ECOLOGY, BEHAVIOR AND BIONOMICS

Adult Carbohydrate Feeding Affects Reproduction of *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

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ABSTRACT - Reproduction of most insects depend on nutrients accumulated during the larval stage, but many lepidopteran species will also depend on nutrients obtained at the adult stage. Feeding at the adult stage allows the intake of carbohydrate and amino acid rich solutions, which may have an effect on the species reproduction and population growth. The objectives of the current study were to characterize the effects of sugar consumption by adults of the potato tuber moth, *Phthorimaea operculella* (Zeller), on its reproduction. To initially test the food intake by adults, a 10% honey solution or water (control), both containing a liquid dye were offered to adult insects 24h after emergence, and the presence of the dye was observed by analysis of their digestive system. The effects of adult feeding on a 10% honey solution on the reproductive performance of *P. operculella* were evaluated by assessing the oviposition rate, fecundity and fertility. Adult feeding was proved by the presence of the dye within the digestive system of adults of *P. operculella*. Although the oviposition rate and fertility were not affected by adult feeding, female fecundity was higher in honey-fed females as compared to the water-fed females.

KEY WORDS: Color dye, digestive system, ovarian development, reproductive system

The potato tuber moth, *Phthorimaea operculella* (Zeller), is considered one of the main causes of potato losses during harvesting and storage. The pest is present all year round and its larvae damage the leaves, twigs and tubers. It is also a pest to several other cultivated Solanaceae species, such as eggplant, pepper, tobacco and tomato (Filgueira 2003a, b). According to Pratissoli *et al* (2003), the adults oviposit generally up to ten days after mating, and the adult life cycle may be extended to 43 days, with females surviving for longer periods.

Although food resources for reproduction of most insects depend on the nutrients accumulated during the larval stage, many lepidopteran species show feeding habits in the adult stage (Chapman 1998). Food intake at the adult stage allows the utilization of carbohydrate and amino acid rich solutions which may be necessary to complete the development of the reproductive system. In the field, flower nectar is the main carbohydrate source, although there may be other sources, such as the extrafloral nectar and hemipteran honeydew (Romeis & Wäckers 2002). No information on how *P. operculella* ingests carbohydrate-rich sources is available. It is assumed that, as in other species of the same order, sugar ingestion by adults comes mainly from the nectar of flowers.

Several techniques for insect marking are utilized in ecological studies. They play fundamental roles in field assays, such as dispersion analyses, capture and recapture and in nutritional and phenological studies. These techniques include the use of fluorescent markers or dyes, which allow easier identification, but in addition they must have the lowest possible influence over the performed studies (Hagler & Jackson 2001).

The reproductive potential of lepidopterans is influenced by the insect's lifecycle, nutritional status and development of reproductive organs, all of them influenced by physiological events regulated by hormonal levels (Parra *et al* 1999, Cole *et al* 2002).

Hormones play a major role in regulating protein synthesis in the reproductive tissues of insects, including Lepidoptera (Sorge *et al* 2000), and the possibility to alter the reproductive capacity of adults by feeding them hormone analogs, such as the ecdysteroid agonist tebufenozide, has been exploited (Smagghe & Degheele 1994).

The objectives of this study were to verify if adults of *P. operculella* feed on sugar solutions and the effect of sugar consumption on their reproductive fitness, as a step towards the development of hormone-based baits to control de reproductive capacity of this pest in field conditions.

Material and Methods

Insect rearing. A stock colony was initiated with insects

obtained from infested potatoes, *Solanum tuberosum* L., collected after harvesting. Rearing was conducted following procedures established by Pratissoli *et al* (2003), under controlled conditions (25 ± 1°C, 70 ± 10% RH, and 14h photophase). The obtained pupae were transferred into cages covered with a white organza cloth until adult emergence. Then, 24h-old adults were transferred to a cylindrical PVC rearing cage (15 cm diameter, 10 cm height) covered by a circular piece of filter paper sitting on top of a fine mesh net. A potato slice was placed on top of the filter paper, and replaced every other day to stimulate oviposition. A 10% honey solution added of 0.03% hydroxybenzoate methyl ester as an anticontaminant was offered to the insects by capillarity via a dental roll wick.

