Kairomone of the Egg-Larval Parasitoid, *Ascogaster reticulatus* WATANABE (Hymenoptera : Braconidae)

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The host-finding behavior of the egg-larval parasitoid, *Ascogaster reticulatus* WATANABE (Braconidae), was observed and the chemical properties of ovipositional stimulant (kairomone) were examined. Once the female wasp contacted a host egg mass of the smaller tea tortrix (STT) moth, *Adoxophyes* sp., the antennal-host-searching and ovipositor-host-searching behavior were sequentially observed. The chemicals, soluble in polar solvents, on the egg mass were found to be the stimuli to release this behavior. The kairomonal activity was found in a female pupa; meconium, adult excretion, wings, and haemolymph in both sexes but with stronger activity in females. Female abdominal contents and the ovary were most active. The kairomone, extracted with water and 70% ethanol, was heat-stable and consisted of at least two components with different molecular weights. Besides the water-soluble chemical(s), a rather weakly active hexane-soluble chemical(s) was found in the egg-mass extract.

INTRODUCTION

Ascogaster reticulatus WATANABE is an egg-larval parasitoid of the smaller tea tortrix (STT) moth, *Adoxophyes* sp. (Tortricidae), which is an important pest of tea plant. This is the second most dominant species in Shizuoka Pref. among the hymenopterous parasitoids of STT (TAKAGI, 1974). Little work has been reported on the oviposition behavior or the cues leading the female wasp to the host, or on egg masses of STT. In our observations on the oviposition behavior of female *A. reticulatus*, she responded with the same behavior to an egg-mass as to a glass surface coated with the surface material of an egg mass. These data suggest that the female wasp responds to the chemical(s) (kairomone) on the egg-mass surface of STT. On an egg-larval parasitoid, *Chelonus texanus* CRESSON, VINSON (1975) reported the presence

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of chemical(s) associated with host eggs, which elicit antennal-host-searching and ovipositor-host-searching behavior.

In this study with *A. reticulatus*, the host-finding and oviposition behavior were observed, and the sources of active material(s) (kairomone) were investigated. The chemical properties of kairomone were also preliminarily analyzed.

MATERIALS AND METHODS

Insects. Female wasps and host egg masses used in the experiments were obtained from a laboratory colony reared under $25\pm 1^{\circ}\text{C}$, 30–50% R. H., 16–8 LD photoperiodic conditions, by the modified method of TAMAKI (1966).

Behavioral observation. The host-finding and oviposition behavioral patterns were observed in a 9-cm petri dish. An egg mass of STT was placed at the center of the petri dish and a female wasp was introduced apart from the egg mass, in order to observe her behavior.

Bioassay of kairomone. Sample solution (ca. 5 μl) was absorbed onto a filter paper disc (6 mm in diam., Toyo filter paper® No. 2), and dried with a hair dryer. The filter paper disc was introduced into a 15-ml sample vial (2.4 cm in diam., 5 cm long) and placed at the center of the side wall of the vial with a female wasp (3 to 5 days old); a cotton plug was inserted as a stopper. When the wasp contacted the filter paper disc, her behavior was observed for two minutes and classified as antennal-host-searching (score=1), ovipositor-host-searching (score=2), or no response (score=0). The activity of the sample was expressed in terms of the average score of several parasitoids' response. Bioassays were conducted under $25\pm 1^{\circ}\text{C}$, 30–50% R.H. with fluorescent light conditions, at the middle of the light period (from 4 hrs after light-on to 4 hrs before light-off), when the searching and ovipositional activities of the wasp were high (HIGUCHI and TAMAKI, unpublished).

Solubility experiment. Eight solvents of different polarity such as distilled water, 70% aqueous ethanol, butanol, isopropanol, tetrahydrofuran, acetone, chloroform and methylene chloride were used. Ten egg masses were extracted with 1 ml of each solvent for two days and the supernatant was bioassayed at a concentration of 5.0×10^{-2} egg-mass equivalent (EME) for one paper disc.

Sources of kairomone. Various parts of the host from larval to adult stages were bioassayed to locate the source(s) of the kairomone. A last-instar larva was killed with ether and directly presented to the wasp. The haemolymph of a larva was absorbed onto the filter paper disc and bioassayed. One milliliter of larval frass collected from feeding boxes was dissolved in 1 ml of distilled water and the supernatant was bioassayed. A pupa was directly given to the wasp, and the haemolymph was absorbed onto a filter paper disc, dried and bioassayed. Meconium and adult excretion were collected on a filter paper (9 cm in diam.) placed on the bottom of a 9-cm petri dish with a pupa. After emergence, the colored part with the excretion was cut (6 mm in diam.) and bioassayed. Wings cut from adults on the 1st and 3rd day after emergence were directly bioassayed. Adult haemolymph was collected by amputation between head and thorax and absorbed onto a filter paper disc. An adult female's abdomen was cut and the ovary was removed. The abdominal tip contents and ovary were separately excised, rinsed with water, dried and bioassayed. Male abdominal tip contents were excised and similarly treated for bioassay.

