New Indices and Method to Measure the Sexual Compatibility and Mating Performance of *Ceratitis capitata* (Diptera: Tephritidae) Laboratory-Reared Strains Under Field Cage Conditions

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**ABSTRACT**

The method to assess the sexual compatibility and mating performance of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), mass-reared strains has been revised. Three new indices (isolation index, ISI; male relative performance index, MRPI; female relative performance index, FRPI) that look at the relative impact of both male and female population on the sexual isolation between laboratory and wild strains are described. The methodology and the indices were used in Argentina to test the SEIB 6–96 genetic sexing strain in competition with wild *C. capitata* population from the Patagonia region. The experiments show that the 2 populations are sexually compatible (ISI = 0.309). SEIB 6–96 sterile males compete with wild males, achieving 1 out of 4 Patagonian female mates. Using the current method as a decision tool, SEIB 6–96 sterile males are released weekly in Patagonia since September 1997. The accuracy of the 3 new indices is compared with the indices previously available. The possible application of the method and the indices in SIT operational programs world-wide is discussed.

**KEY WORDS** *Ceratitis capitata*, sexual compatibility, mating performance, genetic sexing strain

**THE DEMAND FOR** pesticide free fresh fruit is increasing rapidly in developed countries (Hendrichs et al. 1995). As a result, many developing countries, needing to participate in export markets, focus their efforts on the use of environmentally friendly and target-specific techniques such as the sterile insect technique (SIT) to control the *C. capitata*.

For the successful implementation of the SIT, it is essential to produce insects that will compete successfully with wild males for mating with wild females (Orozco and Lopez 1993). Laboratory tests have been developed that measure mating ability and sperm competitiveness (Haisch 1970), assess overall quality (Boller and Chambers 1977, Boller et al. 1981), and monitor competitiveness (Fried 1971). However, laboratory tests cannot assess the full behavioral repertoire of mass-produced insects and therefore field cage tests are essential. Field cage tests generally require that sexually mature males and females from 2 strains be released into a small cage containing a small host tree. Mating pairs are removed from the cage and the identity of each fly assessed. This data can then be used to calculate various mating indices. Zapien et al. (1983) were the first to use field cage tests as a quality control test to monitor the effectiveness of *C. capitata* laboratory strains under field-like conditions.

The indices currently being used to measure sexual isolation between *C. capitata* populations have limitations (Chambers et al. 1983, McInnis et al. 1996). Three new indices are presented that also measure sexual isolation, but take into account the relative mating performance of males and females. They were used to interpret data from field cage tests in Argentina. The tests were run using a genetic sexing strain (GSS), SEIB 6–96, currently mass reared at Mendoza for the release of sterile males in a large operational program and a wild population from the Patagonia Region. SEIB 6–96 is likely to be used for sterile male releases in a *C. capitata* eradication program in Patagonia.

The methodology used to measure the sexual compatibility and the mating performance of GSS sterile flies under field cage conditions is presented and the accuracy of the 3 new indices is compared with those previously available.

**Materials and Methods**

**Biological Material.** SEIB 6–96 is a genetic sexing strain carrying a white pupa (*wp*) mutation (Rössler 1979) in combination with the translocation T(Y;5)2–22 (Franz et al. 1994). The genetic background of the strain is from Egypt. The SEIB 6–96 pupae produced at the KMS facility (Mendoza, Argentina) were irradiated in hypoxia 2 d before emergence (“IMCO 20” Co60 irradiator, minimum absorbed dose = 100 Gy, maximum absorbed dose = 150 Gy) and packed and sent to the test location in Northern...
Argentina. The Patagonian population was collected as pupae from figs and peaches. Pupae from both strains were placed in separate ventilated Plexiglas cages (30 by 30 by 40 cm) until emergence. After emergence, females were kept in separate rooms from males to avoid contact with the male pheromone before the tests. All flies were maintained under low stress conditions at 24°C, 75% RH and kept in plastic containers (40 flies in each 1-liter plastic container) with water and food (protein and sugar). At least 48 h before the test, healthy flies were selected and marked with a dot of water-based paint (Deka) on the notum. Two colors were used (green and red) to mark alternatively wild or GSS flies (i.e., only 1 of the 2 types of flies in the same cage was marked).