The eggs oviposited on the filter paper were kept in Petri dishes under controlled conditions, and were further used to inoculate surface-drilled potatoes upon larva eclosion to maintain the colony.

Insects to be used in the experiments were isolated at their pupal stage in small test tubes (10 mm x 75 mm), covered with transparent PVC plastic film, and adults were sexed upon emergence according to the morphology of the external genitalia.

Food consumption. In order to observe the occurrence of carbohydrate consumption by *P. operculella*, 20 virgin males and 20 virgin females collected 24h after emergence were placed into the rearing cage and fed a 10% honey solution containing glucose, fructose, 0.03% p-hydroxybenzoic acid methyl ester (propyl paraben) and a liquid food dye (blue anise). Evaluations were carried out three days after releasing the insects into the rearing cage. Confirmation of consumption was assessed by analysis of the digestive system contents, guided by the presence or absence of the dye.

Reproductive performance. In order to assess the reproductive performance of *P. operculella*, a completely randomized experimental design was chosen, with two treatments and 25 replicates. Each replicate consisted of a rearing cage built with a semi-transparent 500 ml plastic cup, similar to the PVC cylinder. After 24h post-emergence, one male and one virgin female were placed in each cup, and offered either distilled water (T1) or a 10% honey solution with 0.03% hydroxybenzoate methyl ester (T2).

The following parameters were analyzed during a 10 days period after mating: oviposition rate, fecundity and egg viability. Fecundity was calculated from the mean number of eggs laid during the entire lifecycle of the females, while egg viability was assessed from the mean percentage of hatched larvae in the T_1 and T_2 treatments.

Statistical analyses. Data normality was checked by the Shapiro-Wilk test (Siegel 1975) at a 5% significance level. Due to the rejection of the null hypothesis of normality, non-parametric tests were applied and data were then submitted to the Wilcoxon Rank Sum Test (Campos 1983) at 5% significance level. The analyses were performed with the R software (The R project for statistical computing 2007).

Results and Discussion

Food consumption. The liquid dye used was adequate for the studies of the digestive system of *P. operculella*. It was useful as a marker and caused no physiological alterations that influenced the data obtained. Sugar consumption by the insects became evident three days after contact with the honey solution and dye. The presence of dye could be detected either by external inspection of the adult abdomen (Fig 1a) or by examining the digestive system (Fig 1b). The external inspection of the abdomen indicated the dye presence in 16 males (80%) and 17 females (85%). However, the exam of the digestive system after dissection demonstrated that 100% of the adults ingested the dye. The lower number of individuals having dye in the external abdomen was probably due to the presence of the typical lepidopteran tegument covered by scales, which difficult dye visualization.

Reproductive performance. According to Pratissoli *et al* (2003), the largest number of eggs was laid soon after the beginning of the oviposition period, as expected, in both groups. Despite similar female oviposition rates under both feeding diets, mates fed only on distilled water laid fewer eggs than those fed on honey solution (Wilcoxon Rank Sum Test, $W = 485$; $P = 0.0019$). Females fed on honey solution had a slower decrease in the number of eggs laid after the oviposition peak observed at day 2 as compared to females fed only water. This indicate that sugars aid to maintain egg development during adult ageing (Fig 2).

Values of 50% laid eggs per female were 10-56 for the group fed with distilled water, while for the mates fed with honey, 50% of the eggs laid were 42-86. Median egg number was 26 for water-fed females and 70 for those fed on honey solution (Fig 3a).

