



# Attraction of the parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) to odors from grain and stored product pests in a Y-tube olfactometer

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## ABSTRACT

Pests that affect stored products can be very harmful; they can affect stock, damage products by reducing their weight or by contaminating them, and consequently affect a brand's reputation. As the range of permitted chemical products is being progressively reduced, biological control is a tool that is being increasingly developed. *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) is a well-known ectoparasitoid that attacks late-instar larvae of several stored product pests. The current study evaluates the host preference of female parasitoids, regarding host species, stages and host food products, using a vertical Y-tube olfactometer. Parasitoids were offered late-instar larvae and adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Tribolium confusum* (Jacqueline duVal) (Coleoptera: Tenebrionidae) and *Lasioderma serricornis* (F.) (Coleoptera: Anobiidae) and host food products (rice and flour). They preferred uninfested host food products (rice or flour) to empty controls and uninfested paddy rice to uninfested brown rice, while wheat flour was clearly more attractive to them than brown rice. For all the host species, larvae-infested products were more attractive than uninfested ones and than adults of the same species, except in the case of *T. confusum*. When larvae-infested products of different species were compared among themselves, different levels of attractiveness were observed. Adult stages were not attractive in any of the trials. This preference evaluation aimed to assess the potential of using parasitoids as effective biological control agents in stored product companies.

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## 1. Introduction

Stored product pests can be very damaging to grain and grain-based commodities for a variety of reasons. They can cause direct losses in product weight and also indirect losses: through contamination via their feces; by them acting as vectors for microorganisms; and by them warming the grain, which can lead to problems with moulds (Gorham, 1979). Merely finding live or dead insects in a product can be an important issue. Insect infestations can damage a company's image by giving its brand a bad reputation. This can negatively affect its custom and income, and also cause legal problems (through non-compliance with legislation) (Benz, 1987). These problems, together with the development of insecticide resistance (Haliscak and Beeman, 1983; Zettler and Cuperus, 1990; Zettler and Beeman, 1991) and the progressive reduction in the number of chemical products permitted for controlling these pests, make biological control an increasingly desirable strategy (Brower et al., 1991).

*Anisopteromalus calandrae* (Howard) is a well-known cosmopolitan parasitoid of Coleoptera which infests stored products (Schöller et al., 2006). *A. calandrae* was chosen for these experiments due

to its natural occurrence, its abundance in the sampled locations (Riudavets et al., 2002), and the fact that it is known to attack a wide variety of hosts, including, amongst others: *Sitophilus oryzae* (L.) (rice weevil) (Cline et al., 1985; Press and Mullen, 1992; Lucas and Riudavets, 2002), *Sitophilus granarius* (L.) (granary weevil) (Ghani and Sweetman, 1955), *Sitophilus zeamais* Motsch (maize weevil) (Wen and Brower, 1994), *Rhyzopertha dominica* (F.) (lesser grain borer) (Ahmed, 1996; Menon et al., 2002), and *Lasioderma serricornis* (F.) (Ahmed and Khatun, 1988). This idiobiont, primary ectoparasitoid attacks late-instar larvae (Shin et al., 1994) that grow internally or concealed within the host food (Ghimire and Phillips, 2007). By piercing the host larva with her ovipositor, the female parasitoid paralyzes it and lays one egg outside the integument, which will develop on the body fluids of the host larvae (Ahmed, 1996). Usually only one parasitoid develops from each host larva (Arbogast and Mullen, 1990). Preference studies have been carried out with other parasitoids of the Pteromalidae family, such as *Lariophagus distinguendus* (Steidle and Schöller, 1997; Steidle et al., 2001, 2003) and *Choetosiphila elegans* (Williams and Floyd, 1971), which are parasitizing different stored product pests.

Parasitoid behavior, with respect to searching for a host, is influenced by several factors in a cereal storage ecosystem. These factors range from environmental conditions (Hong and Ryoo, 1991) to host density and food availability (Steidle and Schöller, 2002;

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Shin et al., 1994). In a stored product facility, hosts remain in silos, grain bulks or piles, so they are relatively easy to locate and attack. Temperatures remain constant, which also facilitates parasitoid survival in the storage ecosystem. Among Pteromalidae species, the cues used to find hosts range from chemical stimuli (Ambriz et al., 1996) to visual, vibrational (Meyhöfer and Casas, 1999) and tactile signals (Vinson, 1976; Steidle, 2000). Parasitoids can respond to cues coming from their herbivorous host species or from the different plants on which their hosts feed (Steidle and Schöller, 1997; Steiner et al., 2007). There can be long-distance or short-distance cues (Rojas et al., 2006). On one hand, long-distance or long range cues would tend to be the first cues used by the parasitoid in its orientation towards its host and they are usually emitted by the host food or host plant (Vinson, 1976; Jember et al., 2003) providing information about the status of the host plant (Vet and Dicke, 1992). Parasitoids can also use short-distance cues. These typically come from the host and are used to locate a specific, or preferred, host stage and attack it (Vet and Dicke, 1992; Ambriz et al., 1996).

Different settings can be used to understand how parasitoids locate their hosts. These include: still air olfactometers (Van Tol et al., 2002), one-way olfactometers (Burkholder, 1970), Y-tube olfactometers (Rojas et al., 2006; Wei and Kang, 2006), four arm olfactometers (Giles et al., 1996; Steidle et al., 2001), wind tunnels (Steinberg et al., 1992) and other techniques (Mohan and Fields, 2002). The most appropriate setting to use depends on the insect species and its behavior (Steinberg et al., 1992), and on the type of cues that are to be studied (Vet et al., 1983). The choice of the Y-tube olfactometer for the current set of experiments was mainly determined by the behavior of *A. calandreae*. The horizontal setting used in many other studies was changed to a vertical setting.

