromone was obtained for each of the two species. In each instance, approximately 100 virgin females obtained from field-collected pupae were placed in large flasks and left for 48 hours. The females were then removed and the flasks rinsed with ether. Bioassays were conducted as described by Sanders (*in press*). Briefly, the technique was as follows. Drops of the solution to be tested were placed on filter paper and the ether was allowed to evaporate. The filter paper was then introduced into the airstream blowing into a clear plastic box containing males. The males were between 2 and 3 days old, and were held under continuous illumination. When sexually excited, the males responded by "buzzing" (rapidly beating their wings while circling on the substrate). The number buzzing was recorded before and after the introduction of the filter paper. No attempt has yet been made to test for inhibition of male response of one species by the sex pheromone of the other as has been reported in other Lepidoptera (Roelofs and Comeau 1969); however, tests involving both pheromones applied simultaneously and one after the other were conducted in the laboratory. In no instance during these bioassays did males show any response to extracts of the pheromone of the alien species. Nor was there any indication of inhibition of response by the pheromone of the alien species, for when air was passed over the two pheromones alternately into a box containing males of both species, only the males appropriate to each pheromone responded and there was no reduction in response when both extracts were tested simultaneously.

#### **Discussion**

In *C. pinus* the peak male catches were well synchronized with the calling period of the *C. pinus* females. In *C. fumiferana* correlation was less exact, for although the early evening peak corresponded with the start of female calling, the reduced catches shortly before sunset and the morning peak did not appear to be related to female activity. A morning peak, corresponding to that found here, occurred in the basic activity and responsiveness of male *C. fumiferana* to female pheromone in laboratory studies (Sanders *in press*); this suggests that factors other than the release of pheromone by the females have a strong influence on the activity of male *C. fumiferana*.

The difference in the length of the photoperiod affecting the two species means that although peak activity during these observations was at 22:00 in both species (Figs. 1, 2), in contemporaneous populations, which would be necessary for hybridization, the *C. pinus* would peak slightly later than *C. fumiferana*. Therefore, *C. pinus* is evidently a more nocturnal and a less crepuscular species than *C. fumiferana*, and this implies that mating will be later than in *C. fumiferana* as was found by Smith (1954). However, the differences are not large and the overlap in

the activity of the two species is considerable; the calling periods of the females extend throughout the night and peak male activity in both species is shortly after sunset. Thus, where the two species are to be found as adults at the same time, the daily activity patterns alone are insufficient to prevent hybridization.

The results of the field trappings and the laboratory bioassays show clearly that the sex pheromones are species specific and it is therefore concluded that while the opportunity for hybridization between *C. fumiferana* and *C. pinus* is reduced by ecological isolation and by differences in seasonal and daily activity patterns, it is the specificity of the sex pheromones that is the ultimate barrier to hybridization in the field.

Since hybrid matings do occur in cages, it is also concluded that the close proximity between individuals in the confines of a cage results in the normal restrictions placed on the insects by their behavioural limitations breaking down, and that the males, flying around inside the cage, contact the females by chance and having done so are able to copulate with females of either species. If there is discrimination by male or female, it is not reliable enough to prevent hybridization.

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