There was no significant difference between treatments in relation to egg viability, as indicated by the Wilcoxon Rank Sum Test ($W = 284$; $P = 0.0544$). Dispersed points represented atypical values of high egg viability, which possibly refer to larvae hatched from eggs laid by low fecundity females (Fig 3b).

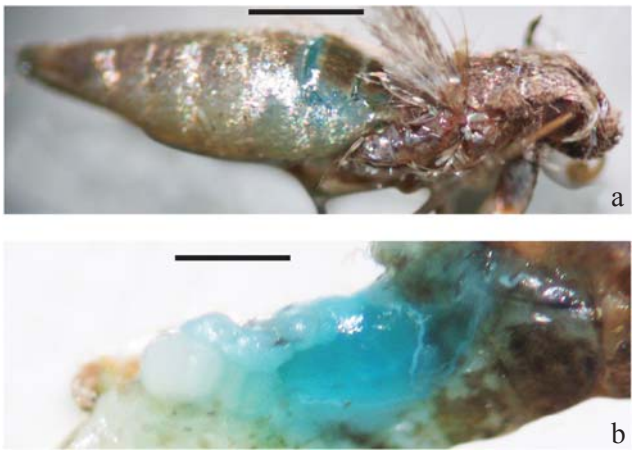

Fig 1 Dye-marked digestive system of adult *Phthorimaea operculella*. a) External view of frontal abdominal region; (b) Abdomen with the exposed digestive system; scale 1 mm.

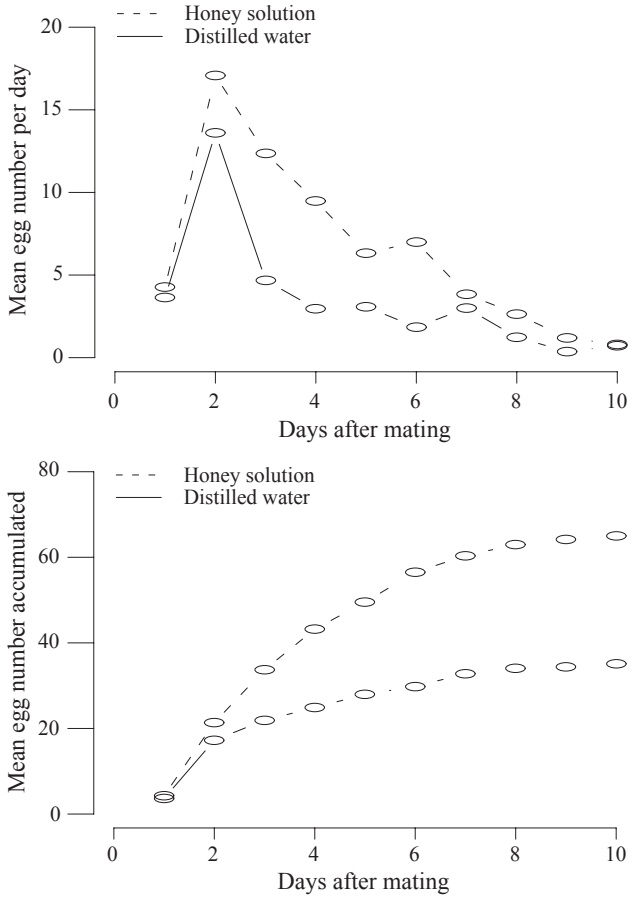

Fig 2 Daily oviposition rate and cumulative mean number of egg laid per *Phthorimaea operculella* females during a 10 days period , when fed either distilled water or a 10% honey solution (25 ± 1°C; 70 ± 10% RH; 14h photophase).

