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POTENTIAL FOR REDUCING OVERFLOODING RATIOS OF STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) WITH THE USE OF GINGER ROOT OIL

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ABSTRACT

The mating behavior of sterile, laboratory-reared, male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), was evaluated in field-cage mating competition tests with wild flies after exposing the laboratory males to ginger root oil extract. Without exposure to ginger root oil, sterile males obtained 12.6%, 69.0%, and 72.8% of the total matings with wild females when present in 1:1, 5:1, and 10:1 ratios of sterile males to wild males, respectively. Sterile males, exposed to ginger root oil for 3 h, 1 d before mating trials, in a 1:1 ratio with wild males, achieved 62.3% of the matings with wild females. These data suggest that exposure to ginger oil can elevate sterile male mating competitiveness to a similar degree as elevated ratios of sterile to wild males. Incorporating the use of ginger root oil extract into sterile release programs may thus increase the effectiveness of the sterile insect technique, and/or allow a reduction in the number of sterile flies that are released.

Key Words: sterile insect technique, *Ceratitis capitata*, mating behavior, alpha-copaene

RESUMEN

El comportamiento de copulacion en las moscas de la fruta, *Ceratitis capitata* (Wiedemann), mediterráneas estériles, machos criadus en el laboratorio, fue evaluado en pruebas de competencia de copula en jaulas de campo fuente con moscas salvajes. Estos preubas se iniciaron después de que los insectos del laboratorio fueron expuestos al extracto del aceite del raíz de jengibre. Sin la exposición al aceite, los machos estériles obtuvieron 12,6%, 69,0%, y 72,8% de las copulas totales con hembras salvajes cuando fueron presentes en proporciones de 1:1, 5:1, y 10:1 de machos estériles a machos salvajes, respectivamente. Los machos estériles, expuestos 1 d antes de copula al olor del aceite de jengibre para 3 h, en un proporcion de 1:1 con machos salvajes, alcanzaron 62,3% de las copulas con hembras salvajes. Estos datos sugieren que la exposición al aceite pueda elevar la competencia sexual del macho estéril a un grado igual a proporciones elevados (ca. 5:1) de machos estériles contra machos salvajes. Incorporarando el uso del extracto del aceite en programas de accion de liberar moscas estériles, puede aumentar así la eficiencia de la técnica estéril del insecto, y/o permitira una reducción en el número de moscas estériles liberadas. Translation provided by author.

The Mediterranean fruit fly, *Ceratitis captitata* (Wiedemann) (medfly), is a multivoltine, polyphagous (250+ plant species) insect pest that could have a devastating economic impact if it were to become established in California or Florida (Metcalf 1994). In these states, the sterile insect technique (SIT) is currently used to inhibit Mediterranean fruit fly colonization. The goal of these programs is for sterile males to mate with any introduced wild females, resulting in the production of infertile eggs (Dowell et al. 2000).

There is an ongoing interest in improving the quality of sterile males used in eradication and control programs for the Mediterranean fruit fly. One way to assess fly quality is through the use of mating competition tests, where sterile males compete with wild males for wild females. In most cases, mating observations and tests have shown that laboratory-reared male flies are at a disadvantage with wild males when competing for females (Robinson et al. 1986, Shelly et al. 1994, Cayol et al. 1999, Lance et al. 2000, but see Taylor et al. 2001 which found no advantage for wild males). In one case in Hawaii, following apparent intense selection in the field, there was almost complete behavioral resistance by wild females to laboratory reared sterile males (McInnis et al. 1996).

Reduced mating performance of sterile laboratory males is attributed to the mass-rearing pro-

cess, which through artificial selection, may result in the production of flies with qualities different from their wild counterparts (Cayol 2000). There are two options available to increase the mating success of SIT flies. The first is the release of greater numbers of sterile flies so that they vastly outnumber wild males. Recommended sterile/ wild fly release ratios vary with each strain, but have been suggested to be 125:1 for the Petapa strain and 100:1 for the Vienna-4/Tol-94 strain based on field cage tests using sterile/wild fly ratios of 1:1, 5:1, 25:1, and 125:1 (Garcia et al. 1999). A second option would be to use "male-only" strains, containing 95%+ males, which have resulted in a significant improvement over releases containing both sterile males and females (McInnis et al. 1986, McInnis et al. 1994, Hendrichs et al. 1995, Rendon et al. 2000). The benefits of "male-only" strains, derived from the virtual elimination of the sterile male and sterile female interaction, are widely accepted and as such, these strains are now used in many rearing and release programs. Both of these options result in improving the success of SIT by altering the probability of interactions between sterile and wild flies. If, in addition, sterile fly mating competitiveness could be improved, then releasing a lower number of sterile flies would result in an even more efficient SIT program

