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Sterile Medfly Males of the *TSL* Vienna 8 Genetic Sexing Strain Display Improved Mating Performance With Ginger Root Oil

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ABSTRACT: A key point of the Sterile Insect Technique (SIT) applied to the medfly, *Ceratitis capitata*, is that the sterile males produced in the laboratory should have at least a minimal sexual compatibility with wild females. Among several genetic sexing *tsl* (Temperature Sensitive Lethal) strains of *C. capitata* mass-reared around the world, the Biofábrica Moscamed Brasil has chosen the most recent mass produced tsl strain, Vienna 8 (V8), which has been evaluated in the San Francisco River Valley, Brazil, since April, 2005. The tests were accomplished in field cages, with different treatments for V8 males, sterile or fertile, exposed to the aroma of ginger root oil (GRO) or not, versus wild males and females. Males of one strain (V8 or wild) were painted white on the thorax the day before the mating tests. All the insects were virgin, and early in the morning (7-8 A.M.) males were released inside the field cages, 10 min. before females. Mating pairs were collected in glass vials, until early afternoon. From this raw data, both the type of male mating and the time in copula were recorded for each pair. Then, the total percentage of mated females, the RSI (Relative Sterility Index), and Fried´s competitiveness values (C), were calculated for each field cage. The percentage of females mated was statistically higher to sterile males exposed to GRO than to non exposed to GRO. Time in copula was significantly higher for wild flies than for laboratory flies, except for the case of fertile V8 males exposed to GRO x wild females. The RSI and C values were significantly higher for V8 males (irradiated and fertile) treated with GRO than for V8 males not treated with GRO. The results indicate that there is adequate sexual compatibility between sterile males of the *tsl* Vienna 8 strain and wild *C. capitata* females from the San Francisco River Valley, Brazil. Also, the radiation dose of 95 Gy, used to sterilize the males, did not affect their sexual activity. Ginger root oil acted as a beneficial stimulatory chemical used to boost medfly male mating activity, thereby increasing the efficiency of the SIT.

Key Words: SIT, sexual behavior, semiochemical, aromatherapy, *Ceratitis capitata*.

INTRODUCTION

Each of the steps in a sterile insect technique (SIT) program, from mass-rearing to the release of sterile males in the field, is critical in order to obtain successful pest control. Today, mass-rearing technology for the medfly, *Ceratitis capitata,* has improved significantly to provide all-male (*tsl*) flies for production facilities around the world. However, through the rearing and release process, sterile males lose quality, such that many of them do not compete adequately with wild males for wild females in the field (Hendrichs et al., 2002). Over generations of rearing in the laboratory, it is known that sterile males become less competitive (Cayol, 2000 and Lance et al., 2000). Therefore, recent research efforts have focused on finding simple but effective means to improve the quality of fruit fly adults destined for field release in SIT programs worldwide. One encouraging effort has been the development of aromatherapy with ginger root oil (GRO) as a strong booster of sterile male mating performance (Shelly, 2001; McInnis et al., 2002; Shelly et al., 2005). This oil contains 0.4% of the known male attractant, α-copaene, along with other sesquiterpenes of unknown attractancy (Shelly & McInnis, 2001).

The objective of this work was to evaluate the GRO aromatherapy on mating compatibility and competitiveness of sterile medfly males *tsl*, Vienna 8, with wild females of *Ceratitis capitata* from the San Francisco River Valley, Brazil.

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MATERIALS AND METHODS

Experiments were carried out at Embrapa Semi-Árido research station, Petrolina-PE, Brazil, from April 2005 to July 2006. Five field cages were prepared for the mating tests in

a well shaded outdoor area. The cages were each ca. 3 m on a side and 2 m high covered with a fine screen cloth. Two potted plants, each ca. 1.5-2 m tall of *Ficus benjamina*, were placed inside each cage. Field cages were run to test several treatments or controls involving flies of the Vienna 8 *tsl* genetic sexing strain and wild flies collected from various medfly host fruits in the São Francisco River Valley. Virgin wild flies were between 10-19 days old when tested and were maintained in 1.5 L plastic cages with food (sugar and protein) and water prior to each test. Virgin laboratory flies (Vienna 8 strain) were 5-9 days old and, along with wild flies, were maintained indoors in a well lit room at 25-27°C and ca. 50% RH. On the day prior to a test, either lab or wild male flies were marked with a spot of white paint on the thorax using a screened bag without anesthesia. To obtain sterile males, 24 h before emergence, pupae received 95 Gy of gamma radiation from a Co^{60} source. Also, if the treatment involved ginger root oil (GRO), 50 males of the V-8 strain were exposed to 40 µl of the aromatic oil on filter paper for 5 hours in a well ventilated, isolated room. At the end of the exposure period, males were returned to the holding room with the rest of the flies.

