Male Approach to the Female and the Role of Two Pheromone Components in the Potato Tuber Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae)¹

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Approaching behavior of the potato tuber moth, *Phthorimaea operculella*, to pheromone source was analyzed. The process of searching by walking was essential for effective finding of female. Searching by walking was characterized by rapid walking while wing fanning and frequent turning near pheromone source. This strategy seemed to be an efficient mechanism for locating females in their habitat. The male's flight or rapid walking while wing fanning, induced by (*E*, *Z*, *Z*)-4, 7, 10-tridecatrienyl acetate (trien), changed to continual walking while wing fanning by the presence of (*E*, *Z*)-4, 7-tridecadienyl acetate (dien). In addition, males spent more time on the lure of dien in combination with trien.

INTRODUCTION

There are many works dealing with the mating behavior of Lepidoptera in relation to sex pheromones. Generally, the behavioral response of males can be divided into two processes, long range and close range orientation (BARTELL, 1977; CARDÉ, 1979).

Males usually change the behavioral pattern to close range orientation within the vicinity of females. In some species the flight of male changes to hovering (searching flight) (HIDAKA, 1972), and in the others males land on the substrate near the female and walk while wing fanning (mating dance) (CARDÉ et al., 1975; BAKER et al., 1976). Both of these behaviors result in location of the calling female. OBARA (1979) has shown that wing fanning of male silk moths, *Bombyx mori*, responding to sex pheromone produced an air current along their body's longitudinal axis, and that this played an essential role in the male's location of the female.

Recent studies of sex pheromones show that the pheromones of almost all moth species have several components (TAMAKI, 1977). However, with the exception of several species (e.g., BAKER et al., 1976; BAKER and CARDÉ, 1979; KAWASAKI, 1981; HAINES, 1983; PALANISWAMY et al., 1983) the role of each component was not clarified. The sex pheromone of the potato tuber moth, *Phthorimaea operculella*, is composed of two components, (*E*, *Z*)-4, 7-tridecadienyl acetate (dien) and (*E*, *Z*, *Z*)-4, 7, 10-tridecatrienyl acetate (trien) (ROELOFS et al., 1975; PERSOONS et al., 1976; YAMAOKA et al., 1976). PERSOONS et al. (1976) isolated about equal amounts of two components from female

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moths. Recently, Tóth et al. (1984) have described some behavioral effects of each pheromone component.

The present paper describes male approach to females and discusses the role of pheromone components in relation to the mating behavior of the potato tuber moth.

MATERIALS AND METHODS

Field observations. Field observations were conducted by releasing males (2–3 days old) reared in the laboratory (25°C, 16 light–8 dark regime). A horizontal wooden plate (1 × 1 m) grided in 100 sections of square was positioned 20 cm above the ground, and either a rubber septum or caged females were fixed in the center. The behavior of males attracted on the plate were observed. One hundred to 200 males were released about 1 hr before set of lure at 5 m downwind. The observations were held from May to September (1981, 1982). The time of observation was 3–4 hr around sunset. Portable cassette recorders were used to record observations, which later were transcribed. In addition, an infrared video camera (Sony AVC-1300s) was used for the tests examining time spent on the septum. The video recordings were played back for analysis.

Glass house observations. Light and temperature conditions in the glass house (160 × 160 × 220 cm) were not controlled, but it was ventilated continuously. Male moths were released into it about 1 hr before each observation and male orientation behavior to a small lure (a piece of filter paper, 5 × 5 mm, impregnated with extract of 4 female equivalents, FE, methylene chloride extract) suspended 1 m above the floor by a thin thread from the ceiling was compared with the same source fixed on the side wall at the same height. The observations were conducted during the day.

Laboratory observations. A plastic box (24 × 30 × 8 cm), a glass container (20 × 25 × 30 cm), and a wind tunnel (30 × 30 × 150 cm) were utilized for behavioral observations. The wind tunnel was made of plastic plates and had a fan on each end. The wind speed was about 1 m/sec. All of these laboratory tests were conducted at 15–25°C. The detailed procedures of experiment will be described in each section.

RESULTS

Description of approaching behavior in the field

A rubber septum with female extract (100 FE) was used as a lure. Males responded immediately, and approached the plate. Almost all approach in this situation was not continuous flight, but short flights approximately 50–100 cm and about 30 cm above the ground, and landing followed by walking for a short distance.

