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RAPID COMMUNICATION

Olfactory responses of the parasitoid wasp, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) to odors from hosts and stored grain

Masoomeh Moosavi, Nooshin Zandi-Sohani*, Ali Rajabpour

Plant Protection Department, Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran

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*Corresponding address:
zandi@asnrukh.ac.ir

Abstract

The ability of parasitoids in locating hosts determines their success in suppressing the pest population. Chemical stimuli emitted from food products and hosts provoke the searching behavior of parasitoids. *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) is a generalist idiobiont ectoparasitoid of coleopteran pests in stored products. In the current study, the behavioral responses of *A. calandrae* females were evaluated regarding host food and different life stages of the host, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae), using a Y-tube olfactometer. The parasitoid was offered uninfested chickpea kernels, damaged chickpea without larvae of *C. maculatus*, damaged chickpea with preferred stage (4th instar) larvae of *C. maculatus*, uninfested chickpea + *C. maculatus* adults, and eggs of *C. maculatus* on chickpea. In another test, the preference of *A. calandrae* for either damaged chickpea without larva of *C. maculatus* or damaged chickpea with non-preferred stage (1st instar) larvae of *C. maculatus* was studied. The results showed that the females did not prefer uninfested chickpea kernels and adults of *C. maculatus*. However, they were attracted to damaged kernels with or without larvae, and the kernels containing eggs of *C. maculatus*. When the female parasitoids had a choice between damaged chickpea without larva of *C. maculatus* and damaged chickpea with 1st instar larva, they did not prefer one over the other. The results of this investigation can be helpful for using *A. calandrae* as a biological control agent in stored products.

Keywords: attraction, *Callosobruchus maculatus*, parasitoid, volatiles

Introduction

Post-harvest insect pests can seriously affect stored products and reduce their quantity and quality in different ways (Lee *et al.* 2003). In grain-based commodities, quality reduction occurs through contamination of the product via feces, transmission of microorganisms, and grain warming, all of which provide suitable conditions for molds (Gorham 1979). Fumigation with phosphin and methyl bromide have been two common methods for stored-product protection during previous years (Rajendran and Sriranjini 2008). However, after reporting some problems such as insect resistance and ozon-depleting activity for both insecticides, the consumption of grains treated with these insecticides has been restricted (El-Aziz 2011). Biological control is one of the desirable strategies to reduce reliance on traditional chemical insecticides (Schöller *et al.* 2018).

Several species of parasitoids and predators develop in stored product ecosystems (Brower *et al.* 1996) and they have many advantages over traditional chemical insecticides. After being released, natural enemies usually continue to reproduce on their hosts and do not leave any detrimental residue. They also can be eliminated from grains by normal cleaning methods (Schöller *et al.* 2006). Wasps from the families Braconidae, Ichneumonidae, Pteromalidae, and Bethylidae are prevalent parasitoids among stored product pests (El-Aziz 2011). *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) is a widely distributed species which is frequently found in stored grain. Various species of stored product pests including *Sitophilus granarius* L., *S. zaemais* (Motschulsky), *Rhyzopertha domonica* (F.), *Acanthoscelides obtectus* (Say), *Lasioderma serricorne* (F.) and *Callosobruchus maculatus* (F.) are attacked and parasitized by *A. calandrae* (Schöller *et al.* 2018). This parasitoid is an idibiont, primary ectoparasitoid and the females attack and paralyze late-instar larvae of the host to lay an egg on their cuticle (Shin *et al.* 1994).

Natural enemies usually use semiochemicals as necessary signals to predict the location of their host or prey habitat over long distances and their microhabitat over short distances (Tinzaara *et al.* 2005; Dicke and Baldwin 2010). Chemical orientation has a key role in host-habitat location and host location by female parasitoids. The searching process may be provoked by olfactory cues derived from the host plant, the host, or a combination of both (Reed *et al.* 1995). Host plant or host food volatiles may attract some parasitoids including Pteromalidae species even if there is no host, host damage, or host derived material. Therefore, the parasitoids seeking hosts' habitat may use volatile signals from uninfested plants as well as from the infested plants (Nordlund *et al.* 1988). However, the ability to differentiate olfactory cues of infested plants from healthy ones has been reported in some parasitoids (Steidle and Schöller 1997).

