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Behavioral and physiological responses of the bean weevil *Zabrotes subfasciatus* to intraspecific competition

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ABSTRACT

Zabrotes subfasciatus uses *Phaseolus vulgaris* seeds as its main host and reacts to variations in the availability of seeds by adjusting egg size and number. When faced to choose among different hosts, however, this insect shows the following preference hierarchy: bean > soy > lentil > chickpea, meaning that it is quite able to use species initially rejected as hosts for oviposition. Here, we investigated how this insect would react when placed in environments with different insect densities and allowed to choose between two types of seeds with contrasting levels of acceptability as substrates for oviposition. The experimental setting compared four levels of resource competition (density) by placing 1, 2, 4, or 6 couples within a Petri dish with 2 grains of *P. vulgaris* and 2 grains of *Cicer arietinum*. During the 7-day oviposition period, the following four behaviors were identified and quantified: fleeing, mating, inspecting *P. vulgaris*, and inspecting *C. arietinum*. We correlated these behaviors with fecundity. The most common behavior revealed by correspondence analyses plots was fleeing (searching for a new resource), followed by inspecting and mating ($p < 0.001$). Increasing competition diminished the inspecting behavior (of both hosts), reduced oviposition on *P. vulgaris* (the preferred host) and increased oviposition on *C. arietinum* (the less-preferred host), promoted fleeing and augmented the number of eggs glued to Petri dishes (egg dumping, $p < 0.0001$). Mating occurred mainly in situations of intermediate density. We previously demonstrated the existence of a preference hierarchy for different hosts and showed that artificial selection increases the oviposition preference for the less-preferred host. Here, we found that fleeing and egg dumping are the main strategies for avoiding competition and that these behaviors may support niche broadening in *Z. subfasciatus*.

1. Introduction

Competition among animals rises when they have common needs and limited recourses. Because competition influences life-history strategies, it also acts as a driving force in behavior evolution. Competition for the same resources may involve members of different species or may occur among individuals of the same species. In some species, intraspecific competition is a key driver of adaptive radiation because it shapes strategic life-history traits (Calsbeek and Cox, 2010). Competition in closed systems promotes additional strategies (Smallegange and Tregenza, 2008). In these circumstances, competitors are restricted to specific niches from which they cannot escape, such as benthic invertebrates that have sessile adult forms and brood feeding in some parasitoid/parasitic insects (Müller et al., 1990; Harvey et al., 2013).

Intraspecific competition is frequently experienced by seed weevils during reproduction and development. Seed weevil larvae have little to no mobility. Because the immatures are forced to feed exclusively on the host chosen by their mothers, the choice of oviposition site is a key aspect of female behavior (Teixeira and Zucoloto, 2012). The bean weevil *Zabrotes subfasciatus* (capital

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breeding) feeds on stored grains and thus has great economic and social relevance because its main host is the common bean *Phaseolus vulgaris,* a major source of protein in Latin America and Africa. *Z. subfasciatus* females lay eggs and attach them to the seed surfaces. After hatching, the young larvae burrow into the seed (Howe and Currie, 1964), where they feed and undergo four larval stages before pupating. After pupation, the new adults use their mandibles to bite a window into the seed to emerge, and after a few hours, females mate and lay eggs; adults normally do not feed (Schwartz and Pastor-Corrales, 1989). Because a single female may lay several eggs on the same seed, the oviposition behavior of *Z. subfasciatus* determines the amount and quality of food the immature and future adults will receive (competition).

Z. subfasciatus uses *P. vulgaris* seeds as its main host, but when given a choice among different host species it shows the following preference hierarchy: bean > soy > lentil > chickpea. It is able to use species initially rejected as hosts for oviposition (Teixeira and Zucoloto, 2003; Teixeira et al., 2008). As expected, *Z. subfasciatus* performs differently in different leguminous seeds (Teixeira et al., 2008). Oliveira et al. (2015) found higher reproductive outputs from adults in situations with lower competition density and found better performance on cranberry beans than on kidney beans.

Phytophagous insects—including seed weevils—vary in their ability to use different host plants. For example, it has been suggested that the genus *Stator* shows conserved ecological interactions. In contrast, another weevil genus, *Curculio*, seems to be characterized by members that show non-conserved ecological interactions (Gómez et al., 2010; see also the case of the genus *Mimosestes*, Kato et al., 2010). Several factors have been identified as possibly influencing insects' choice of hosts. These include the presence of some hydroxycinnamic acid derivatives for *Papilio machaon*, a swallowtail butterfly (Murphy and Feeny, 2006), the insecticidal protein arcelin of *P. vulgaris* for *Acanthoscelides obtectus* (Velten et al., 2008) and also, in certain strains, for *Z. subfasciatus* (Minney et al., 1990), the *C. arietinum* seed surface (texture) and seed coat thickness, as well as lectins that confer resistance to *Callosobruchus maculatus* (Erler et al., 2009; Karbache et al., 2011). Thus, host shifts, host use, or adaptation to new hosts might be permitted by the overexpression of certain detoxifying enzymes, which in the aphid *Myzus persicae* results from gene amplification and mutations in the regulatory region (Bass et al., 2013).