Males landed at about 40 cm from the septum which was fixed on the plate (Fig. 1), though these data may underestimate the number of landing over 50 cm from the source because the observation was held on a 1 m square plate. Hereafter, they walked, often while wing fanning, “searching” for a lure sources.

Function of landing

In the glass house experiment (Table 1), males approached the vicinity of the source in both suspended from ceiling and fixed on the side wall, but a few males (17%) touched the suspended source by hovering search, while many males (68%) easily found the source fixed on the side wall by walk searching.

Fig. 1. Landing position of the male *P. operculella* on the plate on which a lure source (female extract) was set ($n=135$).

Table 1. The approach of male *P. operculella* to a lure source in the glass house

Site of source	No. ♂ approaching within 20 cm from source	No. ♂ touching source (%)
Hanging at 1 m high	42	7 (17)*
Set on the side wall at 1 m high	28	19 (68)

* Significantly different at $p < 0.01$ (χ^2 -test).

Table 2. The approach of male *P. operculella* to a lure source in the wind tunnel

Height of source (cm)	Location	No. ♂ reaching source*	Mean time to contact source (sec)	No. arriving ♂ within 15 cm of source but not contacting source
15	Suspended	7	103	5
15	Placed ^a	15	116	0
5	Suspended	3	270	7
5	Placed	13	160	2

^a A clod of clay was placed on a box ($15 \times 15 \times 10$ cm).

* Significantly different between 15 cm suspended and placed, and between 5 cm suspended and placed at $p < 0.05$ (χ^2 -test).

Table 3. Effect of antennal excision on approaching behavior of male *P. operculella* to a lure source in a wind tunnel

Male	No. circle movements observed		% ♂ reaching source	Mean time to reach lure (sec)
	Right	Left		
Intact	26	20	76	97
Left excised	30	28	24	121
Right excised	40	92*	20	136

* Significantly different at $p < 0.01$ (χ^2 -test).

Table 4. Effect of wing excision on approaching behavior of male *P. operculella* to a lure in a closed box

Male	No. ♂ tested	No. ♂ reaching source	Mean time to reach lure (sec)
Intact	40	16	56
Wingless	24	11	82

Similar observations were conducted in the wind tunnel. The behavior of males released 1 m downwind of a piece of filter paper impregnated with a 10 FE female extract was examined with a suspended lure and with a lure placed on a clod of clay. Number of males observed was 20 in each treatment. Observations ceased if a male was stationary more than 1 min. As in the former experiment in the glass house, males could arrive at a placed source more readily than a suspended one (Table 2). In this case, they also found the placed source by walking.

Male approaching mechanism within the vicinity of females

Tests of males with excised antennae were conducted in the wind tunnel, in which a grided plastic plate (15×65 cm) in 5 cm square section was placed. A lure (female extract, 10 FE) was fixed at the upwind end, and a male was released 50 cm downwind. The wings of all the males tested were removed to observe orientational behavior by walking. Number of males observed was 50 in each condition. Observations ceased if a male was stationary more than 1 min. The results were summarized in Table 3. In the Table, a circle movement means that the track of movement drew a circle within a 5 cm square. Males with one excised antenna made more circle movement, but they were not necessarily biased in one direction. Also, they could reach the lure source to some extent.

The effect of wing excision on the approaching behavior of males was also examined in a closed plastic box, in which a wingless male was released and a lure (female extract, 10 FE) was introduced in a corner (Table 4). Observations ceased if a male was stationary more than 1 min. The wingless male could arrive at the lure source similarly as the intact male in a space without air current. There was no significant difference between winged and wingless males in the time required to reach the lure.