There are several reports on the response of pteromalid parasitoid species to olfactory cues related to the host or host's habitat (Germinara *et al.* 2009; Belda and Riudavets 2010; Niedermayer *et al.* 2016; Germinara *et al.* 2016; Sitthichaiyakul and Amornsak 2017). Host substrate preferences by *Teocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) have been found attacking larvae of the maize weevil (Sitthichaiyakul and Amornsak 2017). The electroantennographic responses of both sexes of *T. elegans* to various volatile compounds exploited from different grains and cereals were also investigated (Germinara *et al.* 2009). *Anisopteromalus calandare* females were evaluated for detection of olfactory cues associated with different host species (*S. oryzae* L., *R. domonica*, *Tribolium confusum* Jacqueline duVal and *L. serricorne*) and host food products (rice and flour) (Belda and Riudavets 2010). The ability of *A. calandrae* to detect populations of *Sitophilus granarius* L. in grain residues was estimated under laboratory and storage-like conditions (Niedermayer *et al.* 2016).

The ability of a parasitoid to find host and host-habitat locations has a direct effect on its success in suppressing the pest population. Therefore, this study was designed to compare the behavioral response of *A. calandrae* females to different odors emitted from uninfested bean seeds and beans infested with eggs, adults, 4th instar larvae of *C. maculatus* and also damaged beans without any larva, using a Y-tube olfactometer. We also investigated which *A. calandrae* choose, damaged beans without any larvae or non-preferred larval stage of the host.

Materials and Methods

Insect host and parasitoid

In this study, we used *C. maculatus* obtained from stock cultures maintained in a laboratory and reared on cowpea *Vigna unguiculata* L. for several generations. Plastic containers (8 cm diameter × 20 cm) half filled with uninfested cowpea with proper ventilation was used for insect rearing. Colonies were maintained at 30 ± 1°C, 65 ± 5% RH (relative humidity) in the dark. *Anisopteromalus calandrae* cultures were obtained from stock culture and maintained on 4th instar larvae of *C. maculatus* in cowpea under the same conditions as for *C. maculatus*. Both insects were mass reared in the entomology laboratory of the Plant Protection Department, Agricultural Sciences and Natural Resources University of Khuzestan, Iran.

Olfactory bioassays

A Y-tube olfactometer was used to evaluate the response of *A. calandrae* females towards odors coming from hosts and host food. The olfactometer was made up of a Y-shaped plastic tube with an inner diameter of 2 cm. The length of the base tube and each arm was 20 cm. For the experiments a plastic container was connected to each end of the arms for putting different odor source treatments. An air flow of 4 liters per minute was transmitted to each arm using an electric pump and a flowmeter was used to transfer the flow evenly between the two arms. The ends of the tubes inside the containers were covered with fine mesh to stop insects entering the tubes (Fig. 1).

Before starting the experiments, 3–4 day-old females of *A. calandrae* with oviposition experience were maintained separately for 10 h, without a host for oviposition. During isolation they were fed an 80% diluted honey solution. To conduct the experiments one female parasitoid was placed at the entrance of the base tube of the olfactometer and the odor source treatments were put inside the containers. After the

