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To Smell you Better: Prior Food Deprivation Increases Herbivore Insect Responsiveness to Host Plant Odor Cues

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Abstract Hunger plays a crucial role in insect feeding behavior, however food deprivation is rarely considered when insect responses to plant host and related chemical stimuli are investigated. Here we assessed, by means of experiments with Y-tube olfactometer, the effect of food deprivation time on the response of a specialist (*Xanthogaleruca luteola*) and a generalist (*Diabrotica speciosa)* herbivore beetle species (Coleoptera: Chrysomelidae) to odor cues of their respective host plants. Increasing food deprivation periods enhanced responses to host plant odor in both species, with insects remaining for longer in the olfactometer arm carrying plant odor than in the control, moving less frequently between olfactometer arms, and being more efficient in moving towards the plant odor as their first choice. These trends were less significant in the generalist species, which also required a longer fasting threshold (48 h) in comparison with the specialist (8 h). Our results, showing that prior food deprivation time can influence insect herbivore responsiveness to plant stimuli and that those effects may vary between species, highlight the risk of neglecting this factor in studies involving insect responses to host or chemical stimuli.

Keywords Odor cues · food deprivation · olfactometer · feeding behavior · herbivore

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Introduction

Insect feeding is regulated by complex interactions of extrinsic and intrinsic factors among which hunger plays a crucial role, with the tendency to feed being greatly reduced immediately after a meal (Browne and Withers 2002). Food deprivation might even supersede other controls of feeding behavior, such as previous experience (Ramírez and Niemeyer 2000; Browne and Withers 2002) or genetic background (Brevault and Quilici 1999). Therefore, hungry individuals are likely to behave differently towards food than satiated conspecifics (Szyszko et al. 2004).

For herbivore insects, food quality is highly variable and frequently suboptimal (Behmer 2009) to the point that food deprivation might be a common physiological state in the group (Carey et al. 2002). In order to feed, herbivore insects must follow a series of stereotyped behavioral modules, among which host plant finding is the first step (Knolhoff and Heckel 2014). Information from chemical (McIntyre and Vaughn 1997; Johnson and Nielsen 2012), visual (Brevault and Quilici 1999; Davidson et al. 2006) or multiple sensorial inputs (Behmer et al. 2005; Patt and Sétamou 2007) is usually involved in this process. Besides extrinsic factors like odor-mediated host plant quality or habitat background odor (e.g. Wäschke et al. 2015), intrinsic factors like hunger seem to determine herbivore responses to host plant volatiles. Insects that have been entirely deprived of food, or fed nutritionally poor or otherwise inadequate diets, frequently alter their host searching behavior in order to increase the probability of finding a suitable food source (Simpson et al. 1995; Browne and Withers 2002). For example, insects may increase their locomotor activity or change their search patterns and direction of movement (Nagata and Nagasawa 2006; Behmer 2009), and the magnitude of those changes may depend on the time elapsed since last feeding (Bernays and Wcislo 1994; Brevault and Quilici 1999; Browne and Withers 2002).

There is evidence that host plant acceptance can be influenced by food deprivation prior to the test (Browne and Withers 2002; Vera et al. 2016), and such influence may be particularly important in short term trials. Nonetheless, insect responses to plant host and related chemical stimuli such as antifeedants are usually investigated without attention to food deprivation time, or using arbitrary periods (e.g. Ninkovic and Pettersson 2003; Nguyen 2008; Hall et al. 2015; Vera et al. 2016). Furthermore, this factor was not addressed in recent reviews of physiological or behavioral aspects of herbivore insect feeding (Chapman 2003; Behmer 2009; Knolhoff and Heckel 2014).

Here we assessed, by means of experiments with Y-tube olfactometer, the effect of food deprivation time on the response of two beetle species (Coleoptera: Chrysomelidae) to odor cues of their respective host plants. We selected for this study two species with different levels of food specificity: the specialized elm leaf beetle *Xanthogaleruca luteola* (Müller), which feeds exclusively on elm trees, and the generalist *Diabrotica speciosa* (Germ.), feeding on at least 21 plant families (Christensen 1943). We expected insects to be more responsive, thus moving more frequently towards the plant odor (i.e. into the olfactometer test arm) at their first choice and settling there, as fasting time increased. Moreover, since specialist herbivores usually respond to appropriate specific host cues whereas generalists need to process a variety of sensorial inputs from the array of potential host plants (Bernays et al. 2000; Akhtar and Isman 2004), we expected behavioral trends linked to food deprivation levels to be clearer in specialist insects in comparison with generalists.

