Oviposition behavior of the sweet potato hornworm, *Agrius convolvuli* (Lepidoptera; Sphingidae), as analysed using an artificial leaf

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Abstract

An artificial leaf used for oviposition by the sweet potato hornworm, *Agrius convolvuli* (Lepidoptera, Sphingidae), was developed based on observations of adult behavior. It consists of a truncated cone disc sandwiched between a sheet of filter paper (top) and a plastic disc (bottom). The filter paper was kept moist by absorption of a leaf extract. Eggs were laid mainly on the lower surface of the plastic disc which was convenient for the collection of eggs. Oviposition behavior was well stimulated when the artificial leaf was supplied with the leaf extracts of fresh sweet potato leaves. Using a diluted ethanol extract (equivalent to 0.06 g fresh leaf/ml), 400–600 eggs/female were laid as many as when a potted plant was used. Observations using the artificial leaf revealed that the phytochemically-mediated oviposition behavior of this moth can be described as follows: 1) oviposition in both mated and unmated females is stimulated by the contact with phytochemicals of the host plant; 2) in addition to high air humidity (>70% RH), moisture at the oviposition site is necessary to induce oviposition; 3) egg-laying can be induced by host-plant volatiles alone although oviposition behavior is remarkably stimulated by both volatile and non-volatile compounds; 4) chemoreceptors for the oviposition stimuli are located not only on the tarsi but also on the antennae; 5) the tarsi are important to stimulate oviposition rather than the antennae; however, 6) the antennae play an important role in the location of oviposition site. It can be concluded that the phytochemically-mediated oviposition sequence is accomplished through synergistic chemoreception by antennae and tarsi in *A. convolvuli*. Because of the high yield and the ease of egg collection, the artificial leaf is useful not only for the study of oviposition behavior, but also for successive maintenance of laboratory colonies of this insect.

Key words: *Agrius convolvuli*, artificial leaf, host selection, oviposition, sweet potato hornworm

INTRODUCTION

In most herbivorous insects, the larvae feed and grow on a limited range of plant species and consequently, host-plant selection by ovipositing females is a crucial behavioral process for survival of progeny. A variety of sensory modalities such as mechanical, visual, olfactory and gustatory, have been postulated to play significant roles in host seeking and host recognition (Prokopy, 1983; Visser, 1986; Ramaswamy, 1988; Thompson and Pellmyr, 1991; Renwick and Chew, 1994). Host-specific phytochemicals (usually secondary plant metabolites) are considered to be the most important sensory cues (reviewed in Honda, 1995). In butterflies, a considerable number of oviposition stimuli derived from host plants have

been identified, and the sequence of oviposition behaviors has been characterized related to chemoreception. However, in another lepidopteran group, moths, relatively fewer oviposition stimuli have been elucidated than in butterflies although oviposition behavior of moths have also been extensively studied. This is because the oviposition behavior of butterflies is typically evoked by contact-chemoreception; and the stimulant activity can be easily assayed using a sheet of filter paper containing the relevant phytochemical (Nishida, 1977; Ichinosé and Honda, 1978; Honda, 1986, 1995; Nishida et al., 1987). In contrast, the oviposition of moths is difficult to assay using this method; and additionally, the oviposition sequence needs to be observed in the dark. Therefore, it is necessary to establish a method of observation in the

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dark; and also to develop an artificial leaf able to stimulate oviposition behavior, in order to demonstrate the role of phytochemical mediators in moths (Ramaswamy, 1988; Thompson and Pellmyr, 1991; Landau et al., 1994; Renwick and Chew, 1994).

Recently, a year-round rearing system using artificial diets was developed for the sweet potato hornworm, *Agrius convolvuli* L. (Lepidoptera, Sphingidae) (Kiguchi and Shimoda, 1994; Kiguchi et al., 1994). Its growth and behavioral characteristics were studied using laboratory colonies (Shimoda et al., 1994, 1995; Shimoda and Kiguchi, 1995; Shimoda and Kiuchi, 1997). *A. convolvuli* is now widely used and recognized as a useful lepidopteran experimental model, especially in the fields of insect endocrinology and neurobiology. The conditions necessary for indoor egg collection from this hawkmoth were elucidated using a potted sweet potato plant (Kiguchi and Shimoda, 1994). Studies of sex pheromones and male reactions were carried out in a wind tunnel (Wakamura et al., 1996). Diel distributions and daily changes in behavioral activities (e.g. flight, feeding, calling, oviposition) of this hawkmoth were determined using a night-vision device (Shimoda and Kiguchi, 1995). In the present study, we developed an artificial leaf able to stimulate the oviposition behavior of *A. convolvuli*, and the sequence of host discrimination behavior in ovipositing females was investigated.