Role of pheromone components in male approaching behavior

Field tests were conducted by using rubber septa with each component and a small screen cage ($2.5 \times 2.5 \times 10$ cm, divided into 5 compartments) containing 5 females as lures. The rubber septa contained the following treatments of the pheromone components or female extract respectively, 300 μ g dien (Pherocon® cap for the potato tuber moth, Zoecon Corporation), 300 μ g trien (synthesized by Dr. YAMAOKA, purity was 99% or more), 300 μ g each of dien and trien on the same septum (added 300 μ g trien on Pherocon® cap), and 100 FE female extract. The observations were carried out on the same wooden plate as the former field observations on which a lure source was fixed. The results are summarized as follows (Table 5): (1) dien alone was not an effective attractant, (2) males landed within the vicinity of the source in all treatments, so neither of the components acted as a landing inducer, (3) males engaged in short flights (10–20 cm) frequently to trien, but the addition of dien evidently suppressed this behavior, (4) males approached caged females with a relatively straight course, as evidenced by the number of grid sections covered in walking to the pheromone source.

Males approached a pheromone source by walking. The time spent on the septum was compared for each treatment. Measurement of time spent on a septum was difficult because many moths gathered on the septum and discrimination of individual moths was impossible. Therefore, the numbers of moths staying on a septum and of those staying within the vicinity of the lure (20 cm square including a septum) were counted every one minute. These numbers were added and the percentages of moths

Table 5. Approach of male *P. operculella* to lures in the field

Lure	No. ♂ observed	No. ♂ reaching source (%)	Mean distance of landing from the source (cm)	% ♂ showing short flight in approaching	Mean No. section passing through from landing to reaching the source
♀	135	93 (69)	39.9	22.2	5.0***
Dien	67	17 (25)*	44.1	20.9	7.9
Trien	148	66 (45)	41.1	34.5**	7.7
Dien + trien	160	50 (31)	44.5	20.6	7.1

* Significantly different from trien ($p < 0.05$, χ^2 -test) and female ($p < 0.01$).

** Significantly different at $p < 0.05$ (χ^2 -test).

*** Significantly different at $p < 0.05$ (t -test).

Table 6. Numbers of male *P. operculella* on the septum of each pheromone component in the field

Lure	Total time of observation (min)	Summated No. ♂ observed		B/A (%)
		Within 20 cm square including septum (A)	On the septum (B)	
Trien	111	42	9	21*
Dien + trien	119	197	101	51
♀ extract	115	78	39	50

* Significantly different at $p < 0.01$ (χ^2 -test).

staying on the septum were calculated. The results (Table 6) showed that the number of males on the septum with dien added were increased over the treatment of trien alone. This suggests the time spent on the septum with trien and dien together is enhanced over trien alone.

Laboratory tests were conducted using a glass container on which a line was drawn at half depth. The males were released in it, and air puffs (5 puffs) containing synthetic pheromone (both dien and trien compounds were synthesized by Dr. YAMAOKA; purity was 99% or more) were introduced at 0 min. Air puffs were made by pipetting after taking up and discharging about 1 ml of each sample in a pipette and allowing the solvent to evaporate. The next air puffs (5 puffs) were introduced after about 55 sec. The behavior of males staying at the lower half of the container was observed. The numbers of males showing each behavior were counted every 10 sec for 0–2 min of the observation period, and every 30 sec for the next 3 min. When dien was introduced after excitation to trien, male behavior was clearly changed (Fig. 2a–c). Many males landed on the wall of the container, especially on the bottom, and they showed continual walking while wing fanning. This was characterized the walking without jump or short flight in contrast with the response to trien alone, in which males often showed jump or short flight. Dien could induce the behavioral change even in lower concentrations (10^{-6} mg/ml) (Fig. 2c). This concentration is lower than the threshold (10^{-5} mg/ml) in glass rod test (YAMAOKA, personal communication), and also did not induce any behavioral responses by itself in this assay method. The behavioral change was continued for about 1 min in the cases of Fig. 2a and b.

Fig. 2. Responses to the male *P. operculella* to each component of sex pheromone. Arrows indicate the introduction of air puffs. a: trien (10^{-6} mg/ml)—dien (10^{-4} mg/ml) (first sample—second sample), b: trien (10^{-6} mg/ml)—dien (10^{-5} mg/ml), c: trien (10^{-6} mg/ml)—dien (10^{-6} mg/ml), d: dien (10^{-3} mg/ml)—trien (10^{-6} mg/ml), e: trien (10^{-6} mg/ml)—trien (10^{-6} mg/ml), f: dien (10^{-4} mg/ml)—dien (10^{-4} mg/ml), g: trien (10^{-6} mg/ml)—air, h: air—air.