Materials and Methods

Insects and Food Deprivation Treatments

Adults of *D. speciosa* and *X. luteola*, fed with lettuce (*Lactuca sativa* L) and elm (*Ulmus pumila* L.) leaves, respectively, were kept in cubic cages (wood, glass and voile, 30 cm on each side) at 26 ± 2 °C temperature, with photoperiod 12:12 (L:D) and 75 ± 5 % relative humidity, prior to their use in the experiments. Food deprivation treatments consisted of groups of ten to twelve individuals randomly selected from the cages and placed in plastic containers (20 cm high × 7 cm diameter), with imbibed cotton wool pieces to ensure water provision but without food, during 3, 8, 24, 48 or 72 h or until most individuals responded positively to food odor in the olfactometer test (see below). Similar groups, placed in identical conditions but with ad lib food provision (lettuce or elm leaves), acted as non-fasted controls.

Bioassays in Y-Tube Olfactometer

We used a glass Y-tube olfactometer (5 cm long lateral arms, 13 cm long central arm, 1 cm internal diameter) to study insect responses to host plant odor in relation to food deprivation time. A strip of filter paper (1.5 × 0.5 cm) impregnated with ground leaves in order to avoid visual attractant stimuli (Gish and Inbar 2006), was placed within an acetate tube (2.5 cm long, 1 cm internal diameter, closed with a mesh plug to avoid contact with the insect) at the end of one lateral arm, from here on called test (or plant) arm. A strip of paper imbibed with distilled water was held within another acetate tube and fitted at the apical end of the opposite arm, hereafter control arm. An air current (1000 cm^3 / min.) was induced by an extracting bomb at the base of the central arm, through a mesh plug. The olfactometer was positioned horizontally on a laboratory countertop (at 23 ± 2 °C) with uniform illumination from bright fluorescent lights; experiments were conducted between 10:00 and 17:00 h. After the filter paper strips were treated and placed inside the corresponding lateral arm, we released a beetle into the central arm of the olfactometer by removing the mesh plug, which was then repositioned and the extracting bomb was turned on to induce the air flow through the apparatus. Designated test and control arms of the olfactometer were alternated following each trial, after washing and drying the apparatus and each individual insect was tested only once, in order to avoid pseudoreplication.

We estimated the following variables: i) time (in seconds) that each individual beetle spent in each olfactometer arm during a 10-min period; ii) total number of times each insect changed from one arm to the other during the observation period, as a mobility indicator and iii) the percentage of individuals initially moving to the test arm, as an indicator of efficiency in decision making.

Statistical Analyses

In order to search for a possible food deprivation threshold in insect response to host odor, we compared the time that beetles spent in test versus control arms of the Y-tube olfactometer, at each food deprivation level, by using paired *t*-test, or Wilcoxon signed-rank test when data were not normally distributed. Mobility, i.e. the frequency of armchanging movements, was compared among different levels of food deprivation by means of Generalized Linear Models, using a log-link function and quasi-poisson error distribution to deal with data overdispersion. We evaluated the significance of the estimated parameters using likelihood ratio tests, performing a posteriori Tukey tests to evaluate differences between treatments. These analyses were performed using R 3.0.2 (R Development Core Team 2013). To assess whether efficiency, i.e. the percentage of beetles selecting the test arm in their first choice, increased with the level of food deprivation, we used the Cochran Armitage trend test and calculated exact p values using StatXact 8 software (Cytel Software Corporation 2007).

Results

Individuals of *X. luteola* unfed for three hours showed a marginally significant tendency to stay for longer in the control than in the test arm ($t = 2.28$, $p = 0.056$). Instead, after 8 h of food deprivation, the beetles remained 8 times longer in test than in control arms ($Z = 2.10$, $p = 0.035$) while those fasted for 24 h spent their time almost exclusively ($Z = 2.54$, $p = 0.011$) in the olfactometer test arm where the host odor was released (Fig. 1). Individuals of *D. speciosa* spent as much time in test as in control arms when unfed for 3 h ($t = -0.026$, $p = 0.979$), 8 h ($t = 0.62$, $p = 0.540$) and even 24 h ($t = -0.98$, $p = 0.347$). However, individuals deprived of food for 48 or 72 h stayed almost three times longer in test than in control arms (Fig. 1) although there was large