On each test day, at ca. 8 A.M., wild and V-8 males were released onto the foliage near the bottom of the cage. Flies that could not fly or were dead were replaced with extra flies, such that the normal starting numbers were 50 lab males and 50 wild flies of each sex in each cage. On rare occasions, less than 50 males were available per strain; in those cases the number of males was balanced to make for equal competition. After ca. 15 min to allow the males to establish territories, 50 wild females were released and dead or non-flying flies were replaced as before. Over the next 4-6 hours, mating pairs were collected in glass vials from each of the cages. The starting and stopping time of each pair was recorded along with the location in the cage. The test was stopped when no mating pair was observed for a period of over 30 minutes.

To observe the ability of *tsl* males to transfer sperm, the mated flies collect in the field cages, separately from each treatment, were taken to the laboratory and placed inside small cages for oviposition using seedless green grapes as the host fruit. Ten fruits were hung from the top of the screened cage. Flies were allowed to remate and females allowed to oviposit *ad libidum* for 24-48 hours. Eggs were dissected from the grapes and seeded on moist filter paper in a Petri dish for 3 days prior to being scored for larval eclosion. The number of eggs and the hatch rate were recorded.

Prior to complete statistical analysis, the data were summarized for the following statistics: RSI (Relative Sterility Index), which represents the proportion of matings in a cage made by laboratory males (sterile or non-irradiated) with wild females, percentage of females mated in each cage, time in copula, number of eggs and percent egg hatch, and Fried's male competitiveness value (C). Data of RSI and percent of mated females were transformed to arc sine of x root.

The C-value indicates the competitiveness of irradiated males with respect to their ability to induce sterility compared to wild males, where: $C=(w/s) \times [(Hw - Hc)/(Hc - Hs)]$, with w= number of wild males released in the field cage, s= number of sterile males released in the field cage, Hw= % of egg hatch from wild females following mating with wild males exclusively, Hc= % of egg hatch from wild females in the test cages, made in laboratory conditions, Hs=% of egg hatch from wild females following mating with sterile males. Values around 1 indicate equivalent mating performance between laboratories and wild males, and less than 0.2 is a reason for concern about the competitiveness of *C. capitata* sterile males (FAO/IAEA/USDA, 2003).

RESULTS

There was statistic difference in the RSI obtained among treatments with different types of males (V-8; V-8 GRO; V-8 irrad and V-8 irrad GRO) ($P \geq 0.0054$; $F = 5.54$). The averages obtained for RSI were statistically different between treatments with males exposed and not exposed to GRO, but we did not find statistic difference between fertile (0.21; n=7) and irradiated V-8 strain males (0.22; n=7) no treated with GRO and also between fertile (0.35; n=5) and irradiated V-8 strain males (0.36; n=8) treated with GRO, in competition with wild males for wild females (Tukey's test, $p = 0.05$) (Fig. 1).

Considering all the field cages carried out with V-8 males, % females mating, independently of whether males were irradiated or fertile, mating frequency was significantly higher in treatments where V-8 males were exposed to GRO (75.6%, n=14) than in treatments where they were not exposed (61.1%, n=15) ($P \geq 0.0177$; $F = 6.28$) (Fig. 2). Aromatherapy with GRO increased female mating by 23.7%.

There was statistic difference in mating duration among different mating types ($P \geq 0.0001$; $F = 17.43$). Mating duration, between wild males and wild females averaged 2.30 hours (n=209). This value was higher and statistically similar for matings involving V-8 fertile males exposed to GRO (2.03 hours, n=67), while statistically different from all other mating types (Tukey's test, $p = 0.05$) (Fig. 3). A shorter mating time was found to V-8 irradiated males, but no statistical difference was detected with V-8 irradiated exposed to GRO or V-8 fertile males (Tukey's test, $p = 0.05$) (Fig. 3).

The competitiveness of V-8 irradiated males exposed to GRO ($\cong 1.00$, n=7) was significantly higher than V-8 irradiated males not exposed to GRO (0.21, n=6) in mating with wild females ($P \geq 0.014$; $F = 8.41$) (Fig. 4). The results obtained with GRO aromatherapy were very close to that for wild males.

