

## Note

## Adding guarana powder to medfly diets: an alternative for improving the Sterile Insect Technique

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**ABSTRACT:** *Ceratitis capitata* (medfly) is a globally important horticultural pest that can be controlled using the sterile insect technique (SIT), but the success of SIT depends on the sexual performance of mass-reared males when released into the field. We added “guarana” (*Paullinia cupana*) powder (derived from an Amazonian fruit that is considered to be a stimulant with aphrodisiac effects, capable of improving human physical stamina) to the diets of adult male medflies to determine whether it increased their sexual performance. The basic diet of a protein extract + sucrose (1:3) was enriched with guarana powder (3 % on a volume basis). Experiments examining sexual competitiveness were performed using lab-reared males fed with the enriched diet vs. lab-reared males fed on the basic diet (and lab-reared females fed on the basic diet), as well as lab-reared males fed with the enriched diet vs. wild males fed on the basic diet (and wild females fed on the basic diet). The results of both experiments indicated that males maintained on diets enriched with guarana powder showed higher copulation successes than males fed only with the basic diet. Guarana powder therefore contributed to the greater mating success of lab-reared males (probably because of its stimulant properties), and may represent a new and viable option for increasing SIT effectiveness.

**Keywords:** *Ceratitis capitata*, *Paullinia cupana*, Tephritidae, artificial diet

### Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is a globally important agricultural pest in fruit orchards (Liquido et al., 1990; Norrbom, 2004). The Sterile Insect Technique (SIT; Knipling, 1955) has been a successful option for controlling fruit flies in many parts of the world and has a low ecological impact (Enkerlin, 2005; Klassen and Curtis, 2005). The success of SIT is dependent, however, on the successful mating of wild females with males from mass-rearing facilities. Matings require large energy outlays by the males, including the emission of pheromones and the performance of courtship behavior and territorial defense within a non-resource-based male mating aggregation (Whittier et al., 1994; Blay and Yuval, 1997; Yuval et al., 1998).

Studies involving exposure to volatile compounds, aromatherapy with ginger oil (Morelli et al., 2010), dietary supplements (Yuval et al., 2007), and/or dietary modifications during early adult life (Pereira et al., 2011; Shelly, 2001), have been tested in attempts to improve the sexual competitiveness of mass-reared males under field conditions. In experiments with wild flies, Arita and Kaneshiro (1988) reported that *C. capitata* males that emerged from coffee berries copulated more frequently than males emerging from Jerusalem cherries (*Solanum pseudocapsicum* L.) – even though the latter insects were larger. One possible explanation for this improved male sexual competitiveness might be the stimulatory effects of caffeine in the coffee berries. The guarana (*Paullinia*

*cupana*) fruit is frequently used by humans as a stimulant (much like coffee). This Amazonian vine is cultivated exclusively in Brazil, with the state of Bahia being the world's largest production region (Fraife Filho and Ramos, 2014). The fruits contain large quantities of caffeine, and their seeds are roasted and ground to produce a powder – the most commonly commercialized product. Caffeine levels in guarana powder can be up to four times greater than those found in ground coffee (CEPLAC, 2011; Tfouni et al., 2007). The effects attributed to the consumption of guarana powder by humans include aphrodisiac properties and increased metabolic rates (Kuri, 2008; Webb, 2006). As such, fruit flies fed on a diet with added guarana powder might be expected to show greater copulatory success. Flies fed on coffee berries during the larvae stage of *C. capitata* demonstrated greater mating success compared with cherry emerged flies (Arita and Kaneshiro, 1988).

The present work sought to determine if the inclusion of guarana powder in the diets of lab-reared males of *C. capitata* would improve their sexual performance and thus potentially increase the effectiveness of SIT.

### Materials and Methods

**Experiment 1:** The male flies used in this experiment were obtained from a line of *C. capitata* maintained in the laboratory for 15 years and fed as larvae on a yeast-based diet (Carvalho et al., 1998). After emergence, the lab-reared males were divided into two groups and fed for five days on different diets.