Field Cage. The field cages were made of saran screen (20 by 20 mesh), they were cylindrical, with flat floor and ceiling (2.9 m diameter and 2.0 m high) (Chambers et al. 1983). Each cage was supported by a PVC frame as described by Calkins and Webb (1983). Each field cage was set over a citrus tree, which filled much of the cage. Pruning was sometimes necessary to facilitate observation in the cage. Six field cages were used. To compensate for the greenhouse effect caused by the field cage, a black shading material that filtered ~80% of the sunlight was added to the top of each field cage.

Field Cage Testing Protocol. The testing period covered the time of maximum sexual activity of both wild and mass-produced flies. Flies were released into the field cages at dawn (07.00 hours, local time) and the test lasted until 1400 hours (local time). Male flies were released 30 min before the females so that they could start forming leks (Prokopy and Hendrichs 1979). In the complete set of tests, flies were released at the age of sexual maturity, 5–10 and 6–8 d for wild and sterile flies, respectively. In the bisexual test, where 30 flies of each sex and each population were released, information was provided on sexual compatibility between the 2 populations. In the unisexual test, where 30 wild males, 30 wild males, and 30 SEIB 6–96 males were released, mating performance of SEIB 6–96 sterile males when competing with wild males for wild female mates was measured. Temperature, relative humidity, and light intensity in the field cages were recorded every 30 min. The number and type of calling males (marked or unmarked) were recorded every 15 min. The mating pairs were checked on a regular basis (every 30 min) and recorded according to its vertical position (bottom, middle, and top). The mated flies were not replaced or released back into the cage after separation (Chambers et al. 1983). A different observer was dedicated to each cage every day, and wild or SEIB 6–96 flies were marked randomly in different cages without informing the observers. Three replicates of each type of test were run on the same day.

Environmental Conditions. The environmental conditions recorded throughout the 7-d period of testing were suitable for C. capitata activities. The temperature ranged from 17°C early in the morning to 29°C at 1400 hours (local time) (Fig. 1) and relative humidity from 45 to 85% (Fig. 1). The mean light intensity ranged from 1,500 lux at 0800 hours to 8,500 lux at 1100 hours despite some cloudy days.

Proportion of Flies Mating. The proportion of flies mating measures the suitability of the flies and the environment for mating. It represents the overall mating activity of the flies, both wild and SEIB 6–96, and it is defined as follows:

$$ PM = \frac{\text{No. of pairs collected}}{\text{No. of females released}} \quad [1] $$

If the proportion of flies mating is <0.2, then meaningful data cannot be collected (IAEA 1997). In the current tests, the overall mean proportion of flies mating was ~0.5 (Table 1), indicating that ~50% of the total number of possible matings took place.

Measurement of Sexual Compatibility. Sexual compatibility is assessed in the bisexual test where each insect has an equal chance to mate. It is based on the preference shown by both type of flies (wild and SEIB 6–96) to mate with an individual of the same strain or another strain and has been calculated using 3 new indices. First, the isolation index (ISI) takes into account the difference existing between homotypic (within strain) and heterotypic (between strain) matings:

$$ ISI = \frac{(WW + LL) - (WL + LW)}{(LL + WW + LW + WL)} \quad [2] $$

where WW is the number of matings between wild males and wild females, LL between SEIB 6–96 males and SEIB 6–96 females, LW between SEIB 6–96 males and wild females, and WL between wild males and

![Fig. 1. Mean temperature and relative humidity recorded inside the field cages throughout the day (normal line, relative humidity; bold line, temperature).](image-url)
SEIB 6–96 females. The index ranges from -1 (negative assortative mating, as found in some Drosophila species [Hoikkala and Kaneshiro 1993]) to +1 to positive assortative mating or total sexual isolation). A value of 0 represents random mating (equal proportion of the 4 possibilities of mating), and sexual compatibility. The mean ISI value obtained (ISI = 0.309, variance = 0.034; Table 1) showed that there was a slight tendency for homotypic matings. To clarify this value, 2 other indices are needed. First, the male relative performance index (MRPI) which highlights any relative difference between males of the SEIB 6–96 strain and wild population in terms of overall mating performance:

\[
MRPI = \frac{(LW + LL) - (WL + WW)}{(LL + WW + LW + WL)} \cdot \frac{1}{\text{duration of the matings involving wild males, reflecting}}
\]

It ranges from -1 (all matings achieved by wild females) to +1 (all matings achieved by SEIB 6–96 males). A value of 0 represents an equal mating performance between sterile SEIB 6–96 and wild males. The mean MRPI value in the tests (MRPI = 0.089, variance = 0.052; Table 1) is close to 0 and shows that the SEIB 6–96 and wild males performed mating equally well. Secondly, the Female Relative Performance Index (FRPI) highlights any relative difference between females of the SEIB 6–96 strain and wild population in terms of overall mating performance:

\[
FRPI = \frac{(WL + LL) - (LW + WW)}{(LL + WW + LW + WL)}.
\]

The interpretation is similar to MRPI. The mean FRPI value (FRPI = 0.368, variance = 0.025; Table 1) shows that the SEIB 6–96 females participated in more matings than the wild females. This explains the departure from 0 of the mean ISI value and reflects a higher acceptance to mate for the SEIB 6–96 females.