The main pest species that occur naturally, abundantly and often together in a grain storage ecosystem are: *S. oryzae*, *R. dominica*, *L. serricornis* (F.) and *Tribolium confusum* (Jacqueline duVal) (Riudavets et al., 2002). The first three species are known hosts, but *T. confusum* had not previously been reported as a host for *A. calandreae*. Determining parasitoid performance in front of this particular range of species can be critical for the use of *A. calandreae* in biological control. Moreover, assessing the influence of the host on which the parasitoid is reared is also important, because this is known to affect the response towards other species (Steidle and Schöller, 2002; Cournoyer and Boivin, 2004). Many studies can be found on coleopteran pests with other Pteromalidae parasitoid species (Steidle and Schöller, 1997, 2002; Steidle, 2000; Steidle et al., 2003; Steiner et al., 2007), but not on the species tested in this study with *A. calandreae* or in a Y-tube olfactometer.

The main objective was to evaluate whether *A. calandreae* females could detect specific olfactory cues associated with different host species, life stages, and host food products in the vertical setting of the Y-tube olfactometer. Host species used were *S. oryzae*, *R. dominica*, *L. serricornis* (F.) and *T. confusum* (Jacqueline duVal). These species were chosen in order to assess whether the parasitoid strain that we were rearing in our laboratory would respond to them in the setting of a vertical Y-tube olfactometer, as all of these species except *T. confusum* were known hosts for this parasitoid.

## 2. Materials and methods

### 2.1. Insect hosts

The four stored product pest species used in these trials were obtained from stock cultures maintained in the laboratory. The host stages used in the trials were late-instar larvae, as this was the stage preferred by the parasitoids (Shin et al., 1994; Ahmed, 1996), although adults were also included. Adult stages were tested to evaluate, on one hand, whether the presence of the adult stage would modify the parasitoid response, and on the other, to

determine whether the parasitoid would be able to use adult volatiles as an indirect way of locating larval stages once the host food product had been located. *S. oryzae* and *R. dominica* were reared on brown rice, while *T. confusum* and *L. serricornis* were reared on a mixture of white wheat flour and yeast (7%). All the colonies were kept under controlled conditions of  $25 \pm 2$  °C and  $70 \pm 10\%$  RH, with a 16:8 (L:D) photoperiod.

### 2.2. Parasitoids

*Anisopteromalus calandreae* adult females were obtained from stock cultures maintained in the laboratory, which had originally been collected from grain silos near Barcelona, Spain. *A. calandreae* were reared at  $25 \pm 2$  °C and  $70 \pm 10\%$  RH on rice weevils (*S. oryzae*) in brown rice. The ages of the females used in the test ranged from 1 to 8 days. They were reared on *S. oryzae* and previous oviposition on this species was supposed for all wasps. Prior to starting the trials, adult *A. calandreae* females were kept in isolation, i.e. without host for ovipositing, for 10–12 h. This condition could have affected parasitoid starvation. The parasitoids were therefore given sugary water but deprived of hosts. In synovigenic females, responsiveness to hosts or product volatiles may vary according to the time that has passed since their last oviposition (Gauthier and Monge, 1999). As previous oviposition had been impossible for 12 h due to isolation, the predisposition of the females to oviposit was presumed high and it was expected that the parasitoids would be motivated to search for hosts.

### 2.3. Vertical Y-tube bioassay

The intrinsic performance of adult *A. calandreae* females towards odors coming from host and host food products was assessed in the vertical setting of a Y-tube olfactometer. Preliminary experiments showed that when the olfactometer was placed horizontally, the insects did not respond and often moved round in circles in the same place. Based on these results and on other reports in the literature (Steidle and Schöller, 2002; Press, 1988), we decided to repeat the test with the olfactometer in the vertical position. The results obtained showed that wasps then moved, and moved faster, so the vertical position was used for the trials. The Y-tube olfactometer consisted of a Y-shaped glass tube with arms whose inner diameter was 3.5 cm; each arm was 17 cm long. The angle between the two arms of the olfactometer was 75°, and the angle between each arm and the main body was 145°. Each of the upper arms of the olfactometer was connected to an air pump (Nathura, ECIS, Bessanvido, Italy) which produced a constant air current flowing from the arms of the tube to the base. Air flow was controlled, using a hot wire anemometer (TESTO, Barcelona, Spain), at the ends of both pump tubes prior to the air entering the arms of the olfactometer and was maintained at  $2.70 \pm 0.10$  m/s. The air flow that exited the base of the olfactometer, having passed through the olfactometer, was maintained at  $0.20 \pm 0.02$  m/s. A glass jar was connected to each arm of the olfactometer using plastic tubing (0.5 cm diameter). The lid of each jar contained an air inlet tube, which connected the air pump to the jar, and an outlet tube, which connected the jar to the olfactometer arm. The outlet tubes were covered with gauze (0.3 mm mesh size) inside the jar lid to prevent insects from entering the tubes. Different odor source treatments (four host species, two life stages, and host food: rice or flour) were placed inside the jars for the experiments (Tables 1–3).

To start the trial, an *A. calandreae* female was allowed to walk onto a meshed lid placed at the base of the olfactometer. We then waited until the female had walked off the lid and into the olfactometer arm. The lid was then removed for the rest of the trial and the chronometer was started. Each individual was observed until either it had moved a third of the way up one of the olfac-

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