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Evaluation of field dispersal and survival capacity of the genetic sexing strain Tapachula-7 of *Anastrepha ludens* (Diptera: Tephritidae)

*Salvador Flores, Sergio Campos, Enoc Gómez, Emigdio Espinoza, Willy Wilson and Pablo Montoya**

Abstract

The sterile insect technique (SIT) is an ecologically oriented strategy for pest control and a very important tool for establishing low pest prevalence and/or areas free of fruit flies (Diptera: Tephritidae). This technique involves releasing highly competitive sterile adults into an area with the aim to induce sterility in the wild population. Because genetic sexing strains are an economical and efficient improvement for SIT, the Moscafrut Program in Mexico developed the Tapachula-7 (Tap-7) strain of *Anastrepha ludens* (Loew) from which the female flies emerge from black pupae and can be separated mechanically allowing release of predominantly male flies. This study compared the field dispersal and survival of Tap-7 adult males with those of standard mass-reared adult males (SMR strain) after irradiation, packaging, and an aerial release of chilled adults. The Tap-7 strain exhibited a statistically larger dispersal pattern and slightly lower, although not statistically significant, survival compared with the SMR strain. These results show that both strains should perform similarly in the field and suggest that the Tap-7 strain could replace the standard one for field release of sterile flies against *A. ludens* wild populations in the near future, reducing costs in the use of the SIT.

Key Words: sterile insect technique; aerial release; longevity

Resumen

La Técnica del Insecto Estéril (TIE) es una estrategia para el control de plagas orientada ecológicamente, y una herramienta importante para el establecimiento de áreas libres y/o de baja prevalencia de moscas de la fruta (Diptera: Tephritidae). Esta técnica implica la liberación de adultos estériles altamente competitivos con el objetivo de inducir esterilidad en una población silvestre. Debido a que las cepas sexadas genéticamente representan una mayor eficiencia y economía para la TIE, el Programa Moscafrut en México desarrolló la cepa Tapachula 7 (Tap-7) de *Anastrepha ludens* (Loew), en la cual las hembras emergen de pupas negras y se pueden separar mecánicamente para liberar predominantemente machos. En este estudio se comparó la dispersión en campo y la sobrevivencia de machos Tap-7 con machos de la cepa estándar criados masivamente (SMR), después de la irradiación, empaque y liberación aérea utilizando la técnica del adulto frio. La cepa Tap-7 exhibió un patrón de dispersión mayor y una sobrevivencia ligeramente inferior en comparación con la cepa estándar, aunque no se encontraron diferencias estadísticas entre ambas cepas. Estos resultados muestran que ambas cepas pueden desempeñarse similarmente en el campo, y sugieren que la cepa Tap-7 podría sustituir en un futuro próximo a la cepa estándar en las liberaciones de insectos estériles contra poblaciones silvestres de *A. ludens,* reduciendo costos en la aplicación de la TIE.

Palabras Claves: Técnica del Insecto Estéril; liberación aérea; longevidad

Fruit flies of the genus *Anastrepha* (Diptera: Tephritidae) are devastating pests to the fruit industry in Latin America (Aluja 1994). In Mexico, the 4 species considered economically important are *Anastrepha ludens* (Loew), the main pest of citrus; *A. obliqua* (Macquart), which infests mangos and plums; *A. serpentina* (Wiedemann), infesting Sapotaceae; and *A. striata* Schiner, which feeds on guava (Aluja et al. 1996; Reyes et al. 2000).

The sterile insect technique (SIT), also known as autocidal control, is a technology that is currently used to control pestiferous tephritid species worldwide (Hendrichs et al. 2002; Klassen 2005). This technique is also the core component of the National Fruit Fly Program (PNMF, in Spanish) implemented by the Mexican government in collaboration with fruit producer associations. The aim of this program is to establish fruit fly–free areas in order to promote the national and international trade of the fruit crops grown in these areas (Reyes et al. 2000; Gutiérrez 2010). The released sterile insects must have biological attributes to compete successfully with the wild insects (e.g., survival, dispersal capability, and sexual competitiveness) to induce sterility in wild-type populations (Hernández et al. 2007; Meats 2007). Sterile flies, however, are typically less competitive than wild flies (Hendrichs et al. 2002), a characteristic mainly attributed to artificial selection during prolonged periods of mass rearing as well as irradiation, shipping, and handling procedures (Cayol 2000).