Collection and extraction of egg masses. Aqueous ethanol (70%) was used as an extracting solvent for egg masses. Egg masses of STT, collected on glass plates, were dipped into 70% ethanol in an extracting bath for one or two days under room temperature (ca. 20°C). The extract (35,200 egg masses/5-l 70% EtOH) was concentrated to 150 ml under reduced pressure. Five grams of Celite 545® were mixed with the extract and the mixture was filtered. The filtrate was concentrated and filled up to 100 ml (EM extract I). The residue with Celite 545® was dried at room temperature, and 1 g (=6,560 EME) was suspended in 328 ml of distilled water. The supernatant (0.10 EME/disc) and the concentrated egg-mass extract (EM extract I) serially diluted with distilled water (1.76×10^{-5} to 10^{-1} EME/disc) were bioassayed.

Long term extraction. Since the egg-mass residue was still active (score=2.0) after extraction with 70% ethanol for a few days, long term extraction was adopted. Egg masses on sheets of paraffin paper were dipped into 70% ethanol for one or two months at room temperature (ca. 20°C), the extract (10,690 EME/2-l 70% EtOH) was filtered, and the filtrate concentrated to 50 ml (EM extract II). This concentrated extract was serially diluted (1.07×10^{-5} to 10^{-1} EME/disc) for bioassay and the activity was compared with EM extract I.

Hexane extraction. Egg masses (14,840) were extracted with about 1.5 l of 70% ethanol for 10 to 12 months at room temperature (ca. 25°C). The extract was concentrated to 60 ml under reduced pressure and shaken with 60 ml of hexane in a separatory funnel. The hexane layer was further shaken three times with the same volume of distilled water. A small amount of anhydrous Na_2SO_4 was added to the hexane layer and kept overnight in a refrigerator (5°C). The hexane extract was filled up in a 10-ml volumetric flask and subjected to a serial dilution for bioassay (7.42×10^{-3} to 10^0 EME/disc). The first aqueous layer was also concentrated to 20 ml and serially diluted for bioassay (3.71×10^{-4} to 10^{-2} EME/disc).

Thermal stability experiment. Twenty egg masses of STT on paraffin paper were dipped into 10 ml of distilled water in a test tube and kept at 100°C in a water bath for 1 hr. The extract was bioassayed (1.0×10^{-2} EME/disc). The egg-mass residue after washing with water and drying with a hair dryer was directly given to the wasp for bioassay.

In another experiment, 10 ml of water solution of EM extract I (3.52 EME/ml) was put into two test tubes. One was kept at 100°C in a water bath for 1 hr, the other was kept at room temperature (ca. 20°C) as control. After the sample cooled, the activity of each sample was compared in bioassay.

Sephadex G-25 column chromatography. One milliliter of EM extract I (=352 EME) was loaded on a Sephadex G-25 (fine) column (2.5×100 cm) and 650 ml of distilled water was passed through the column (flow rate: 0.4 ml/min). The eluate was collected with a fraction collector (5 ml/fraction), and every second or third fraction was bioassayed. Optical density at 280 nm was determined using every other fraction with a spectrophotometer (Hitachi Model 101). The recovery was examined in a bioassay by collecting the whole eluate.

RESULTS

Behavioral observation

From the observation on the host-finding behavior of a female wasp confined in

a 9-cm petri dish, she seemed to walk randomly with an active movement of antennae until she contacted an egg mass. There seemed to be no attraction of the wasp to the egg mass from a distance by means of visual or chemical stimuli.

Once the wasp contacted an egg mass with her antennae, she walked back and forth over it several times, palpating it with her antennae (antennal-host-searching), then she moved slowly forward, probing it with her ovipositor in a downward movement until reaching a suitable oviposition site (ovipositor-host-searching). The ovipositor-host-searching and oviposition were carried out with her antennae extended forward. She spent about one hour ovipositing on each egg mass.

Solubility experiment

As shown in Fig. 1, female wasps showed different degrees of response, i.e., antennal-host-searching and ovipositor-host-searching behavior to filter paper discs treated with various egg-mass extracts. The polar solvent extracts had stronger activity than the non-polar solvent extracts.