The first group received a diet based on yeast extract + sucrose (1:3) – the diet regularly used in maintaining the adults of these populations (Silva Neto et al., 2012) – and referred to hereafter as the basic diet. The second group received the basic diet but with the addition of guarana powder (3 % vol/vol). On the sixth day, 10 male flies from each dietary group were placed together with 10 five-day-old lab-reared virgin females (fed on basic diet) in a screen-mesh cage (68 × 68 × 90 cm) containing a small pitanga tree (*Eugenia uniflora*) approximately 60 cm tall. Each group of males was encased in tulle to prevent them moving and marked on the dorsal region of the thorax with a spot of non-toxic ink for identification. Observations of mating behavior were performed from 07h00-12h00, and each copulating pair was gently removed from the cage. The latency time for copulation (the time from the release of the individuals into the cage at the beginning of the experiment until the beginning of copulation), as well as copulation duration (the time from the insertion of the aedeagus to the moment the flies separated) were recorded. The experiments were undertaken under controlled laboratory conditions (temperature  $25 \pm 1$  °C, 70 % relative humidity, and an illumination regime of 1500 lux from a fluorescent light on a 12:12 L:D cycle). Fifteen replicates were performed.

Experiment 2: Lab-reared males fed on a diet of yeast extract + sucrose (1:3) enriched with guarana powder (3 % vol/vol) were tested for their sexual competitiveness in relation to wild males (obtained from infested *Terminalia cattapa* fruits) that were fed on the basic diet after emergence (without added guarana powder). The sexual competition conditions followed those used in experiment 1, with a number of modifications. Tests were conducted in field cages (230 × 150 × 230 cm) containing a pitanga (*Eugenia uniflora*) bush to simulate the natural environment. Twenty males from each group (previously marked with non-toxic paint) were liberated together with 20 wild females that had been fed on a basic diet. In this experiment, the wild males and wild females were 15 days old due to their longer sexual maturity; the laboratory males used were six days old, as in the previous experiment. All of the flies were fed with their respective diets until the day before initiating the mating competition tests. Five repetitions were performed. In a previous experiment, sexual competitiveness was compared for males reared in laboratory, fed with basic diet (sugar + yeast extract - 3: 1), and wild males also fed with the basic diet, in field cages. In that experiment, wild females chose to mate with significantly more wild males (at least twice more) than lab-reared males (unpaired t-test;  $p < 0.0001$ ) (Aquino and Joachim-Bravo, 2014). This experiment was used as reference for comparison of the new data we present here, since it used the same lineage flies and the same methodology and laboratory conditions. In order to test the influence of guarana-diet on the length of the male calling behavior (the first step of a sequence of behavioral courtship units, in which the male releases a pheromone drop by eversion of the anal epithelium and flaps its wings to aid

dissemination), a complementary experiment was performed. Sexually mature wild males fed with or without guarana-diet (one of each group) were painted with different colors and placed in glass boxes (90 × 90 × 70 mm) for observation during 10 min to compare the duration of calling behavior between them. The experiment was conducted in the morning with 10 replicates.

Data analysis: Sexual competitiveness, mating latency, copulation duration, and calling behavior tests were compared between guarana-fed and control males, using the Student T-test, after evaluating the data for variance normality and homogeneity. The nonparametric Mann-Whitney test was used when these assumptions were not met.

## Results

Experiment 1: Lab-reared *C. capitata* males maintained on a diet enriched with guarana powder showed greater copulatory success than lab-reared males maintained on a basic diet (T-test;  $p < 0.0001$ ) (Figure 1). There were no significant differences in copulation latency between the two groups (Mann-Whitney,  $U = 1532$ ,  $p = 0.457$ ), but males fed with guarana demonstrated greater copulation dura-

Table 1 – Latency to copula and copula duration of *Ceratitis capitata* males fed on diets with and without guarana powder. The data represent the medians (with ranges given) of latency to copula and copula duration. All flies used in this experiment were from a laboratory strain.