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Because only the males induce sterility in wild populations, the use of genetic sexing strains, which operationally eliminate females prior to irradiation and field release, can substantially improve the results achieved via SIT (Hendrichs et al. 2002; Rendón et al. 2004). Male-only strains also permit lower shipping, packaging, and release costs and reduce the fruit damage caused by sterile females when attempting oviposition. An additional advantage of using this type of strain can be the potential for decreasing the irradiation dose when the males are more sensitive to radiation than the females (Caceres 2002), and a reduced dose may improve the performance of released males (Rull et al. 2012 a,b).

Successful production of genetic sexing strains has already been achieved through conventional techniques and a filter system to remove genetic cross-overs as in the case of the *Ceratitis capitata* (Wiedemann) *tsl* strain (a temperature-sensitive lethal mutant) (Franz 2005; Robinson 2005). By using this strain, only males are produced and released as part of control actions in eradication programs that have been implemented in different regions of the world (see Rendón et al. 2004). Orozco et al. (2013a) and Flores et al. (2014) found that release of sterile males without sterile females significantly improved the efficiency of the SIT in the control of *A. ludens*.

Recently, the fruit fly rearing and sterilizing facility Moscafrut SAGARPA-IICA, located in Metapa de Domínguez, Chiapas, Mexico, began the mass production of the new genetic sexing strain of *A. ludens* named "Tapachula-7" (Tap-7 strain, henceforth). In this strain, the males emerge from normal-colored pupae (brown color), whereas the females emerge from black-colored pupae (Zepeda 2010; Orozco et al. 2013b). This characteristic allows the sorting of pupae by Sortex Z+-Buhler® machines (Buhler Sortex Inc., Stockton, California, USA), which select for the waste pupae (black in this case) by using an optical system and leave only the desired brown male pupae. After mechanical separation, the Tap-7 males must undergo irradiation and holding for emergence before the release of adults that are chilled at temperatures between 0 and 3 °C for ease of handling (Hernández et al. 2010). Tests in field cages indicated that Tap-7 sterile males did not differ in time to sexual maturity, sexual compatibility, and sexual competitiveness when compared with sterile males of the bisexual strain and wild males (Orozco et al. 2013b).

Some other quality parameters—such as dispersal and survival capacity—of the released insects could be affected during holding and release processes (Zavala et al. 2010). The ability of sterile flies to survive in the field and to move from the release point to feeding, mating, and resting sites is obviously a critical issue for the success of SIT programs (FAO/IAEA/USDA 2014). Therefore, we aim in this study to evaluate and compare the field dispersal, longevity, and survival of *A. ludens* Tap-7 strain males with the standard mass-reared (SMR, henceforth) strain males, both produced under mass-rearing conditions. These results will allow more rigorous assessment of the likely effectiveness of Tap-7 males in SIT programs.

Materials and Methods

This study was conducted in 2 phases. In the first phase, the dispersal ability of Tap-7 strain *A. ludens* males was determined by conducting ground releases in mango orchards. In the second phase, the longevity of aerially-released Tap-7 strain males was determined in the field.

BIOLOGICAL MATERIAL

Pupal lots (each lot = 14,000 pupae) of sterile *A. ludens* of the SMR and Tap-7 strains were provided by the Moscafrut facility located in Metapa de Domínguez, Chiapas, Mexico. The strains were produced following the procedures described by Domínguez et al. (2010) for the SMR strain and by Orozco et al. (2013b) for the Tap-7 strain. Before irradiation, each pupal lot was marked with a different dye color (Day-Glo® Color Corp, California, USA; i.e., fire orange and aurora pink for the SMR strain, and signal green and Saturn yellow for the Tap-7 strain; colors were alternated every 14 d) so that the origin of the adults captured in the field could be determined.

GROUND RELEASE EXPERIMENT

This experiment was conducted from Sep to Dec 2012 in a 64 ha mango ('Ataulfo') orchard Carrocera in Chiapas, Mexico (N 14°41'53" W 92°16'52"; 20 m asl; 35 °C mean temperature; 2,300 mm annual precipitation). For recapture of sterile flies, 64 Multilure® traps (1 trap per hectare) baited with Biolure® (ammonium acetate + putrescine) and water + propylene glycol (10%, as a preserving agent) were distributed 100 m apart in an 8 lines ´ 8 rows "grid" design with the release point at the center of the grid, as described in Hernández et al. (2007) .