Mating Performance of SEIB 6–96 Sterile Males. This was measured in the unisexual experiment using the Relative Sterility Index (RSI) (McInnis et al. 1996):

\[
RSI = \frac{LW}{(LW + WW)}.
\]

It is the proportion of matings achieved by SEIB 6–96 males when competing with wild males for wild females. RSI is an estimation of the sterility that would be induced by sterile male releases into a wild population at a particular ratio of sterile to wild males. In the case of a GSS, where only sterile males will be released in the field, RSI must be calculated in the unisexual-type experiment. When a bisexual strain is to be used for field releases, RSI must be calculated in the bisexual-type experiment. RSI ranges between 0 and +1 through an equilibrium value of 0.5, which represents an equal mating performance of wild and sterile males. Any value of RSI < 0.5 highlights a better performance of wild males, any value of RSI > 0.5 represents a better performance of sterile males. The mean RSI value obtained in the unisexual experiment (RSI = 0.264, variance = 0.027; Table 1) shows that SEIB 6–96 males achieved 26% of all matings with the Patagonian females. This indicates that the sterile SEIB 6–96 males perform well in competing for wild Patagonian females.

Male Calling Activity. For the success of an SIT program, it is important that released sterile males attract the wild females and that they follow the same sexual activity rhythm as the wild males. Counting the number of calling males throughout the day is a way to immediately identify any differences in activity rhythms between the SEIB 6–96 and the wild males. Fig. 2 shows the proportion of SEIB 6–96 and wild males calling during the day in the bisexual and unisexual tests. No qualitative differences were found in the male calling activity pattern of the 2 strains, implying that SEIB 6–96 males were well adapted to the local environment. However, ~2 times more SEIB 6–96 than wild males engaged in calling.

Duration and Starting Time of Mating. The duration of mating is the time spent by the flies in copula (excluding the duration of courtship). The duration of mating initially expressed as hours and minutes has been transformed into fractions of a day, using the TIMEVALUE function of Microsoft Excel 5.0, and analyzed by analysis of variance (ANOVA) and the Tukey honestly significant difference (HSD) tests using Systat for Windows (Systat 1996). The analysis (Table 2) shows that, in the bisexual test, the mean duration of the matings involving SEIB 6–96 males is significantly shorter (Tukey HSD test; \( P < 0.05 \)) than the duration of the matings involving wild males, regardless of the type of female. This was also confirmed in the unisexual test with only wild females.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Indices used</th>
<th>Indices previously available</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PM</td>
<td>RSI</td>
</tr>
<tr>
<td>Bisexual*</td>
<td>0.488</td>
<td>0.332</td>
</tr>
<tr>
<td></td>
<td>0.019</td>
<td>0.047</td>
</tr>
<tr>
<td>Unisexual*</td>
<td>0.492</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>0.037</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Means, variance in italics; NA, not applicable; PM, proportion of flies mating; ISI, isolation index; MRPI, male relative performance index; FRPI, female relative performance index; RSI, relative sterility index; RII, relative isolation index (McInnis et al. 1996); \( I_U \), isolation index for unisexual type test; \( I_B \), isolation index for bisexual type test (Stalker 1949); I, isolation index (Chambers et al. 1983).

* Based on 21 replicates, 520 pairs.

Based on 18 replicates, 520 pairs.
The starting time of mating is a representation of the time spent by the flies between the female release and the mating. It is important to verify that the matings of females are not limited by the nonavailability of 1 type of male. The analysis of the bisexual test (Table 3) shows that the SEIB 6–96 females mated significantly earlier than the wild females, regardless of the type of male. However, no significant difference between the starting time of mating of SEIB 6–96 and wild males was found. It suggests that when the SEIB 6–96 males are successful in mating with wild females they have been selected by the females within the same time range as the successful wild males. It also confirms that the starting time of mating is a parameter controlled by the type of female not the male.