Fig. 1. Solubility of the kairomone into eight different solvents. Concentration: 5.0×10^{-2} EME (egg-mass equivalent)/disc. : antennal-host-searching. : ovipositor-host-searching.

Sources of kairomone

The response of the wasp to each part of the host is shown in Table 1. Female wasps showed no response to any of the body surface of a host larva, a filter paper disc treated with haemolymph, or a water extract of larval frass; nor did the pupal surface elicit any activity. However, haemolymph of a female pupa had slight activity, eliciting antennal-host-searching of one wasp out of five. Meconium and adult excretion had some activity with that of the female occasioning the stronger response of the wasp. Wings of the female moth had greater activity than the male's and this activity increased with age. Female abdominal tip contents and ovary had the greatest activity among all host organs; those of the male abdominal had less activity.

Collection and extraction of egg masses

The water solution of the precipitate with Celite 545® was inactive. The response curves of EM extract I are shown in Fig. 2. Antennal-host-searching behavior

Table 1. Response of the female *A. reticulatus* to various body parts of the host (STT) at different stages

Materials	Sex	Days after emergence	No. wasps tested	Mean score ^a
Last instar larva	—	—	10	0
Haemolymph	—	—	10	0
Frass extract	—	—	5	0
Pupa	♀	—	5	0
	♂	—	5	0
Haemolymph	♀	—	5	0.20
	♂	—	5	0
Meconium or adult excretion	♀	—	18	1.33
	♂	—	14	0.86
Wings	♀	1	10	1.10
		3	10	1.30
	♂	1	9	0.11
		3	9	0.89
Adult haemolymph	♀	1	10	0.80
		3	10	1.70
	♂	1	9	0
		3	9	0.44
Abdominal tip (without ovary)	♀	1	10	2.0
		3	10	2.0
Ovary	♀	1	10	1.60
		3	10	2.0
Abdominal tip	♂	1	9	0.44
		3	9	0.33
Egg mass	—	—	10	2.0

^a No response: score=0, antennal-host-searching: score=1, ovipositor-host-searching: score=2.

was elicited by the diluted egg-mass extract at 1.76×10^{-4} EME/disc and 100% response was obtained at 1.76×10^{-2} EME/disc. Fifty percent behavioral response (BR₅₀) was obtained at ca. 8.5×10^{-4} EME/disc. Ovipositor-host-searching behavior was elicited at 1.76×10^{-3} EME/disc and reached 100% at 1.76×10^{-1} EME/disc. BR₅₀ was ca. 9.8×10^{-3} EME/disc.

Long term extraction

A comparison of the activity at antennal-host-searching level (score=1) on the response curves of EM extract I and II, showed the latter to be approximately 3.2 times as active as the former.

Hexane extraction

The response curve of the hexane layer and that of the aqueous layer are shown in Fig. 3. The hexane-soluble substance(s) in 70% ethanol extract of egg masses elicited low response in the wasp, mostly antennal-host-searching. Only one wasp out of eleven showed ovipositor-host-searching behavior at 7.42 EME/disc. The slope of response curve of the hexane layer differed from that of the aqueous layer. In another experiment with different egg-mass extract, the activity of the hexane layer was not

Fig. 2. Response curves of the egg-mass extract (EM extract I). $\blacktriangle \cdots \blacktriangle$: antenno-host-searching. $\bullet \cdots \bullet$: ovipositor-host-searching. Numeral in parenthesis indicates number of insects tested.

Fig. 3. Response curves of the hexane-soluble active substance(s) (hexane layer) and the water-soluble one(s) (aqueous layer).

transferred to the aqueous layer even after washing 7 times with distilled water.

Thermal stability experiment

The egg-mass extract with hot water was rather active (average score=1.6, n=5) and the egg-mass residue did not lose activity (average score=2.0, n=5). Egg-mass extract solution heated at 100°C for 1 hr remained highly active (average score=2.0, n=5) and did not lose any activity compared with the unheated control sample (average score=2.0, n=5).

Sephadex G-25 column chromatography

The activity of eluates from Sephadex G-25 column chromatography is shown with the UV absorbance in Fig. 4. There were two peaks of activity, the second being correlated with the peak optical density at 280 nm and the yellowish color peak

Fig. 4. Sephadex G-25 column chromatography of the crude egg-mass extract. Column : Sephadex G-25 (fine), 2.5×100 cm. Sample : EM extract I, 1 ml. Eluent : distilled water. Flow rate : 0.4 ml/min. Parasitoid for bioassay : 3-6 females/fraction.

at fraction No. 65. The recovery of the whole eluate was very low (ca. 23%) compared with the loaded sample at the same concentration level (3.51×10^{-3} EME/disc).