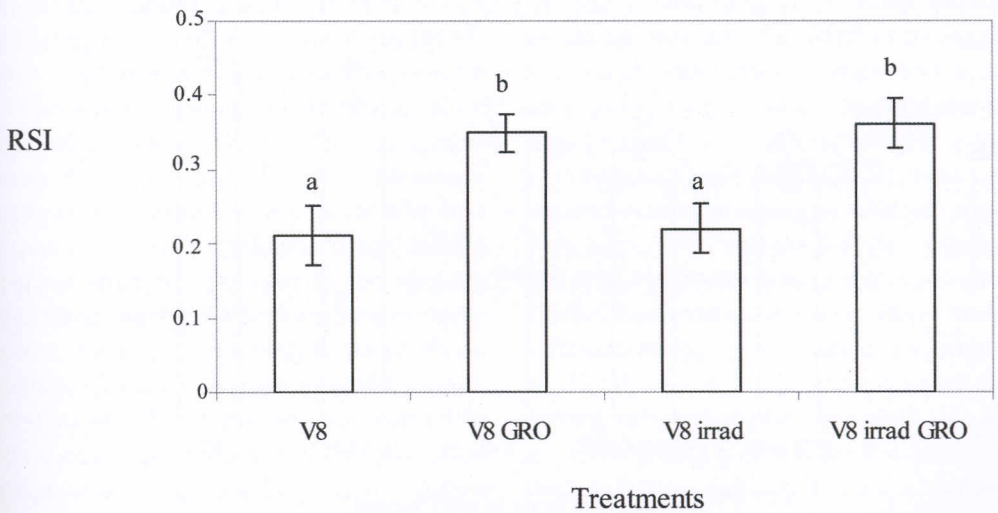

Figure 1. Relative sterility index (RSI) obtained in field cages among V-8 males, fertile or irradiated, and treated or not treated with ginger root oil (GRO), in mating competition with wild males for wild females. Bars with same letter do not differ significantly from each other (Tukey's test, $p = 0.05$).

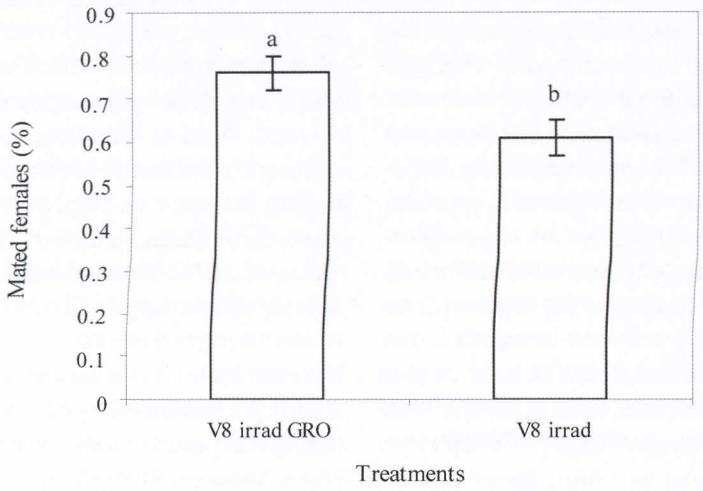

Figure 2. Percentage mated females in field cage experiments with V-8 males, exposed and not exposed to ginger root oil (GRO), in competition with wild males. Bars with different letters are statistically different (Tukey's test, p= 0.05).