Every 2 wk, pupal lots were packed at a density of 1,200 pupae per Kraft No. 20 paper bag. Each bag contained a 25 × 25 cm strip of brown paper saturated with Mb® (Mubarqui® Aerial Services, Ciudad Victoria, Tamaulipas, México) food (contains approximately 9% protein) and a strip of brown paper (1.0 m L × 25 cm W) that could be used as a resting site for the adults. The bags were kept at 25 ± 1 °C and 60 to 80% RH for 7 d and then transported to the field for ground release.

The mean emergence of adults was 80% and thus according to the standard for mass-reared *A. ludens* flies (FAO/IAEA/USDA 2014), which produced an average of 11,000 adults of each strain that were released at the central point. The traps were checked on days 1, 2, 3, 4, 6, 8, and 10, and the captured specimens were placed into glass bottles with 70% ethanol for transportation to the laboratory for identification (strain, sex, and mark color) using an epi-fluorescent microscope (SMZ 1500 Nikon, Japan) . Six replicates, each represented by one release every 14 d, were conducted.

AERIAL RELEASE EXPERIMENT

The releases were conducted over a 600 ha polygon located in Mazatán, Chiapas, Mexico (14°52'00"N, 92°27'00"W; 22 m asl; 28.9 °C mean temperature; 3,915 mm annual precipitation), at a density of 3,000 sterile adults per hectare. The released adults were monitored using a network of 41 Multilure® traps baited with Biolure® and georeferenced using a GPS (Garmin III plus).

Batches of colored sterile *A. ludens* pupae (1,500,000 SMR strain and 750,000 Tap-7 strain) were obtained every 2 wk from the Moscafrut facility. Pupae were packed in "Mexico"-type towers (Hernández et al. 2010) at a density of 18,000 flying adults per level for their emergence. Adults were fed a 1:24 mix of food (1 g protein and 24 g sugar) until they reached sexual maturity as recommended by Liedo et al. (2013). The sexually mature adults of both strains were exposed to a temperature of 3 to 4 °C for 40 min (Hernández et al. 2010) for easy collection and placement into aerial release boxes in a Cessna 206 airplane. Based on the estimate that the samples consisted of 80% flying adults, a total of 1,800,000 sterile adults (600,000 Tap-7 males mixed with 1,200,000 SMR males) were released. Traps were checked on days 2, 4, 7, 9, 13, and 16 post release (there were no fly captures after 16 d), and all of the captured adults were preserved in alcohol (70%) and transported to the laboratory for analysis under an epi-fluorescent microscope. As in the previous experiment, the sex, strain of origin, and mark color were determined and 6 replicates, each represented by one release every 14 d, were performed.

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DATA ANALYSIS

Ground Release

For each fly strain, adult movement was considered to be the standard distance calculated as mean distance from the release point and as standard deviation of this mean according to Thomas (2010). This author considers that dispersal of released and captured flies does not fit a normal distribution but a leptokurtic distribution, so the arithmetic mean may not be the adequate standard parameter. The total number of recaptured males was compared by using a factorial analysis in which the strain and recapture day were the factors considered. All analyses were performed by using the statistical software JMP (SAS Institute 2002).

Aerial Release

Longevity and life expectancy were compared between strains by using a *t*-test for paired data. Recapture curves were compared by using a log rank test adjusted to an exponential regression. The life expectancy on the release day (e_0) for each strain was calculated by using formulas from the life table (Carey 1989; Hernández et al. 2007). This method is based on the following two assumptions: 1) All ages have the same probability of being captured, and 2) flies remain in the trap area. The number of flies captured on a given day (Y_x) was used to calculate the accumulated number of flies captured from a given day, and the following equations were used:

$$n_0 = \Sigma Y_x \text{ and}$$
$$n_{x+1} = n_x - Y_x$$

Survival rate to a given day was calculated as:

$$l_x = n_x / n_0$$

Average life expectancy on the release day was estimated as:

$$e_0 = ½ + (l_1 + l_2 + \ldots + l_w)\, l_0$$

Where *w* stands for the last day of capture and l_0 was equal to 1.