DISCUSSION

Among various stimuli involved in the host selection of parasitoids, chemicals appear to play a major role in each level of the process, as pointed out by VINSON (1976). In this study, only stimuli in the host-location process in the steps divided by DOUTT (1959) were dealt with.

From the observation of host-finding and oviposition of *A. reticulatus* females, the host egg mass seemed to have no specific color or odor which attracted the parasitoids. The chemical(s) on egg masses were shown to elicit the sequence of behavior, i.e., antennal-host-searching and ovipositor-host-searching in the solubility experiment. Color and texture of the egg-mass surface were not important, since the wasp showed ovipositor-host-searching behavior on a filter paper disc coated with the egg-mass extract. However, the oviposition behavior in response to the filter paper disc lasted only a few minutes, even when the disc was treated with a high concentration of the egg-mass extract. This may be due to the lack of physical and/or chemical stimuli on or in the egg mass. Sensilla on the ovipositor or on its sheath may be responsible for the perception of these stimuli. Some work has been reported on the sense organs on the ovipositor of parasitoids (DETHIER, 1947; KING and RAFAI, 1970; GANESALINGAM, 1972; HAWKE et al., 1973; GREANY et al., 1977).

The kairomone in a host's body parts emerged from the pupal stage of a female *Adoxophyes* sp. and increased with maturity. Judging from the highest activity in the abdominal tip contents of a female moth, the kairomone on the egg mass seemed to have originated from the organs around the ovary. Similar results were reported by VINSON (1975) with the egg-larval parasitoid, *Chelonus texanus*. Organs specific to a female host may not however be the sources of the kairomone, since the activity was also found in male body parts.

The activity of the body parts of an adult moth increased from the 1st to 3rd day

after emergence. This increase in the amount of active chemical(s) may correlate with maturity of the adult organ(s) associated with egg production. Since scales of STT were proved to have some activity (KAINOH and TAMAKI, 1982), the adult excretion and scales deposited at the time of oviposition may become the clues for parasitoids to locate the vicinity of the host egg mass.

Scales from the adult female *Heliothis* were proved to have some activity, eliciting increased walking and turning activity and rapid antennal movements by *C. texanus* (VINSON, 1975). The scales left by the host moth were demonstrated to have a host-seeking stimulant for the egg parasite, *Trichogramma evanescens* (LEWIS et al., 1972). In the case of *A. reticulatus*, the chemical(s) on scales may be the same as that on egg masses, since both are derived from an adult female moth and are alike in solubility (KAINOH, unpublished).

From the response curves of EM extract I, both the antennal-host-searching and ovipositor-host-searching behavior were suggested to be sequentially triggered depending on the concentration of the egg-mass extract.

Hexane-soluble active substance(s) was also found to exist in egg masses. The different slopes of the response curves indicate two different active substances, i.e., the water-soluble and the hexane-soluble. The function of this hexane-soluble substance(s) is not known, since the activity was very low in the 15-ml sample vial bioassay.

From the solubility experiment and the thermal stability experiment, the main chemical(s) which elicit oviposition behavior of *A. reticulatus* are water-soluble and heat-stable, and are comprised of at least two active components with different molecular weights. Functions of these two components are not known. Both the antennal-host-searching and ovipositor-host-searching behavior were elicited by the concentrated eluate of each active fraction. The low recovery of eluate may be due to adsorption or inactivation of the active chemical(s) through chromatography.

There are some chemicals identified as kairomones for parasitoids involved in the host-location and host-acceptance steps, though they are mostly for larval parasitoids. These are, for example, 13-methylhentriacontane from the larval frass of *Heliothis zea* for *Microplitis croceipes* (JONES et al., 1971), heptanoic acid from the frass of the potato tuberworm for *Orgilus lepidus* (HENDRY et al., 1973), and methyl-branched, saturated hydrocarbons from the mandibular gland of *Heliothis virescens* for *Cardiochiles nigriceps* (VINSON et al., 1975).

As for the egg parasitoids, tricosane was identified from moth scales as a kairomone for *Trichogramma evanescens* (JONES et al., 1973). However, little work has been reported on kairomones for egg-larval parasitoids except for *Chelonus texanus* (VINSON, 1975) in which some chemical properties were described. Since the kairomones of *C. texanus* are as water-soluble and heat-stable as that of *A. reticulatus*, these chemicals may be analogous; this hypothesis is supported by the kairomone sources of both species. Further purification is required to identify the chemicals.

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