DISCUSSION

In many species, males approach a female from a “long” distance by flight which is guided by pheromones. Long distance flight was not observed in *P. operculella*. Instead, males showed intermittent flight from a distant site, and changed to walking near the source. The intermittent flight may, therefore, correspond to long range flight.

Walking approach is an essential process for locating females in *P. operculella*. The function of walking seems to be similar to that of hovering in other species. Both of these behaviors result in localized “searching” by slow movement compared with high speed long range flight. In addition, when the walking is accompanied with wing fanning, both walking and hovering may introduce air currents along the male’s body. A walking approach may be more important than hovering for the species living in narrow or complicated habitats, while hovering may be more important for those active

in open spaces. But, in some instances both behaviors may occur. A few males of *P. operculella* show typical hovering within the vicinity of pheromone source in wind tunnel observations. A similar phenomenon has been observed by MURUS and BETTANY (1977) in the Egyptian cotton leafworm moth, *Spodoptera littoralis*, and LINGREN et al. (1980) in the pink bollworm moth, *Pectinophora gossypiella*, indicating that the two strategies may be interchangeable in some species.

Male *B. mori* responding to pheromone can orient to a lure by tropotaxis (OBARA, personal communication). In this species, males showed circle movement which was directed to the side of intact antenna when one antenna was removed. The results of this observation showed that the orientation behavior of *P. operculella* may include another mechanism. Male with one antenna showed many circle movements, but the direction was not always biased (Table 3). Moreover, removal of wings (Table 4) has little effect on the approach behavior, differing from *B. mori* (OBARA, 1979) in which wingless males did not reach the female. Male response to sex pheromone in close range is characterized by walking while wing fanning and frequent turning. This searching behavior does not seem to orient a female (or lure) by "plume" following. Moving speed and/or frequency of turning may be increased by high concentration pheromone within the vicinity of the source. The probability of encountering a female (or lure) would be enhanced by this behavior. Males may be able to find a source by this mechanism, even if one of their antennae is excised and their wings are removed.

Habitat of *P. operculella* is on potato plants, which are densely foliated near the ground. The dispersion of pheromone in such an environment would be very different than in an open wind field: well-defined "plumes" typical of unobstructed habitats may be replaced by broad active spaces enveloping the area of the plant near the female. These approaching behaviors mentioned here seem, therefore, to be an efficient search mechanism of females of *P. operculella*. KENNEDY (1977) has suggested the possible involvement of klinokinesis by males responding to odor stimuli. It may be possible that this mechanism is included in the approaching behavior of *P. operculella*.

The pheromone of *P. operculella* has been identified as two compounds, (*E*, *Z*)-4, 7-tridecadienyl acetate and (*E*, *Z*, *Z*)-4, 7, 10-tridecatrienyl acetate (ROELOFS et al., 1975; PERSOONS et al., 1976; YAMAOKA et al., 1976). Each of the components can evoke wing fanning response in male moths, though the threshold concentration is about 10,000 times higher in dien (10^{-5} mg/ml) than trien (10^{-9} mg/ml) in glass rod tests (YAMAOKA, personal communication). TÓTH et al. (1984) have studied on the role of each component of *P. operculella* pheromone, and shown that males spent longer time within the vicinity of pheromone source by adding of dien.

From the results of Fig. 2, dien evidently can induce some behavioral change to males. But, at least, dien added to trien does not seem to be involved in inducing landing because in the field observations males landed in similar manner on the plate for all treatments (Table 5). The results of Tables 5, 6 and Fig. 2 indicate the following conclusions. Dien changes male's flight or rapid walking while wing fanning induced by trien alone, and the behavior of males result in continual walking while wing fanning, which is an adaptive behavior in the searching process as mentioned above. In addition, males spend more time on the lure of dien in combination with trien, in agreement with the conclusions of TÓTH et al. (1984).

In the present observations, males showed somewhat different responses to female extract and caged female moths from other lures. For example, males approached a

source of female extract in a relatively straight course (Table 5), and males showed very strong "arrestment" to caged females (not shown in RESULTS). These responses may indicate the presence of some unknown pheromone component, though we can not compare these results directly because there may be some differences in pheromone amount, concentrations, ratios or timing of release.

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