Results

GROUND RELEASE EXPERIMENT

The average number of males recaptured per check date was 15.1 for the SMR strain and 12.9 for the Tap-7 strain, which did not differ (F = 1.13; df = 1, 70; P = 0.291). Furthermore, while there was an overall decrease in the number of flies recaptured over time (F = 7.88; df = 6, 70; P < 0.001). There was no significant difference between the two strains in the rate at which their respective numbers declined (F = 0.46; df = 6, 70; P = 0.836),

Males of the SMR and Tap-7 strains had similar distributions in the study plot (Fig. 1), although more Tap-7 males were captured close to the release point. The average dispersal distance for the SMR strain was 152.7 m and was significantly shorter than the average distance for the Tap-7 strain, which was 171.0 m (F = 3.65; df = 1, 5; P = 0.003). The standard deviation was 91.5 m for the Tap-7 strain and 85.7 m for the SMR strain, which did not differ (F = 2.17; df = 1, 5; P = 0.201). Considering that the observed fly dispersion tended to fit a leptokurtic distribution (Thomas 2010), dispersal of the two strains was measured by the standard deviation of the mean distance of captured flies from the release point, observing two fly groups, one with low dispersion and the other with higher dispersion. We drew connecting lines through these data points and found that the dispersal area of the SMR strain was completely overlapped by the dispersal area of the Tap-7 strain (Fig. 2).

AERIAL RELEASE EXPERIMENT

More males of the SMR than Tap-7 strain (111.7 versus 55.7) were recaptured per check date, which was significantly different between strains (F = 10.72; df = 1, 54; P = 0.002). There appeared to be no association between strain and the check date (F = 1.01; df = 5, 54; P = 0.366), but the decrease in the recapture number over time was significant (F = 21.82; df = 4, 54; P < 0.001). There was significant difference in the survival curves for the two strains (Fig. 3) (log-rank, χ^2 = 90.92; df = 1; P < 0.001). The average life time in the field, which was calculated based on the recapture numbers, was 4.2 d for the SMR strain and 3.6

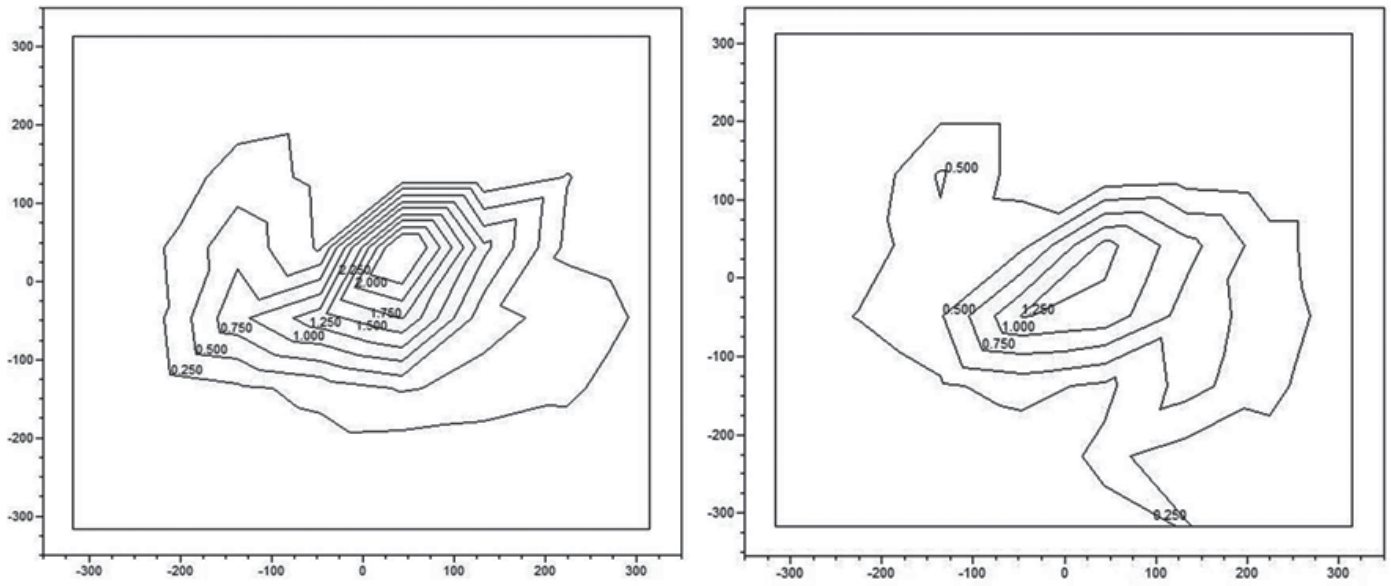


Fig. 1. Contours of displacement of the SMR strain (left) and Tap-7 strain (right) of *Anastrepha ludens* inside the field plot. The density of the flies at each contour is indicated by the number.