MATERIALS AND METHODS

Insects and rearing methods. *A. convolvuli* used were derived from a laboratory colony established from pupae collected at Tsukuba, Ibaraki, Japan in 1989 and maintained successively on artificial diets (Kiguchi and Shimoda, 1994). First to second instar larvae were reared in a plastic dish (14 cm in diameter $\times$ 2.5 cm, 50 larvae/dish), third to fourth instar larvae in a plastic cage (22 $\times$ 30 $\times$ 6 cm, 20–40 larvae/cage), and fifth (final) instar larvae in a plastic container (38 $\times$ 57 $\times$ 13 cm, 20–30 larvae/container) at 27°C under a 16 h L–8 h D photoperiod. First to fourth instar larvae were fed on an artificial diet containing approximately 20% (dry weight) sweet potato leaf powder (SPLP-20)

and fifth instar larvae were fed on approximately 5% sweet potato leaf powder (SPLP-5) (Kiguchi et al., 1994). Pupae were laid in a 20 $\times$ 28 $\times$ 6 cm plastic box on a wet paper towel and kept at 25°C under a 12 h L–12 h D photoperiod. For uniform egg production from each individual, only pupae weighing 4.5–5.0 g were used.

Oviposition experiments and observation. Pharate adults were transferred to a wooden box cage (73 $\times$ 85 $\times$ 115 cm) with the inside surfaces covered with black paper to prevent extra light reflection (Fig. 1). The emergent moths were kept at 25°C under a 16 h L–8 h D photoperiod. As no fertilized eggs can be obtained in complete darkness (Kiguchi and Shimoda, 1994), the cages were weakly illuminated (about 2–5 lx) during scotophase using a rheostat-controlled 3 W incandescent bulb. High humidity

Fig. 1. Rearing cage for observation of adult *A. convolvuli* (73 $\times$ 85 $\times$ 115 cm). a) Timer-controlled 15 W fluorescent lamp, b) illuminator (rheostat-controlled 3 W incandescent bulb), c) artificial leaf, d) basket for holding pupae, e) mesh floor placed on water-filled containers, f) timer-controlled humidifier, g) screen door, through which the moths are observed using two sets of infrared camera-video recorder systems. Other inner walls are covered with rough black paper.

(>70% RH) was maintained by supplying water beneath the mesh floor and a timer-controlled humidifier. In each experiment, either a potted sweet potato plant or a surrogate leaf was placed in the center of the cage as an oviposition site. The behavior of the moths was observed using two infrared camera-video recorder systems (AV5500, Alps Inc.) as described by Shimoda and Kiguchi (1995).

Sweet potato leaf extract. Because a lot of attractants and oviposition stimuli have been so far found to be polar compounds and are successfully extracted in alcohols (reviewed in Honda, 1995), distilled water and ethanol were used as extraction solvents. For the water extraction, 100 g of fresh sweet potato (*Ipomoea batatas*) leaves was added to 320 ml of distilled water, homogenized using a cooking blender, filtered through a paper filter (15 cm in diameter, No. 1, Toyo Roshi), and stored at -20°C until use. For the ethanol extraction, 100 g of fresh sweet potato leaves were cut into rectangles (0.5 cm wide) and soaked in 320 ml of ethanol for about 3 days at 4°C . The ethanolic solution was filtered using the same type of filter paper, then stored at -20°C until use. The concentration of the sweet potato leaves was about 0.3 g/ml in either extract.

Oviposition on the leaf extract treated-chromatography papers. The activity of the leaf extract was tested for egg laying using a chromatography paper as described in butterflies (Nishida, 1977; Ichinosé and Honda, 1978; Honda, 1986). Five milliliters of undiluted, or a $5\times$ dilution of the aqueous or ethanolic extract, was applied to a 15 cm square of chromatography paper (3MM Chr, Whatman). The sheets were semidried at room temperature and hung on the wall (50–60 cm high from the floor) of a wooden cage. Ten female and ten male moths were put together in the cage and the number of eggs laid was counted everyday until the seventh night after emergence. Because water content of host plant is known to be important for stimulation of oviposition behavior in some lepidopteran species (Wolfson, 1980; Stanton and Cook, 1983; Navasero and Ramaswamy, 1993), another series of experiments supplied distilled water to the test sheet using a rolled, wet tissue paper.