Location of Mating Pairs. Considering that for *C. capitata* it is the female that approaches and selects the male for mating (Briceno et al. 1996), the location of matings inside the cage reflects the initial location of the calling males within the host tree. Data presented in Table 4 show that most of the successful wild males occupied the top of the tree and the successful SEIB 6–96 males were mainly present in the lower part.

During the tests, it was observed that most of the fights involving calling males (of both types), which defended their lekking territory, occurred in the upper part of the tree. This observation leads us to the hypothesis that the difference found in the original location of the mating pairs might illustrate a competition between wild and SEIB 6–96 males to occupy the top of the tree, where most of the matings occurred.

Discussion

The following 4 conclusions can be drawn from this data: (1) SEIB 6–96 is sexually compatible with the wild Patagonian population (ISI = 0.309); (2) SEIB 6–96 sterile males achieved ∼1/4 of the wild female matings (RSI = 0.264); (3) SEIB 6–96 sterile males and the wild males performed matings equally well, regardless of the type of females (MRPI = 0.089); and (4) SEIB 6–96 females were involved in more matings than the wild females (FRPI = 0.368), probably as a result of selection during mass-rearing which might reduce the discrimination of laboratory-reared females when selecting a male for mating.

The additional parameters measured during the experiments proved to be meaningful in interpreting the performance of the SEIB 6–96 GSS. The tests also highlighted some traits, like a higher proportion of males calling or a shorter duration of mating, which characterize the mass-reared *C. capitata* strains. However, one can consider it as a “waste” of biological resources and a disadvantage because it was proven that calling males are more prone to predation by wasps (Hendrichs et al. 1993, 1994). The experiments also showed that the duration of mating was shorter for SEIB 6–96 males than for the wild ones. Eberhard and Briceño (1996) showed that the duration of courtship is shorter for mass-reared males than for wild males, probably because of an adaptation to the crowding in mass-rearing cages. A shorter duration of mating can result from the same adaptation process.

Most of the indices previously used to quantify the mating compatibility between 2 populations of insect were developed on *Drosophila*. Stalker (1942) described 2 isolation (I) indices that could be used for bisexual (I_b) and unisexual-type experiments (I_u) (see Table 1 for values):

$$I_b = \frac{(%WL - %WL)}{(%LL + %WW)} \quad \text{and} \quad I_u = \frac{(%WW - %WL)}{(%WW + %LL)}. \quad [6]$$

Some other indices were also described by Bateman (1949), Levene (1949), Merrell (1950), and Barker

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### Table 2. Duration of mating of wild and GSS flies in field cage tests

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Mating combination</th>
<th>WW_n</th>
<th>WW_mean</th>
<th>LW_n</th>
<th>LW_mean</th>
<th>WL_n</th>
<th>WL_mean</th>
<th>LL_n</th>
<th>LL_mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisexual</td>
<td>WW</td>
<td>131</td>
<td>2.52a</td>
<td>54</td>
<td>1.53b</td>
<td>151</td>
<td>2.38a</td>
<td>238</td>
<td>2.06b</td>
</tr>
<tr>
<td></td>
<td>LW</td>
<td>151</td>
<td>2.38a</td>
<td>54</td>
<td>1.53b</td>
<td>54</td>
<td>1.53b</td>
<td>238</td>
<td>2.06b</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>238</td>
<td>2.06b</td>
<td>54</td>
<td>1.53b</td>
<td>238</td>
<td>2.06b</td>
<td>54</td>
<td>1.53b</td>
</tr>
<tr>
<td>Unisexual</td>
<td>WW</td>
<td>221</td>
<td>2.38a</td>
<td>84</td>
<td>2.23b</td>
<td>221</td>
<td>2.38a</td>
<td>84</td>
<td>2.23b</td>
</tr>
<tr>
<td></td>
<td>LW</td>
<td>84</td>
<td>2.23b</td>
<td>221</td>
<td>2.38a</td>
<td>84</td>
<td>2.23b</td>
<td>221</td>
<td>2.38a</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>221</td>
<td>2.38a</td>
<td>84</td>
<td>2.23b</td>
<td>221</td>
<td>2.38a</td>
<td>84</td>
<td>2.23b</td>
</tr>
</tbody>
</table>

Means followed by the same letter on the same row do not differ significantly according to Tukey HSD test with α < 0.05. n, Number of pairs in the category. Mean duration of mating is in hours and minutes.