A survey in the 1950's identified several attractants of male medflies (Beroza & Green 1963) that were later developed for use with medfly trapping and monitoring programs. One of these attractants, trimedlure, was shown to increase the mating success of males after they were exposed to it (Shelly et al. 1996). In addition, exposure of medfly males to ginger root (*Zingiber officinale* Roscose) oil, which contains alpha-copaene at 0.4% volume, increased their mating competitiveness vs. wild males (Shelly 2001, Shelly & McInnis 2001). Alpha-copaene is a known male attractant that has been identified from angelica oil (Guiotto et al. 1972, Flath et al. 1994a, b, Nishida et al. 2000). Shelly & McInnis (2001) found a several-fold greater mating success of mass-reared, sterile flies exposed to ginger root oil compared with sterile flies not exposed. This study adds to previous research by comparing the mating success of sterile males at different sterile:wild fly ratios, to a 1:1 ratio of sterile (exposed to ginger root oil): wild flies.

Materials and Methods

Study Animals

Wild male and female Mediterranean fruit flies were collected as larvae and eggs from coffee, *Coffea arabica* L., from Kauai, Hawaii and from loquats, *Eriobotrya japonica* Thunb., from Kula, Maui, Hawaii in February and March, 2001. Fruits were placed on screens above vermiculite (22-26°C) that was sifted every 5-7 d for pupae. To obtain virgin flies for mating trials, newly emerged flies were separated by sex <2 d after eclosion. Adult flies were fed honey, sugar, and protein hydrolysate until they were sexually mature (>12 d old). Twenty five adult flies were held collectively in small plastic containers (400 ml) with nylon mesh screening. The source of wild flies for each mating trial was dependent on the availability of host material (coffee and/or loquat) and the number of flies obtained from each host.

Laboratory-reared flies of the Vienna-4 (Toliman) strain (male-only genetic sexing strain, carrying a temperature sensitive lethal (*tsl*) mutation) were obtained from the Tropical Fruit and Vegetable Research Laboratory, USDA-ARS, in Honolulu, HI. The sexing strain was obtained from the mass-rearing facility in Guatemala at El Pino in 1998. The larval rearing protocol followed that of Tanaka et al. (1969) & McInnis et al. (1994). Before irradiation, white pupae (presumed to be females) were removed in order to achieve a higher percentage of males. In preparation for irradiation, which occurred 2 d before eclosion, pupae were placed in hypoxia for 1-2 h. Sterilization was achieved using a dose of 14.5 Kr in a $Cobalt^{60}$ irradiator located at the University of Hawaii, Manoa (McInnis et al. 1996, Rendon et al. 1996). Adult sterile flies were fed honey, sugar, and protein hydrolysate until they were sexually mature (at least 4 d old). Adult flies were held in cages (16 liters, 225-250 flies per container) with nylon mesh screening until testing in the field. (Different size containers were used for wild and sterile, laboratory flies, in part because of the numbers of each type needed for each experiment and also to reduce the mortality of wild flies, which is usually high in the laboratory. Carey et al. (1995) have investigated the affects of different fly densities on mortality.

Mating Tests

On the day before a mating trial, one male type was marked (wild and Vienna-4 were alternated with each replicate) by placing a small drop of enamel paint on the thorax. This allowed later identification of male type in mating pairs. In the ginger root oil treatment, 1 day before the mating trial, 25 laboratory male flies were exposed in each of two small plastic containers (400 ml) for 3 h to 20 µl of ginger root oil (Citrus and Allied Essences Ltd., Lake Success, NY) placed on a 1 cm^2 piece of blotter paper. These flies were exposed in an isolated room that was distant from all other flies.

Circular, nylon-screened, field cages (2.5 m high, 2.5 m diameter, and containing a 2 m tall guava tree, *Psidium guajava* L.) were used for the mating trials (McInnis et al. 1996). Four treat-

ments were randomly assigned to four field cages for each of five replications over time. The treatments were 1:1, 5:1, and 10:1 ratios of sterile laboratory-reared males to wild males, and a 1:1 ratio of sterile laboratory-reared males exposed to ginger oil to wild males. In each cage, there were always 25 wild males and 25 wild females, so different ratios of laboratory to wild males were obtained by altering the number of laboratory-reared males (i.e., the 1:1 ratio had 25 laboratory-reared males, 5:1 had 125, and 10:1 had 250).

On the day of a mating experiment, males were released first into each of the cages, followed by the females after an interval of 5-10 min. Flies that were dead, incapable of flight, or noticeably damaged in any way at the time of release were replaced. Two field observers, alternating between cages every 15 min, located and removed mating pairs without replacement. Observations were made from approximately 0900 until 1400 h. Temperature ranged from 23-29°C and relative humidity ranged from 47-68% (HOBO® datalogger, Pro Temp/ RH, Onset Computer Corporation, Bourne, MA).