Development of the artificial leaf. An artificial leaf was designed (Fig. 2) based on observation of adult behavior as well as on the results for *Manduca sexta* using a surrogate leaf by Sparks (1970). It consisted of a truncated cone disc of styrene foam (1.5 cm thick and 12.5/12 cm in diameter top/bottom) sandwiched between a sheet of filter paper (12.5 cm in diameter, No. 2, Toyo Roshi) and a plastic disc (12 cm in diameter with a 1.5 cm hole at the center). The top of the truncated cone disc was grooved (4 cm in diameter) to hold a gauze cord, so that the leaf extract in the stock vessel could be absorbed through the gauze cord to the filter paper. A disc of carpet (10 cm in diameter, the bottom of which was nylon-sealed) was placed on top, leaving a 1.25 cm-wide band (radius = $12.5/2 - 10/2 = 1.25$ cm) of the filter paper exposed at the outer edge of the artificial leaf. The plastic disc was placed on the bottom for ease of egg collection, since female moths usually lay eggs on the lower surfaces of sweet potato leaves.

To examine the performance of the artificial leaf, egg collection using diluted leaf extracts ($5\times$ and $50\times$ dilution) was compared with collection from a potted sweet potato plant (about

Fig. 2. Artificial leaf for oviposition by adult *A. convulvi*.

15 leaves). Five female and five male moths were put together in the cage and eggs laid were counted everyday until all females died. Mating of females was confirmed by the existence of spermatophores in the *bursa copulatrix*. Each experiment was repeated three times.

In these experiments, 100 ml of the test solution was put into the stock vessel of the artificial leaf at the beginning, and the volume of the solution decreased 2.5–3.5 ml a day in both distilled water and 15% ethanolic extracts. In order to avoid the change of the leaf extract concentration, the filter paper and the test solution were replaced with new ones every 3 days.

Analysis of oviposition behavior using the artificial leaf

1. *Effect of the leaf extract on oviposition.* To examine the effect of leaf phytochemicals on oviposition, oviposition behavior was observed with or without leaf extract as follows. 1) Oviposition of mated moths—one newly emerged female moth was put in a cage and 3–5 males were added on the third night. An artificial leaf containing a 5× diluted leaf extract was placed in the cage on the second night (A), or one containing distilled water was placed in the cage from the second through the fifth night and replaced by a 5× diluted extract on the sixth night (B). 2) Oviposition of unmated moths—one newly emerged female was put in a cage without a male. An artificial leaf was set up as described above. Eggs laid were counted everyday until the female died. Each experiment was repeated two times.

2. *The site of chemoreceptors.* As the chemoreceptors responsible for host discrimination generally exist on antennae and tarsi in butterflies, the sites of chemosensory organs in these hawkmoths were investigated as follows. 1) Ovipositing females were prevented from contact with both volatile and non-volatile phytochemicals by coating antennae (a), tarsi (b) or both sites (c) with fingernail polish. 2) Ovipositing females were allowed contact with only volatile phytochemicals by covering the upper surface of the filter paper with a nylon stocking sandwiched between two plastic nets (12.5 cm in diameter, 1 mm thick, 4 mm mesh), and by sealing the side surface of the artificial leaf with a masking tape (d). In addition to

covering the filter paper, the antennae (e) or the tarsi (f) of ovipositing females were coated with fingernail polish. In these experiments, five female moths were mated on the fourth night as described above, fingernail polish was applied during the following day; and then the artificial leaf containing a 5× diluted leaf extract was placed in the cage. Each experiment was repeated two times.

RESULTS

Oviposition on the leaf extract treated-chromatography papers

Effects of leaf extracts on the oviposition are investigated using a chromatography paper sheet. When the chromatography paper sheets were treated with leaf extracts and semidried, only a few eggs were laid on them, even when treated with the undiluted extract corresponding to 1.6 g fresh leaves/sheet. In contrast, many eggs were laid on the moistened sheets containing leaf, and the aqueous and ethanolic extracts could stimulate oviposition almost equally (80–120 eggs/female). However, the number of eggs laid using a chromatography paper was still fewer than that using a potted host plant (usually, 400–600 eggs/female are expected, Shimoda and Kiguchi, 1995). It seems to be difficult to elicit the oviposition to the full by the chromatography paper method in *A. convolvuli*. As both aqueous and ethanolic extracts were shown to be effective for egg laying, we used the ethanolic extract for subsequent experiments, as it did not require thawing after storage at -20°C .