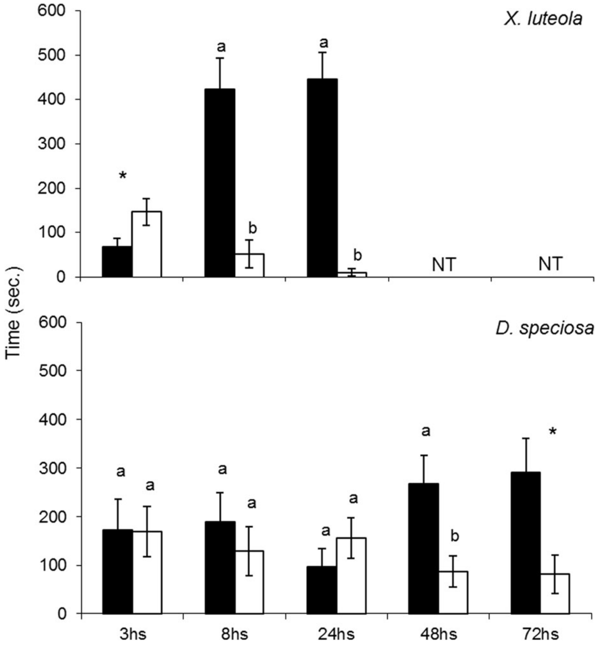

Fig. 1 Mean (and SE) time spent by *X. luteola* (*upper panel*) and *D. speciosa* (lower panel) in host (■) and control (□) arm of olfactometer at different time periods from the last meal. Means with the same letter do not differ significantly (*: marginally nonsignificant, $0.05 < p < 0.1$). NT: not tested

variability among individuals and the difference was only marginally significant in the last case ($t = 2.17$, $p = 0.046$ and $t = 0.11$, $p = 0.057$, respectively).

Mobility was also affected by food deprivation time in *X. luteola* ($F_{2,22} = 13.07$, $p < 0.001$). Beetles that were not offered food during 3 h prior to the test, moved between arms nearly five times more than those deprived of food for 8 or 24 h (Fig. 2). By contrast, no significant effects ($F_{4,59} = 0.821$, $p = 0.517$) of starvation on mobility were observed in *D. speciosa* beetles, despite a tendency to fewer changes between olfactometer arms at increasing time since feeding (Fig. 2).

Efficiency in decision making (Fig. 3) increased with the length of the food deprivation period. The percentage of *X. luteola* individuals choosing the test arm as their first choice increased four-fold after 24 h starvation in comparison with insects unfed for 3 h ($Z^2 = 8$, $p = 0.020$), whereas a more moderate yet significant increase was observed after 72 h starvation in *D. speciosa* ($Z^2 = 50$, $p = 0.017$), in comparison with recently fed specimens (3 h).

Discussion

Internal factors such as hunger may have a strong impact on insect behavior (Ramírez and Niemeyer 2000; Nagata and Nagasawa 2006), however this is rarely considered when testing insect feeding choices. Our study has shown that time elapsed since last

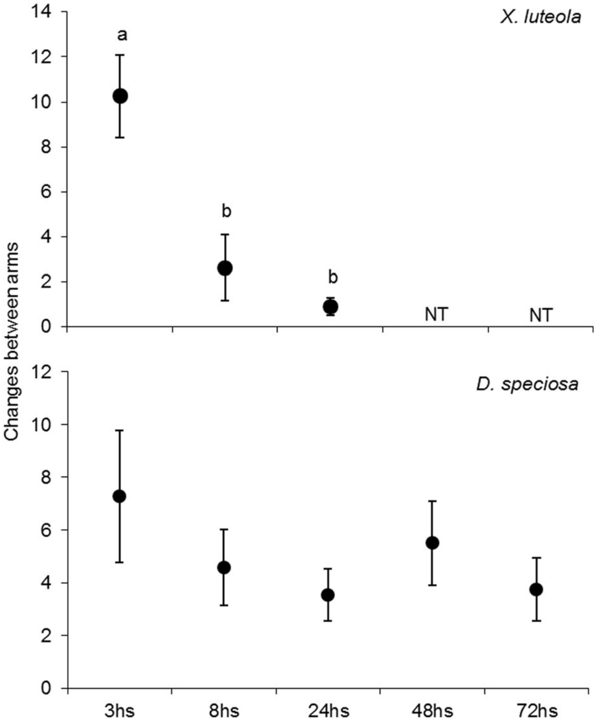

Fig. 2 Mean (and SE) number of changes between olfactometer arms performed by *X. luteola* (*upper panel*) and *D. speciosa* (*lower panel*) within 10 min trials, at different time periods from the last meal. NT: not tested