An ANOVA was performed on data, after arcsine transformation, for the three treatments with sterile males not exposed to ginger root oil to determine if there were significant differences in mating based on the sterile:wild fly ratio. A two-way t-test was used to compare transformed data from all four treatments.

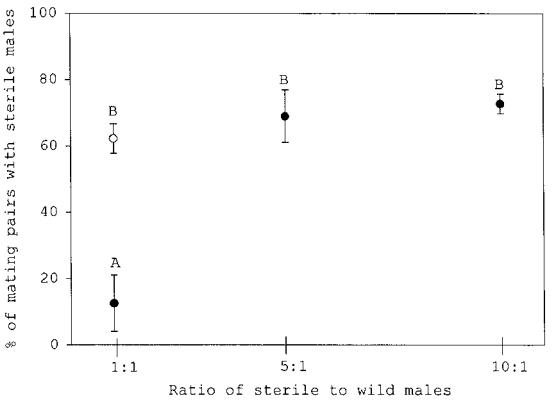

Fig. 1. Mating success (mean ± SE) of Vienna-4 (Toliman) sterile males not exposed (·), or exposed to ginger root oil (o), for 3 h, 1 d before mating competition trials with wild flies. The 1:1 treatment with sterile flies not exposed to ginger oil was significantly different from all other treatments (1:1 to 5:1, $P = 0.0057$, df = 4; 1:1 to 10:1, $P = 0.0068$, df = 4; and 1:1 to 1:1 ginger exposed, $P = 0.013$, df = 4).

Results

Mating Tests

With flies not exposed to ginger root oil, the sterile: wild fly ratio had a significant effect on the percentage of mating pairs involving a sterile male ($F = 14.98$; df 2, 12; $P = 0.001$; Fig. 1). Results with the 1:1 ratio treatment, with sterile flies not exposed to ginger oil, were significantly different from all other treatments (1:1 to 5:1, $P = 0.0057$, df = 4; 1:1 to 10:1, $P = 0.0068$, df = 4; and 1:1 to 1:1 ginger exposed, $P = 0.013$, df = 4). Pair-wise comparisons among the three remaining treatments (1:1 with ginger exposed sterile males, 5:1, and 10:1) were not significant ($P > 0.05$, df = 4).

Discussion

Specially treated mass-reared flies have the potential to outperform wild flies in mating competitiveness trials (Shelly & McInnis 2001). In our study, ginger root oil was responsible for increased mating success with sterile laboratory-reared flies. At a 1:1 ratio of sterile (exposed): wild flies, sterile males had a success (62% of the matings) similar to wild males. In comparison, a 1:1 ratio of sterile (not exposed): wild flies, resulted in sterile males failing to mate in 3 of 5 replicates. Sterile males (not exposed) showed mating success similar to, or above that of, wild males only at elevated ratios of 5:1 and 10:1 (sterile: wild males). These results suggest that exposure to ginger root oil elevates male mating success to a degree comparable with elevated ratios (5:1 or 10:1) of sterile/ wild males.

During a medfly infestation, in areas where eradication is the goal (i.e., Florida and California), it is likely that sterile:wild male ratios will be more skewed than the highest ratio that was tested in this study, 10:1, because of daily releases of sterile flies. Assuming that these cage tests are indicative of what may happen during mass releases, there could be as much as a 1/5 reduction in the number of flies released if they were pretreated with ginger root oil or if numbers of flies remained unchanged, then the flies released would have five times the mating success compared with the present system. The use of ginger root oil should result in a fly that is qualitatively better, regardless of the sterile:wild fly ratio.

In this study, the number of available wild flies was a limiting factor. Using higher numbers of wild flies in each cage would increase the power of the statistical tests, but would also increase the number of sterile flies that would be needed in order to maintain the ratios used. Using more than 300 flies in a field cage of the size we used (see Materials and Methods) could raise concerns about unnaturally high densities of flies, and whether the results could reasonably be extrapolated to open field conditions. Fly density in this study ranged from 15-60 flies/ m^2 (75 to 300 flies in a 4.9 m^2 cage). The Preventative Release Program

(PRP) in Southern California releases approximately 300 million flies each week over an area of approximately 6,446 km^2 (CDFA 2001). This corresponds to a density of approximately 0.048 flies/ m^2 (or 1 fly/ 21 m^2), assuming 0% mortality for released flies, 100% mortality for flies released the previous week, and uniform fly distribution. Testing higher ratios of sterile to wild flies, as well as using more total flies could be better accomplished with the use of larger field cages, however, the ability to find almost all mating pairs then becomes more difficult.

Further study is needed to determine how to incorporate ginger root oil into mass-rearing programs. The impact of ginger root oil on mating performance should also be evaluated against other strains of wild flies and using other sterile male strains. In addition, the economics and practical methodology of incorporating ginger root oil exposure into the large Mediterranean fruit fly SIT programs is now being investigated (TES and DOM, unpublished data).

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