Development of an artificial leaf

As a basis for the design of an artificial leaf, behavior of mated females of *A. convolvuli* was observed and characterized as follows: 1) alighting on and plunging into a bush of sweet potato plants was often observed during the first hour of darkness and preceding oviposition behavior; 2) they oviposited more frequently while hovering than while sitting on the leaves; 3) fore- and mid-tarsus were usually in contact with the leaf edge during oviposition while hovering; 4) female moths laid eggs on the lower surface of leaves by bending their abdomens when the leaves were about parallel with the

Fig. 3. Oviposition behavior of adult *A. convolvuli* stimulated by the artificial leaf treated with a $5 \times$ diluted leaf extract (equivalent to 0.06 g fresh leaves/ml) (A) and the eggs collected on the plastic disc (B). The structure of the artificial leaf is described in Fig. 2.

ground; and 5) when plant leaves covered a plastic pot, many eggs were laid on the surface of the pot, suggesting that the ovipositor may not be important to recognize the phytochemical stimuli.

Based on these observations and the results using a surrogate leaf with *Manduca sexta*

(Sparks, 1970), we designed a disc-shaped artificial leaf as shown in Fig. 2. Leaf extract was supplemented from a stock vessel to the sandwiched filter paper, such that the exposed 1.25 cm-wide outer edge of the filter paper was always moist. When the artificial leaf containing a $5 \times$ diluted leaf extract was placed in the wooden

box cage, mated females often contacted the filter paper using their tarsi before and during oviposition; and oviposition behavior was remarkably stimulated as shown in Fig. 3A. Most of the eggs were laid on the lower surface of the plastic disc of the artificial leaf (Fig. 3B), although some (usually less than 30% of the total) were laid elsewhere on the artificial leaf (Fig. 3A). There seemed to be no deterrent to oviposition on the egg surface since unusual egg masses accumulated on the plastic disc.

Host plant chemicals were not necessary for mating (Fig. 4A). Though the empty artificial leaf had no stimulating effect on oviposition ((a) in Fig. 4B), oviposition was evoked by either distilled water or 15% ethanol (b, c). The number of eggs laid obviously increased in the presence of diluted leaf extract compared to 15% ethanol alone; and the increase was shown to occur in a dose-dependent manner with respect to extract concentration (d, e). The number of eggs (489 ± 73 /female) laid in the presence of the $5 \times$ diluted extract was not significantly different from that (503 ± 54) obtained using a potted plant (e, f) (at 5% level by *t*-test). Moreover, the percentage of the eggs laid on the artificial leaf in the total oviposition also increased similarly (Fig. 4C); the artificial leaf with a $5 \times$ dilution of leaf extract had more eggs ($88 \pm 5\%$) than a potted plant ($79 \pm 16\%$). The difference in oviposition response among individuals may be worth noting. Some females seemed to be highly stimulated even by distilled water or 15% ethanol alone, as dissection of each female after the end of the experiment revealed that there were large differences in the number of eggs remaining in the abdomen.

Analysis of oviposition behavior using the artificial leaf

1. Effect of the leaf extract on oviposition

The effects of the leaf extract on daily oviposition was investigated using the artificial leaf (Fig. 5). In mated females, most of the eggs were laid within two days after mating (Fig. 5A). When the leaf extract was absent for two days after mating, oviposition was strongly suppressed until the leaf extract was supplied, and fewer eggs were laid (Fig. 5B). In most un-

Fig. 4. Oviposition responses of adult *A. convolvuli* to the artificial leaf compared to a potted plant. Each artificial leaf contained (a) nothing, (b) distilled water, (c) 15% ethanol, (d) $50 \times$ diluted ethanolic extract (equivalent to 0.006 g fresh leaves/ml), or (e) $5 \times$ diluted ethanolic extract (equivalent to 0.06 g/ml). A potted sweet potato plant (about 15 leaves) was used in (f). The ethanol concentrations in (c), (d) and (e) were fixed at 15% for comparison in consideration of the leaf-water content (approximately 80%, Shimoda et al., 1996). Five each of female and male moths were reared in a cage and eggs laid were counted until all females died. Each experiment was repeated for three times. Vertical line indicates the standard deviation.

mated females, less than 150 eggs were laid beginning on the third night and continuing gradually as described using a potted plant (Shimoda and Kiguchi, 1995). Oviposition was also delayed in unmated females until the leaf extract was supplied (data not shown). Almost identical oviposition responses were obtained in the repeated experiments.