* Mating combination, first letter male, female second, W and L for wild and GSS *C. capitata*, respectively.
In the 1980s, as mating compatibility and mating performance became critical issues for the use of SIT checked. Fried (1971) described an indirect method to measure the sterile male competitiveness and, consequently, mating compatibility, by calculating the hatchability of eggs laid by the females. However, this method has some difficulties to be applied in a field. It requires an additional device (like agar balls) to collect the eggs and a longer test so that egg hatch can be sure the sterile male competitiveness and, consequently.

Fried (1971) described an indirect method to measure the sterility of male competitiveness and, consequently, mating compatibility, by calculating the hatchability of eggs laid by the females. However, this method has some difficulties to be applied in a field. It requires an additional device (like agar balls) to collect the eggs and a longer test so that egg hatch can be checked.

In the 1980s, as mating compatibility and mating performance became critical issues for the use of SIT in C. capitata eradication/control programs. Chambers et al. (1983) used a modified version of Stalker's index where

$$I = \frac{(LW + WL)}{(LL + WW)}. \quad [7]$$

Like Stalker’s index, the isolation index does not provide any explanation of why a particular value is obtained. It was used by Robinson et al. (1986) in the 1st field cage tests with a C. capitata GSS. McInnis et al. (1996) devised 2 indices specifically for testing C. capitata under field cage conditions, the RSI as used in the present tests, and the relative isolation index (RII), which compares the numbers of homotypic matings with heterotypic matings:

$$RII = \frac{(LL \times WW)}{(LL \times WL)}. \quad [8]$$

The RII ranges between 0 and $+\infty$. A value of 1 indicates random mating between strains while values >1 indicate positive assortative mating. Because it is multiplicative, the RII is very sensitive to changes in a single type of mating. Also, where LW = 0 or WL = 0 (which could be meaningful in case of sexual isolation), RII cannot be calculated. In addition, the high variance associated with this index is not conducive to informative statistical analysis. If the RII value is calculated for the current test (Table 1), the SEIB 6–96 strain should not be used for sterile male release, but the ISI, FRPI, MRPI, and RSI showed clearly that the performance of the strain was satisfactory.

A reliable measurement of sexual isolation must take into consideration the relative contribution of both, the sex and origin of the insects, based on the number (or proportion) of each type of combination. The 4 indices, RSI, ISI, MRPI, and FRPI, represent an attempt to do this. They must be used exclusively with a 1:1 sterile to fertile ratio; nevertheless, the indices could be modified for fluctuating ratios. Being additive rather than multiplicative, these indices are not very sensitive to changes in single type of pair, they can always be computed and a generally very low variance makes them meaningful and reliable. To be used as an easy-to-use field diagnostic tool, the interpretation of sexual isolation measurement must not require any complex mathematical transformation. As stated by Levene (1949) the 3 indices range from −1 to 1 with an equilibrium at 0, which immediately, and without any transformation, give a clear representation of the sexual compatibility to the field cage observers.

The indices described in the current work provide not only a measurement of “sexual isolation” but they also analyze the factors why 2 strains are or are not sexually compatible. The field cage assessment of the sexual compatibility and the mating performance of mass-reared C. capitata strains as described in the current work represents a reliable tool for SIT operational programs and mass-rearing facilities. With very little equipment and human resource investment, the sexual compatibility assessment can be used to decide which strain is more suitable for field release, to assess the sexual compatibility of wild C. capitata populations from different geographic origins, and to test the sexual behavior of recently developed GSS. In addition, the mating performance assessment represents a ma-

<table>
<thead>
<tr>
<th>Type of test</th>
<th>On the tree</th>
<th>On the cage screen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bottom</td>
<td>Middle</td>
</tr>
<tr>
<td>Bisexual</td>
<td>WW</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WL</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>LW</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>15</td>
</tr>
<tr>
<td>Unisexual</td>
<td>WW</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>LW</td>
<td>4</td>
</tr>
</tbody>
</table>

Means followed by the same letter on the same row do not differ significantly according to Tukey HSD test with $P < 0.05$. $n$, Number of pairs in the category. Mean starting time of mating is expressed as time of the day.

*a Mating combination, first letter male, female second, W and L for wild and GSS C. capitata, respectively.

*b For 100 matings occurred in bisexual type experiment.

*c For 100 matings occurred in unisexual type experiment.
ior quality control method to detect any modification in the performance of a mass-reared strain, and it can be used as a reference to decide on the need for strain replacement.

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