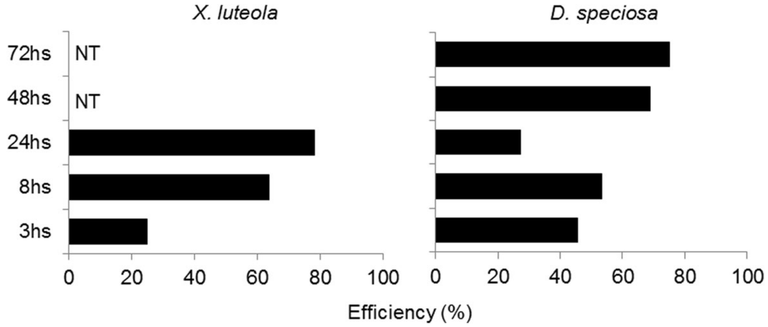

Fig. 3 Efficiency, as indicated by the percentage of individuals of *X. luteola* (*left*) and *D. speciosa* (*right*) selecting the olfactometer host arm as their first choice, at different time periods from the last meal. NT: not tested

feeding can directly influence insect herbivore responsiveness to host plant odor cues, particularly for specialists.

Individuals of both *X. luteola* and *D. speciosa* responded positively to host plant olfactory cues, by remaining for longer in the olfactometer arm carrying plant odor than in the control, but only after a fasting threshold was reached. Such threshold varied between species: responsiveness in the specialized *X. luteola* was shown after barely 8 h of food deprivation, whereas 48 h were required for *D. speciosa.* Thus, food deprivation could trigger an increase in sensitivity to host plant stimuli as proposed by Bernays and Chapman (1994). Instead, insects recently fed may be unresponsive to cues from their hosts (Withers et al. 2000) or even move away from food sources in order to carry out other activities (Bernays and Chapman 1994). The latter possibility could explain the tendency of recently fed elm leaf beetles to remain in the control arm of the olfactometer.

In addition to changes in time allocation to different parts of the olfactometer, fasting influenced the efficiency of decision making in both species. As the length of time since last feeding increased, a larger fraction of insects took the "right" decision by making their first move towards their host plant odor, thus reaffirming the idea of increased responsiveness (Davidson et al. 2006).

Insects deprived of food are expected to increase their locomotion activity in order to improve their chances of finding food, which has been supported by some studies (Bernays and Wcislo 1994; Szyszko et al. 2004; Nagata and Nagasawa 2006) but not by others (McIntyre and Vaughn 1997; Roabacker and Fraser 2001). In our study, fasting for 24 h effectively resulted in an order of magnitude decrease in *X. luteola* mobility, with specimens moving between olfactometer arms just once instead of the average 10 arm changes observed in recently fed individuals. Thus, food deprivation over 8 to 24 h did not only improve elm leaf beetle orientation toward its host plant as mentioned above, but also favored its settling. On the other hand, mobility of *D. speciosa* was not altered by feeding state. In other words, fasting improved these beetles' ability to make their first move towards plant odor and they remained for longer near the odor source, but without actually settling there. Such restlessness could be linked to the lack of visual or contact stimuli following olfactory detection. Besides olfactory cues, host searching frequently involves additional visual stimuli (Gish and Inbar 2006) and contact chemical assessment (Knolhoff and Heckel 2014), which insects in our tests were (by methodological design) unable to carry out, so they continued searching.

The observed differences between *X. luteola* and *D. speciosa* could be related to their relative feeding strategies involving a trade-off between host plant perception and neural processing limitations (Schäpers et al. 2015). Acquiring and processing host plant information would be more effective in specialists like the elm leaf beetle, which need to deal with a narrower range of stimuli (Bernays et al. 2000; Schäpers et al. 2015) and tend to be more efficient decision makers (Nylin et al. 2000; but see Tosh et al. 2003) in comparison with generalists like *D. speciosa*. For example, the specialist *X. luteola* is so highly tuned to its host plant that it can differentiate olfactory cues from individual plants showing different damage levels (Meiners et al. 2005).

Our results, showing that food deprivation may influence herbivore insect responses to plant stimuli and that such effects may vary among species, highlight the risk of neglecting this aspect in studies involving insect feeding behavior. For example, a search on the Scopus database on 2014–2015 publications testing herbivore insect responses to odor cues through olfactometer assays (keywords: olfactometer AND insect*) revealed that, out of 34 studies, only in one case the starvation level at which insects became responsive was previously assessed. Instead, 18 studies employed satiated specimens or their feeding status was unspecified, whereas in 15 studies the insects were starved for an arbitrary time period. We suggest that food deprivation levels need to be considered when interpreting insect responses to host or chemical stimuli, since satiated insects may be unresponsive whereas on the other extreme, too much hunger can have harmful physiological effects on the insect and impact orientation. Particularly, this should become standard practice when assessing feeding deterrent compounds for pest management, since false negative results may arise from lack of response to food stimuli if testing satiated insects.

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