Based on the observation using two sets of

Fig. 5. Effect of the sweet potato leaf extract on the oviposition of a mated *A. convolvuli*. One newly emerged female moth was put in a cage and 3–5 males were added on the third night. (A) an artificial leaf containing a 5× diluted ethanolic leaf extract was placed in the rearing cage; (B) an artificial leaf containing distilled water was placed initially and then replaced by a 5× diluted ethanolic leaf extract on the sixth night. Hatched bar indicates presence of distilled water and shaded bar indicates presence of leaf extract. Eggs laid were counted everyday until the female died. The number of eggs laid by a female and the percentage of eggs laid on the artificial leaf in the total oviposition, including eggs laid on the cage wall, are presented in each panel.

infrared camera-video recorder system, the oviposition behavior of *A. convolvuli* on the artificial leaf was characterized as follows: 1) hovering or zigzag flight near the artificial leaf was often observed preceding oviposition behavior; 2) fore-tarsus was usually in contact with the filter paper containing the leaf extract before and during oviposition, but the tarsal drumming was never observed; 3) antennal rubbing on the filter paper was not observed before oviposition; 4) immediately after the alighting on the artificial leaf, when the tarsus touched the filter paper, the abdomen bending started without ovipositor probing.

2. The site of chemoreceptors

The chemosensory organs responsible for oviposition behavior were investigated by

Fig. 6. Effect of coating antennae or tarsi with fingernail polish on the oviposition of *A. convolvuli*. The experimental conditions are written as follows, mesh: the upper surface of the filter paper was covered with a nylon stocking sandwiched between two plastic nets to prevent the moths from touching the leaf extract; ant-coated: antennae were coated with fingernail polish; tarsi-coated: all tarsi were coated with fingernail polish. +: treated; -: no-treated. Five females were kept in a cage and 10–15 males were added on the fourth night in order to fix the mating day. Fingernail polish was applied the day after mating and an artificial leaf with a 5× diluted ethanolic extract was placed in the cage on the third night. The data of control (Cont.) was derived from Fig. 5A.

preventing mated females from coming into contact with the leaf extract in several ways (Fig. 6). The number of eggs laid decreased by coating either the antennae or tarsi with fingernail polish (Fig. 6, panel A-a, b) compared to the control (A-Cont.); and was strongly suppressed by the coating of both sets of organs (A-c). In addition, only 24% eggs of the total oviposition were laid on the artificial leaf by the antennae-coated females (B-a), whereas oviposition was concentrated (82%) on the leaf by the tarsi-coated females (B-b) like the control (92%, B-Cont.). These results suggested that both the antennae and the tarsi were important for chemoreception of phytochemicals and that the selection of oviposition sites was highly dependent on antennae rather than tarsi. When

the nylon stocking prevented contact of the substrate by the contact chemoreceptors, oviposition behavior could still be stimulated (A-d). Although the antennae- and the tarsi-coated females still laid eggs (A-e, f), the percentage of oviposition on the stocking covered-artificial leaf largely decreased by the fingernail polish coating of the antennae (3%, B-e) in contrast to that of tarsi (70%, B-f). Egg-laying on the stocking covered-artificial leaf was strongly suppressed by the coating of antennae (2 eggs, e) in contrast to that of tarsi (53 eggs, f). Similar oviposition responses were obtained in repeated experiments.