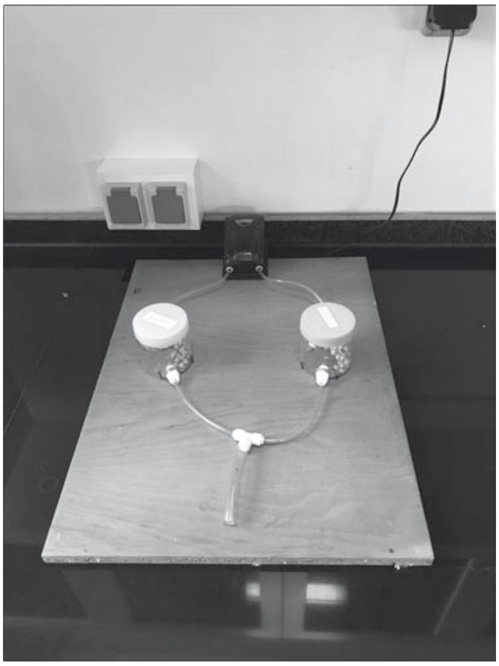

Fig. 1. Y-tube olfactometer used in the study

A. calandrae walked inside, it was given 20 min to choose its favorite container with preferred odor or stay in the base tube. The female was then removed using an aspirator and replaced with another wasp. One hundred females were used in these trials with five replicates and their responses were recorded. After experimenting with 10 wasps the containers were switched to prevent any possible asymmetry in the experimental set-up. All the experiments were conducted at room temperature around 25 ± 5°C and 65 ± 5% RH. The amount of medium used as an odor source for the experiments was 50 g in all trials.

The female wasps could choose from the following odor source treatments:

1. Uninfested chickpea vs. empty control.
2. Uninfested chickpea vs. damaged chickpea without larva of *C. maculatus*.
3. Uninfested chickpea vs. damaged chickpea with preferred stage (4th instar) larva of *C. maculatus*.
4. Uninfested chickpea vs. uninfested chickpea + + *C. maculatus* adults.
5. Uninfested chickpea vs. eggs of *C. maculatus* on chickpea.
6. Damaged chickpea without larva of *C. maculatus* vs. damaged chickpea with non-preferred stage (1st instar) larva of *C. maculatus*.

Statistical analysis

Data from olfactory experiments were analyzed and compared by chi-square test using SPSS software version 20. The wasps which did not respond to odors during the experimental period (20 min) were not statistically analyzed.

Results

Behavioral responses to different odors

Responses of *A. calandrae* females to odors from different sources of stimuli are shown in Table 1. Odors from uninfested chickpea did not attract females of *A. calandrae* compared to the empty control ($p = 0.468$). On the other hand, damaged chickpea without larva of *C. maculatus* ($p < 0.001$), damaged chickpea with preferred stage (4th instar) larva of *C. maculatus* ($p = 0.001$), and chickpea kernels with *C. maculatus* eggs on them ($p < 0.0001$) caused a significant attraction in female wasps when compared to uninfested chickpea. The interaction of *A. calandrae* females when presented with uninfested chickpea *versus C. maculatus* adults on uninfested chickpea showed that they did not have any preference for either of the choices ($p = 0.053$).

In another trial, *A. calandrae* was offered a choice of damaged chickpea without larva of *C. maculatus* or

Table 1. Responses of *Anisopteromalus calandrae* females when choosing between: a – uninfested chickpea, b – empty control, c – damaged chickpea without larva of *Callosobruchus maculatus*, d – damaged chickpea with preferred stage (4th instar) larva of *C. maculatus*, e – uninfested chickpea + *C. maculatus* adults, f – eggs of *C. maculatus* on chickpea

Source of odor		Total no. of insects tested	χ^2	N(A)	N(B)	N(0)	*p*
a	b	100	4.59	41	52	7	0.468
a	c	100	23.80	32	63	5	<0.001
a	d	100	18.30	33	64	3	0.001
a	e	100	9.19	39	53	8	0.053
a	f	100	40.00	9	85	6	<0.0001

N(A) – number of females choosing uninfested chickpea (a); N(B) – number of females choosing treatments (b), (c), (d), (e), and (f); N(0) – number of females without any response to odors

Table 2. Response of *Anisopteromalus calandrae* females when choosing between: a – damaged chickpea without larva of *Callosobruchus maculatus* and b – damaged chickpea with non-preferred stage (1st instar) larva of *C. maculatus*

Source of odor		Total no. of insects tested	χ^2	N(A)	N(B)	N(0)	*P*
a	b	100	9.19	38	53	9	0.053

N(A) – number of females choosing treatment (a); N(B) – number of females choosing treatment (b); N(0) – number of females without any response to odors

damaged chickpea with non-preferred stage (1st instar) larva of *C. maculatus*. The *A. calandrae* females had no strong preference for odors emitted from either arm of the olfactometer ($p = 0.053$). Of 100 females tested, 38 females were attracted to damaged chickpea with no larva inside the kernel, 53 wasps were attracted to chickpeas containing 1st instar larva of host, and 9 females were not able to select a treatment (Table 2).