	Latency to copula (min) Mann-Whitney Test $U = 1532$	Copula duration (min) Mann-Whitney Test $U = 1187$
Males fed on guaraná-diet	25 (19 – 32)	177.5 (149.75 – 212)
Males fed on no-guarana diet	20 (16 – 32)	169 (137 – 183.5)
p-value	0.457	0.01937

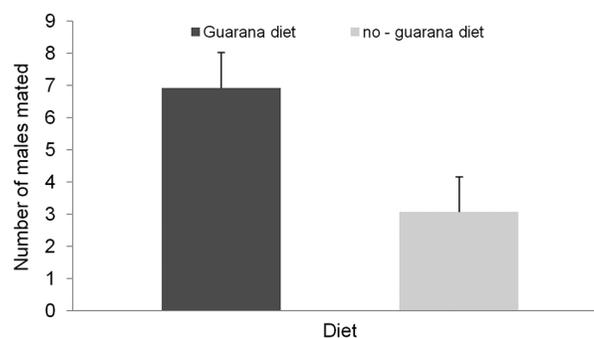


Figure 1 – Number of mated males fed on a basic diet with or without guarana powder. Bar heights represent means (+ SD = standard deviation;  $n = 15$ ), (T-test;  $p < 0.0001$ ). All flies used in this experiment were derived from a laboratory strain and females used were fed with basic diet without guarana.

tions (Mann-Whitney,  $U = 1187$ ,  $p = 0.01937$ ) (Table 1). Experiment 2: In competitive experiments between lab-reared and wild males, the lab-males maintained on the guarana diet had greater mating success than wild males fed with the basic diet (T-test,  $t = 2.144$ ;  $p < 0.05$ ) (Figure 2). The results were based on minimum replicates necessary to allow the t-test, due to the experiment's limitations (the experiment was conducted under field conditions with the use of a large number of wild *C. capitata*, which are difficult to obtain).

There were no significant differences in copulation latency time (T-test,  $t = 0.336$ ,  $p = 0.745$ ) or copulation duration between the groups (T-test,  $t = 0.968$ ,  $p = 0.362$ ) (Table 2).

As regards the duration of calling behavior, males fed on the basic-diet spent more time in calling behavior (mean = 352.5 s; standard deviation - SD = 158) than males fed on the guarana-diet (mean = 62.5 s; standard deviation - SD = 55.5) (T-test;  $p = 0.002$ ) (Figure 3).

## Discussion

The addition of guarana powder improved the sexual performances of lab-reared *C. capitata* males, as they showed greater mating success with wild females - including their preference over wild males. Previous research done by our group using the same medfly strain (wild flies fed with basic diet vs. lab-reared fed with basic diet) and about the same time (Aquino and Joachim-Bravo, 2014) had shown that wild male fruit flies tend to be more successful at mating than lab-reared males -

Table 2 - Latency time to copulation and copulation duration of wild males (fed with non-guarana diet) and lab-reared ones (fed with guarana-diet). Data represent the mean of latency time to copulation (minutes) and duration of copulation (minutes).

	Latency to copula (min) T-test = 0.336	Copulation duration (min) T-test = 0.968
Lab-reared male	107	132
Wild male	128	157
p-value	0.745	0.362

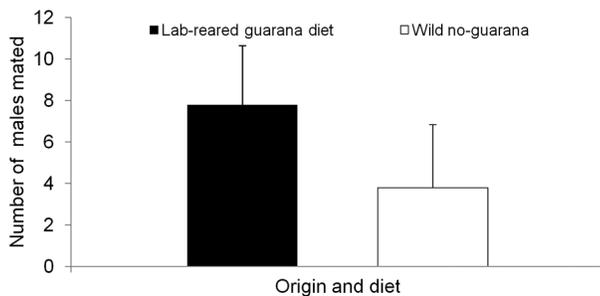


Figure 2 - Number of mated males (lab-reared males fed with guarana-diet and wild males fed with the same basic diet without guarana powder). Wild females fed with basic diet without guarana were used in these experiments. Bar heights represent means (+ SD = standard deviation;  $n = 5$ ), (T-test;  $p < 0.05$ ).