Seed weevils such as *Z. subfasciatus* can be exposed to environments such as seed warehouses that have alternating periods of abundance and scarcity. During periods of low seed availability, the weevils face high levels of competition for oviposition sites, which influences oviposition behavior. We have previously shown by artificial selection that *Z. subfasciatus* has a genetic background that enables it to adjust its life-history traits to exhibit higher fitness in crowded conditions (Teixeira and Zucoloto, 2012). Females react to variations in the availability of *P. vulgaris* seeds by adjusting both egg size and number. Faced with low bean seed density, females lay larger and fewer eggs than when bean seed density is high, when smaller adults are produced (Teixeira et al., 2009).

Here, we aimed to determine the behavioral patterns of *Z. subfasciatus* individuals facing varying levels of host availability. Specifically, we investigated how individuals of *Z. subfasciatus* would react when placed in environments with different insect density levels and given the option to choose between two types of seeds with contrasting levels of acceptability as oviposition substrates. The experimental setup allowed us to identify and quantify four common behaviors during oviposition: fleeing, mating, inspecting *P. vulgaris* and inspecting *C. arietinum.* We also quantified fecundity. These behavioral patterns may represent the physiological underpinnings of niche broadening (host expansion) in this insect species. Understanding these behaviors may also help in designing management strategies for the biological control of this insect, thereby reducing stored grain damage from infestations.

2. Materials and methods

2.1. Weevil populations

Wild *Z. subfasciatus* (Coleoptera; Polyphaga; Cucujiformia; Chrysomeloidea; Chrysomelidae; Bruchinae; Amblycerini) individuals were originally obtained from infested seeds of *P. vulgaris* (variety "carioquinha") collected in houses and stores in the Ribeirão Preto region, São Paulo, Brazil (21° 05′-21° 15′ S and 47° 50′-47° 55′ W). Populations of thousands of individuals were cultured in a laboratory environment for approximately twenty years, periodically (approximately every three months) incorporating new wild individuals. The level of genetic differentiation in the Brazilian populations of this species is low, and its geographic structure is weak (Souza et al., 2008). For more information on the life history of this insect, see Bondar (1937), Teixeira et al. (2009), Teixeira and Gris (2011), and Teixeira and Zucoloto (2012).

2.2. Experimental procedure

Experimental units of *Z. subfasciatus* were established using insects taken from the laboratory population described above and maintained on different levels of host seed availability under controlled temperature (29 ± 2 °C) and humidity (70%) conditions. Newly emerged (within 24 h) females and males of *Z. subfasciatus* were placed in pairs into 90 × 15 mm glass Petri dishes with *P. vulgaris* and *C. arietinum* seeds.

To test the effect of different levels of insect density on *Z. subfasciatus* behavior, we created four different treatment situations by placing two seeds of each host into each Petri dish and increasing the density by varying the number of insect couples (a female and a male), using 1, 2, 4 and 6 couples. We have previously shown that each female may lay all her eggs (approximately 32) on only one seed (we have found 63 eggs on a single seed; Teixeira and Zucoloto, 2012). Each of these experimental conditions had ten replicates (n = 10). Seeds from two host species with contrasting acceptability were used: *P. vulgaris* and *C. arietinum* (Teixeira et al, 2008). The seeds were fresh and had previously been examined for any signs of imperfection. After a previous observation of the females' behavior we selected the following behaviors because of their biological implications:

- Host preference: represented by the number of eggs laid on each host seed species.
- Fleeing: when insects avoided the seeds and tried to escape from the dish.
- Mating: when a male was on a female.
- Inspecting: when insects walked over the seeds.

The frequency of these behaviors was estimated daily by direct observation and counting every 20 min during a 10 h period each day (~30 times per day) during the approximately 7 days of the oviposition period of the insects within each experimental unit of 1, 2, 4 or 6 couples. Increased insect density was expected to increase the level of competition for oviposition sites and to reduce the resources available for larval development (see Teixeira et al., 2008). After the 7th day of the oviposition period, we counted the numbers of eggs laid on seeds and Petri dishes, as a measure of fecundity.