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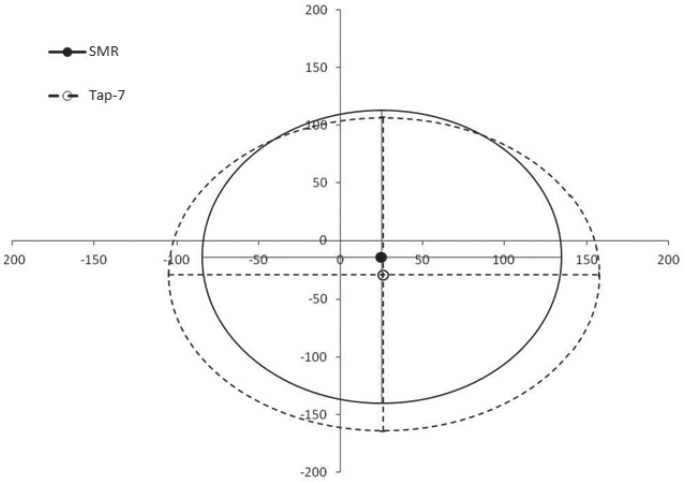


Fig. 2. General displacement of *Anastrepha ludens* SMR strain (solid line) and Tap-7 strain (broken line) in a Cartesian plane.

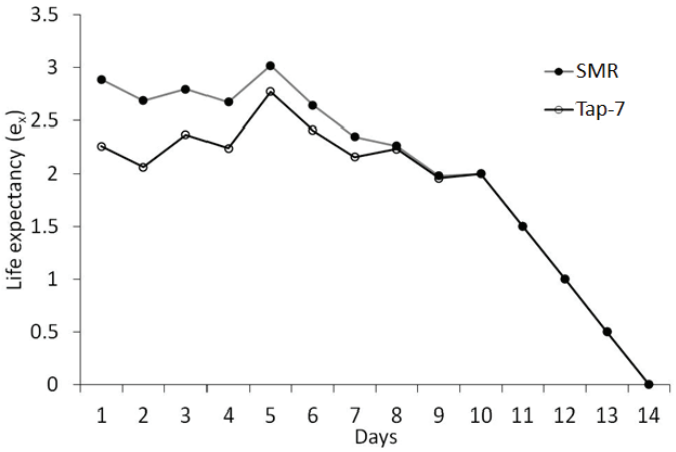


Fig. 4. Life expectancy of SMR and Tap-7 strains of *Anastrepha ludens* after aerial release.

d for the Tap-7 strain, which did not differ (F = 1.26; df = 1, 6; P = 0.299). Life expectancy of the SMR strain was greater than of the Tap-7 strain (F = 10.87; df = 1, 12; P = 0.006) (Fig. 4).

Discussion

According to Thomas (2010), the dispersal of fruit flies is appetitive, implying that flies move mainly in search of food. Furthermore, the phenology of the hosts and other agro-ecological conditions, such as temperature and relative humidity, may play an important role in the movement of these insects (Liu & Ye 2006). In this study, the dispersal of the Tap-7 strain (171 m) was significantly farther than that of the SMR strain (153 m) in a mango orchard with trees in the vegetative phase and under agronomic management (pruning and weed removal), conditions that are predominant in this area from Sep to Dec of each year. During the mango fruiting and harvest season, it is reasonable to expect a low fly dispersal distance given the high concentration of attractive host fruits. For *C. capitata* in southwestern Guatemala, the presence of cherries in a coffee crop is the main factor associated with dispersal (Midgarden & Lira 2008). In addition to host presence, the direction of the prevailing winds could play an important role in the distance and orientation of fruit fly dispersal (Díaz et al. 2008).

Regarding the dispersal pattern of the adult flies for both strains (Figs. 1 and 2), captures were highest near the release point. Plant & Cunningham (1991) and Meats & Smallridge (2007) reported that dispersion of irradiated *C. capitata* populations released from a single point could be modeled as if the population consisted of two subpopulations, one of which was dispersing in a diffusion-like pattern and the other was not dispersing. The non-dispersing subpopulation tended to remain in a circular pattern around the release point, as we observed in our results (Fig. 2). Paranhos et al. (2010) reported a low dispersal and low survival rate for sterile *C. capitata* adults, with 60% of the captures collected on the 1st day in a radius no greater than 25 m from the release point. This low dispersal may have reflected release in a favorable environment, resulting in little need for long movements to locate resources (Iwaizumi & Shiga 1989). Crowded conditions, however, can cause dispersal movements to farther distances (Froerer et al. 2010). The vegetation density at the release site is another factor that may influence the dispersal of adult flies as was shown for *A. ludens* by Utges et al. (2011).