Discussion

The results of the current study showed that *A. calandrae* females were able to find host insects and distinguish between damaged and healthy host products in a Y-tube olfactometer. *Anisopteromalus calandrae* females did not receive any cue from uninfested chickpea in comparison to the empty control. According to Steidle *et al.* (2001) who worked on another Pteromalidae species, *Lariphagus distinguendus* Forster, undamaged rice odors did not attract the females when offered as an alternative to the empty control which is consistent with our results. However, in previous studies conducted on *Aenasius bambawalei* Hayat using other healthy food products like brown rice, paddy rice, and white flour, the females were able to recognize host food products (Belda and Riudavets 2010). The differences of our results with those reported in previous studies may be due to various factors e.g. differences in environmental conditions, pest species, parasitoid geographical strain, and grain volume (Steidle and Schöller 2002). Moreover, different host species and host products may affect the ability of a parasitoid to locate its hosts, because they release different blends of volatiles (Lo Pinto *et al.* 2004). Therefore, it may be possible that either chickpea kernels do not release any specific volatile to attract *A. calandrae* females or the wasp is not able to distinguish and respond to such cues.

In our study, female wasps were attracted to damaged chickpea kernels without *C. maculatus* larvae. This can be explained by the fact that in damaged products the emitted volatiles could have changed and as a result the parasitoid can better detect the damaged product than the healthy one (Powell *et al.* 1998; Turlings *et al.* 1998). Moreover, the presence of larvae feces inside the damaged kernels may be the source of some volatiles which affects the attraction of female parasitoids (Steidle *et al.* 2001; Steiner *et al.* 2007).

The findings of this study also demonstrated the preference of *A. calandrae* females for kernels infested with eggs and 4th instar larvae of *C. maculatus*. The attraction of *A. calandrae* females to larvae of *S. oryzae*, *Ryzopertha dominica* (F.), *T. confusum*, and *Lasioderma sericornae* (F.) are in line with previous studies conducted on *A. calandrae* (Belda and Riudavets 2010), and other parasitoid wasps such as *L. distinguendus* (Steidle and Scholler 1997; Steidle 2000), and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) (Jembere *et al.* 2003). Semiochemicals emitted from the mandibular secretions of larvae, feces and also the exoskeletons of the previous larval stages inside the kernels are the main sources of *A. calandrae* attraction to larvae of *C. maculatus* (Bender *et al.* 2020). It has been shown that in some parasitoid wasps, plant synomones induced by oviposition of the pest, have been used as a cue to find the host (Conti and Colazza 2012). This subject can explain the preference of *A. calandrae* females for chickpea kernels oviposited by *C. maculatus*.

In the experiments with adult stage *C. maculatus* which was compared to the uninfested cowpea, *A. calandrae* females preferred neither *C. maculatus* adults nor uninfested cowpea which is in accordance with the results of Belda and Riudavets (2010). This result indicated that *C. maculatus* adults did not exude any attractive volatile for the female wasps. The Y-tube olfactometer tests on the attractiveness of adults of the Curculionidae family for *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) showed that the female parasitoid was attracted to adults of *Listronotus oregonensis* LeConte. In this case, cues such as kairomones produced during ecdysis and adult pheromones may be used for parasitoid orientation (Cournoyer and Boivin 2004). Considering that *M. hyperodae* is a parasitoid of the adult stage of the curculionidae family, its response to cues from the adult stage is plausible.

In conclusion, *A. calandrae* females may either use the kairomones that are directly released from the herbivore body and its secretions or synomones which are produced by plant materials to find the location of *C. maculatus*. Results of behavioral bioassays confirmed that uninfested products were not attractive to *A. calandrae* females. Damaged kernels with or without larvae, and those with *C. maculatus* eggs on them were more attractive than healthy kernels and the adult stage of *C. maculatus* was not attractive to the wasps.

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