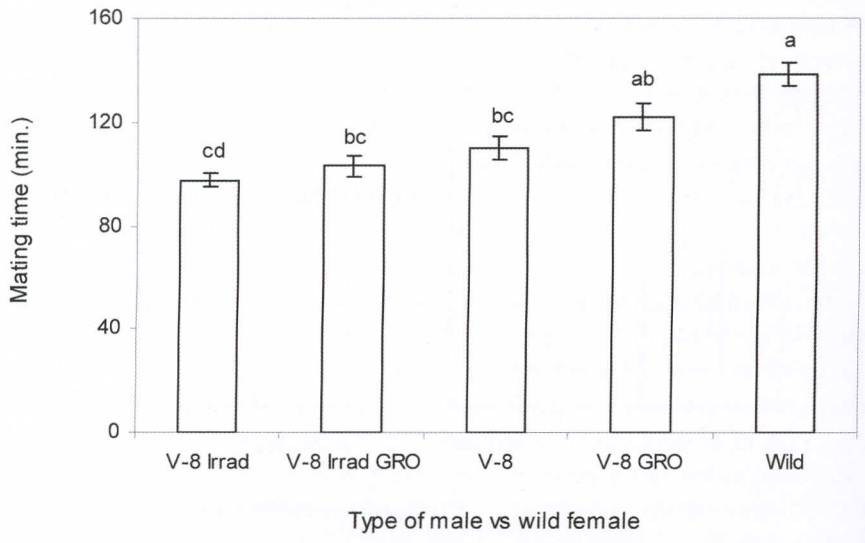

Figure 3. Mating times between wild females and five male types: irradiated V-8 strain (V-8 Irrad, n= 150), irradiated V-8 strain exposed to ginger root oil (GRO) (V-8 Irrad GRO, n= 129), non irradiated V-8 strain (V-8, n= 107), non irradiated V-8 strain exposed to ginger root oil (GRO) (V-8 GRO, n= 67), and wild males (wild, n= 209). Bars with same letter do not differ significantly from each other (Tukey's test, p= 0.05).

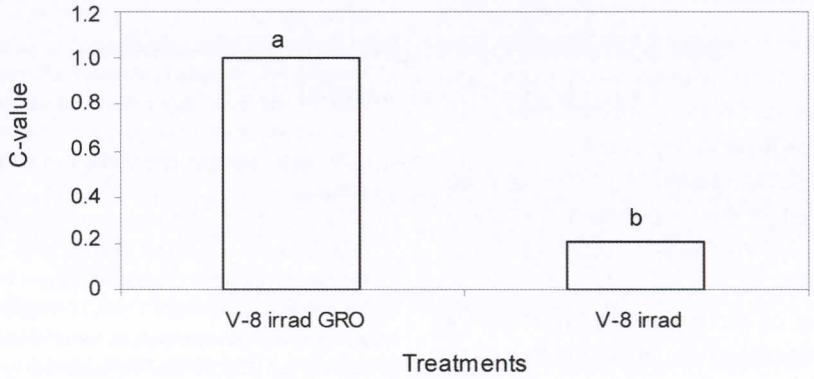

Figure 4. Competitiveness Fried test, C-value, between V-8 irradiated males exposed (V-8 Irrad GRO) and not exposed (V-8 Irrad) to ginger root oil (GRO) in mating competition with wild males for wild females. Bars with different letters are significantly different from each other (Tukey's test, p= 0.05).

DISCUSSION

The RSI found for the V-8 males strain vs. wild females of *Ceratitis capitata* in San Francisco River Valley, Pernambuco-PE, Brazil, was satisfactory according to the quality control standards for the *tsl* strain (FAO/IAEA/USDA, 2003). In contrast to results found by Lux et al. (2002) and Kraaijeveld & Chapman (2004), the gamma radiation from a Co-60 source (95 Gy) used in this experiment did not negatively affect the sexual compatibility between V-8 males and wild females. The lower irradiation doses used in the present study can lead to less damage regarding the mating performance of sterile males. However, a small egg hatch (2-3% on average) from wild females mated by irradiated males, 2-3% on average was found. This could pose a problem in any SIT eradication program of *C. capitata* but not in suppression programs as is the case in Brazil.

In agreement with Shelly and McInnis (2001), McInnis et al. (2002) and Shelly et al. (2005), ginger root oil (GRO) significantly improved the sexual performance of V-8 males exposed to GRO in competition with wild males for wild females in relation to V-8 males not exposed to GRO. Additionally, we found that GRO aromatherapy can increase the average mating time significantly (ca. 6 to 10 mins) for irradiated and fertile V-8 males, respectively. There is no supportive study indicating how many minutes would be necessary for medflies to transfer a complete quantity of semen and accessory gland fluid to avoid or decrease the possibility of remating. According to Seo et al. (1990) and Taylor et al. (2001), mass rearing and irradiated males transfer less sperm than wild males, and the transfer success is not correlated with time in mating. Besides, studies have shown remating cases also in wild population of medflies (Kraaijeveld et al., 2005). Such a result remains to be confirmed in future studies. The GRO also had a positive effect on the C-value, additionally decreasing the egg hatch rate from females where wild males were competing for mates with irradiated males exposed to GRO. Sterility may have been higher partially because few more minutes in copula, but also mainly because the RSI increased from 0.21 to 0.34, a ca. 40% increase with GRO treatment.

CONCLUSIONS

There is sexual compatibility between sterile, mass-produced Vienna 8- tsl males and wild females of *Ceratitis capitata* present in San Francisco River Valley, Brazil.

A dose of 95Gy applied to pupae, 24 h before emergence, does not affect the sexual performance of Vienna 8- tsl males of *C. capitata*.

Ginger root oil aromatherapy improves the mating competitiveness of Vienna 8- tsl sterile males of *C. capitata*.

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