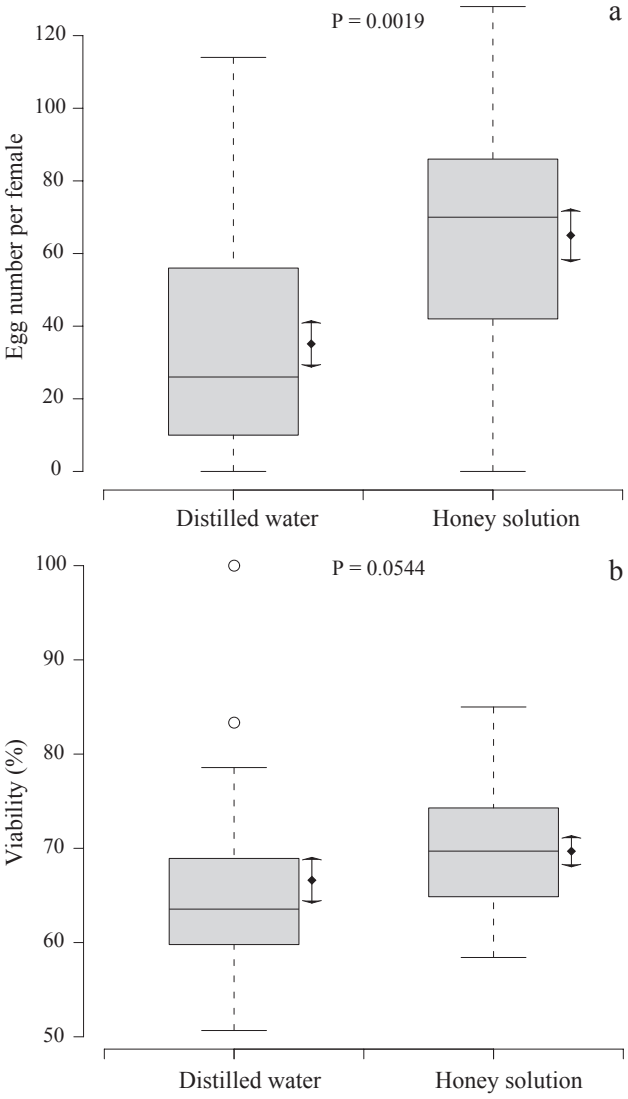

Fig 3 Effects of adult feeding on *Phthorimaea operculella* fecundity (a) and egg viability (b), when adults were fed either distilled water or a 10% honey solution (25 ± 1°C; 70 ± 10% RH; 14h photophase). P is the one-sided p-value of Wilcoxon Rank Sum Test. The line into the box indicates the median, the extremities indicate minimum and maximum numbers and the signal (○) indicate outlier values. The point (●) and vector on the right side of the box indicates de mean value and the estimated standard error, respectively.

The requirements of *P. operculella* for food sources during adult stage for sustaining reproduction was observed for several other lepidopterans able to feed on several sugar sources (Savopoulou-Soultani *et al* 1998, Tisdale & Sappington 2001), although adult feeding is not always required (Milano *et al* 2010) and certain sugar sources may even be toxic to the adult (Parra *et al* 1999). Nevertheless, it has been demonstrated and argued that carbohydrates fed by adults may be an additional food supply that helps the vitellogenin synthesis and egg development, thus increasing fecundity (Savopoulou-Soultani *et al* 1998, Tisdale & Sappington 2001).

Adult feeding, including sugar consumption, can be important to the development of integrated management strategies for *P. operculella*. It can allow for the combination of pesticides, such as insect growth inhibitors, whose hormonal effects may reduce insect fecundity and fertility, thus reducing reproductive fitness (Minakuchi & Riddiford 2006, Wimmer *et al* 2006). Another possible strategy to modify reproductive fitness could be the use of commercial pesticides mixed with sugar solutions, especially those with hormonal effect similar to the juvenile hormone or ecdysis triggering hormone agonists. The use of such feeding solutions should be associated with attractants to keep safe the beneficials, such as predators and parasitoids, allowing for the reduction of the pest population. Such strategies could also aid to alleviate the environmental impacts originated from the traditional ways of insecticide application, since pesticide application could be restricted to strips among the rows and not spread all over the planted area.

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