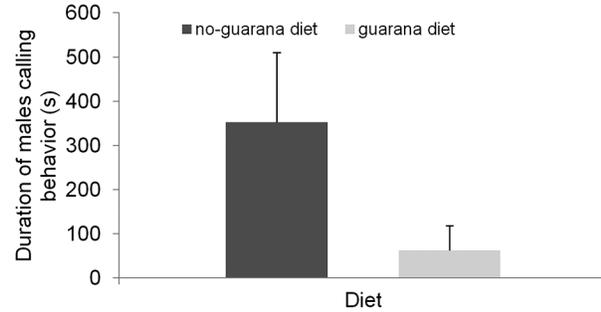


Figure 3 - Duration of calling behavior of lab-reared male flies that as adults were provided diets either with or without 30 % guarana powder included. Bar heights represent means (+ SD = standard deviation;  $n = 10$ ), (T-test;  $p = 0.002$ ).

even when the physical characteristics of the lab-reared males appeared to be more favorable. These experiments indicated that although lab-reared males demonstrated characteristics that would otherwise make them more favorable to females (such as having larger body sizes (Anderson, 1994; Anjos-Duarte et al., 2010)), wild males were more successful at copulations when present.

Many efforts have been made to improve the efficiency of SIT with medflies by attempting to improve the copulatory success of sterile males, including exposure to volatile compounds derived from fruits such as guava, lemons, and mangoes (Vera et al., 2010) and citrus oil (Haq et al., 2010; Kouloussis et al., 2010); sterile *C. capitata* males have also been exposed to ginger oil in attempts to reduce female re-mating rates (Morelli et al., 2010). In addition to aromatherapy, providing food supplements during the adult stage has also shown promising results. Liedo et al., (2010), for example, demonstrated that both wild and sterile *A. obliqua* males fed with mangoes and oranges showed greater mating success than flies fed on normal diets (yeast + sugar). Pérez-Staples et al., (2009) found that sterile males of *Bactrocera tryoni* (Froggatt) that fed on yeast hydrolysate for a brief period were as competitive as wild males. Other workers have shown that the application of methoprene as well as greater access to proteins in adult diets could increase the mating success of the males of some species of Tephritidae; these fruit flies also attained sexual maturity earlier (Gómez et al., 2011; Haq et al., 2010; Haq and Hendrichs, 2011; Pereira et al., 2011; Teal et al., 2011). It was demonstrated that *Bactrocera tryoni* males maintained on hydrolyzed yeast and sugar showed benefits such as: rapid development, longer copulation times, increasing probability of mating and spermatozoa-stocking by the females, sexual inhibition of females once they had copulated, and increased longevity (Pérez-Staples et al., 2007; Vijayasegaran et al., 2002; Weldon and Taylor, 2011).

The reasons for the success of added guarana to the diets of lab-reared males still need to be more closely examined from the physiological and behavioral points of view. The results suggest that the addition of guarana powder to the males' diet should contribute to their mat-

ing success but not by dint of duration of calling behavior. Another interesting result that must be analyzed in further studies concerned the greater duration of copula in guarana-fed males. Although these results cannot be fully explained yet, it is conceivable that the high concentration of caffeine in guarana powder contributed to the males' mating success. One hypothesis is that males fed on a guarana diet could be more agile to "fight" in lek and could have greater ability to reach the most interesting points of the tree. The data about mating success obtained here were similar to the results obtained by Arita and Kaneshiro (1985), who reported the mating success of males obtained from coffee berries (also containing high caffeine levels), although the fruits had been provided during the larval stage in their experiments instead of a food supplement for adults, as reported in the present study. The authors suggested that coffee-fed males could achieve the best positions in lek and could be more chosen by females. Further studies concerning courtship behavior will be performed to investigate the reasons for the mating success of males fed on the guarana diet.

The sexual competitiveness of sterile, mass-produced *C. capitata* fruit flies was improved by adding natural stimulants to their diets, and this utilization of guarana powder may represent a new and viable option for increasing SIT effectiveness for medfly control in Brazil – and a viable alternative to expensive imported products such as methoprene.

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