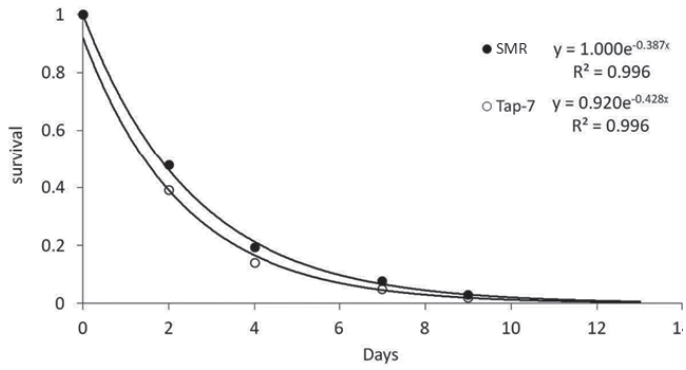


Fig. 3. Survival of SMR and Tap-7 strains of *Anastrepha ludens* after aerial release.

Typically, the mass-reared strains of tephritid flies have been associated with smaller dispersal capacities in the field compared with the wild individuals (Gilchrist et al. 2012). Gilchrist & Meats (2012) reported that an inbred mass-reared *Bactrocera tryoni* (Froggatt) strain had lower dispersal than an outbred strain. This was consistent with the results from Nakamori & Soemori (1981) for *Bactrocera cucurbitae* (Coquillett), as well as with our results because the Tap-7 strain covered a larger dispersal area than the SMR strain. The number of generations under mass rearing of the strains evaluated here (Tap-7 = 54 generations; SMR = 164 generations) could be playing a role in this observed difference, as Cayol (2000) reported that the age of a strain under mass-rearing conditions may affect some of the attributes of the released sterile adults.

The lower recapture rate observed for the Tap-7 than for the SMR strain may be the result of the combined effect of the shorter life expectancy and the greater rate of movement registered for the Tap-7 strain. The traps located on the outskirts of the orchard captured low numbers of Tap-7 males, even 7 d after release, and the central traps captured more SMR males during the 1st day after release. Life span of sterile males can be improved by providing suitable diet after emergence. Sterile adults of *A. ludens* and *A. obliqua* fed prior to release with a combination of sugar and fresh mango fruit pulp showed longer field survival than those fed with protein-enriched diets (Utges et al. 2011). However, Barry et al. (2007) reported that the ability of the sterile flies to locate and feed on protein and carbohydrate sources in the field may be more important for survival than the type of food supplied before the release.

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Survival times in the field after release for *A. ludens* have been reported to be 4.7 d (Utges et al. 2011) and 5.7 d (Hernández et al. 2007), which are within the range reported in this study for both strains. For *C. capitata*, Gavriel et al. (2012) reported that released adults rarely survived more than 5 d. These survival times are the minimum amount of time needed for the sterile males to reach sexual maturity, and the ability of sterile flies to survive until sexual maturity under field conditions is important for the successful use of the SIT (Thomas & Loera-Gallardo 1998; Gómez-Cendra et al. 2007). When the adults survive only a few days, it is recommended that the flies released are already sexually mature so that they are competitive with the wild insects soon after release (Paranhos et al. 2010).

Several studies (e.g., Dominiak 2012) have indicated that the dispersal distance of fruit flies rarely exceeds 1 km and that the scarcity of resources (e.g., water, food, hosts) triggers greater dispersal. The overlap of the dispersal patterns of the wild flies and sterile flies determines the success of the SIT (Iwaizumi & Shiga 1989; Gavriel et al. 2012) and indicates that releases must be planned carefully to include hot spots where the opportunities of sterile males to find wild females are high.

The genetic sexing strains tend to exhibit lower quality control standards than the bisexual strains (FAO/IAEA/USDA 2014). Even though the Tap-7 strain had a lower longevity, its dispersal capacity was slightly higher than that of the SMR strain, which may allow the Tap-7 adults to cover a larger area in their search for food sources and/or places for copulation, which could render better results in the field. In conclusion, our results on survival and dispersal indicate that both strains should perform similarly in the field, and suggest that Tap-7 could soon replace the SMR strain for field releases of sterile flies against *A. ludens* wild populations, which can render lower costs and better efficiency in the use of the SIT.

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