DISCUSSION

A large number of studies on host selection and oviposition have been conducted for butterflies. They are attracted by the leaf shape, color or odor of the host plant (Prokopy, 1983; Visser, 1986; Thompson and Pellmyr, 1991; Renwick and Chew, 1994). The most important of the criteria for host discrimination has been shown to be contact chemoreception of the non-volatile secondary metabolites of the plant. This is achieved by "drumming" on the leaf surface with tarsi endowed with chemosensory receptors (Ma and Schoonhoven, 1973; Nishida, 1977; Ichinosé and Honda, 1978; Honda, 1986; Du et al., 1995; Städler et al., 1995; reviewed in Honda, 1995; van Loon, 1996). On the other hand, only a few oviposition stimuli have been identified for moths, although relevant phytochemicals, especially attractants, have been extensively studied (Ramaswamy, 1988; Honda, 1995). This might be due to the difficulties of observing nocturnal behavior, and to the difficulties involved in bioassaying oviposition stimuli in moths. Specifically, oviposition stimuli of moths could not be easily examined using a sheet of filter paper, as for butterflies; and it is suggested that host discrimination in moths is not as highly dependent on contact chemoreception as it is in butterflies. Electro-antennogram (EAG) analysis is considered the most useful method of identifying volatile mediators from host plants (Shimizu and Usui, 1986; van Loon et al., 1992; Baur et al., 1993). However, behavioral evidence is indispensable in defining the exact role of various phyto-

chemicals.

In this study, we have developed an artificial leaf useful for studying phytochemically-mediated oviposition behaviors in *A. convolvuli*. The chemical factors required to stimulate the oviposition sequence can be extracted from fresh sweet potato leaves using distilled water or ethanol. The artificial leaf containing a 0.06 g leaves/ml ethanolic extract fully stimulated females to lay 400–600 eggs/female (equal to the number stimulated by a potted sweet potato plant); and oviposition was stimulated even using a 0.006 g leaves/ml ethanolic extract. Using the artificial leaf, the sequence of phytochemically-mediated oviposition behavior in *A. convolvuli* was elucidated. Oviposition is neither induced in mated nor unmated females until contact with phytochemical stimuli, and moisture at the oviposition site is indispensable to induce oviposition. By fingernail polish coating experiments, it is clarified that tarsi are more important to stimulate oviposition than the antennae, and the selection of oviposition sites during oviposition behavior is highly dependent on the antennae. Because egg-laying could still be stimulated by the stocking covered-artificial leaf, oviposition behavior can be induced by host-plant volatiles alone, without contact-chemoreceptions. The fact that egg-laying on the stocking covered-artificial leaf was suppressed by the coating of antennae indicates chemoreceptors for the volatile oviposition stimuli are located on the antennae. It can be concluded that the phytochemically-mediated oviposition sequence is accomplished through synergistic chemoreception of volatile and non-volatile plant components using antennae and tarsi in *A. convolvuli*.

Generally, volatile components emitted from host plants are involved as attractants for locating the host plants, and subsequent contact chemoreception of less- or non-volatile components is highly significant for host recognition (Honda, 1995). In phytochemical mediation of oviposition in *A. convolvuli*, both volatile and non-volatile factors seemed to synergistically stimulate oviposition behavior although egg-laying was elicited by either type of compound. Volatiles of sweet potato leaves may have dual functions both as attractants and as oviposition

stimulants in this moth, although it is still not clear if these functions are performed by identical compounds. Little in the literature has documented the involvement of host-plant volatiles as oviposition stimuli of butterflies (Honda, 1995). In moths, such a dual function of volatiles was described in *Choristoneura fumiferana* (Städler, 1974) and *Diaphania nitidalis* (Peterson et al., 1994). Besides the plant-specific chemicals, the correlation between leaf water content and oviposition preference was reported in some lepidopterans (*Pieris rapae*: Wolfson, 1980; *Colias philodice eriphyle*: Stanton and Cook, 1983; *Heriothis virescens*: Navasero and Ramaswamy, 1993). Oviposition of *Anadevidia peponis* was promoted by moisture alone, even if host plant chemicals were completely absent (Ichinosé and Sasaki, 1975); as in the results reported in this study. Moreover, the fact that some eggs were laid on the artificial leaf containing only distilled water indicates that the moth is sensitive to water or aqueous vapor, and that the sensory information against water also participates in the stimulation of egg deposition. In order to determine the essential role of various phytochemical mediators, it will be necessary to purify each factor serving as an attractant, repellent, stimulant or deterrent from the leaf extract and to examine the effect of each on behavior.

Due to the high yield and ease of egg collection, the artificial leaf is useful not only for the study of phytochemical mediation, but also for successive maintenance of laboratory colonies of this moth. Egg collection using live plants requires many plants, and is highly influenced by the quality of the plant. In our laboratory, the artificial leaf and 1 l of leaf extract (equivalent to about 250 g fresh leaves) can substitute for the maintenance of 50 pots of plants grown in a green house and used for daily egg collection. Moreover, the eggs collected on the plastic disc are convenient to handle in the laboratory for rearing, sterilization of eggs, etc.

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