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Author(s): T. Mastrangelo, M. F. Chaudhury, S. R. Skoda, J. B. Welch, A. Sagel, and J.M.M. Walder

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Feasibility of Using a Caribbean Screwworm for SIT Campaigns in Brazil

T. MASTRANGELO,[1,2] M. F. CHAUDHURY,[3] S. R. SKODA,[3] J. B. WELCH,[4] A. SAGEL,[3] AND J.M.M. WALDER[1]

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ABSTRACT The screwworm, *Cochliomyia hominivorax* (Coquerel), remains one of the most damaging parasites of livestock in South America, causing millions of dollars in annual losses to producers. Recently, South American countries demonstrated interest in controlling this pest using the Sterile Insect Technique, and a pilot-project was conducted near the Brazil-Uruguay border in 2009. Since molecular studies have suggested the existence of *C. hominivorax* regional groups, crossing tests were conducted to evaluate mating competitiveness, mating preference and reproductive compatibility between a *C. hominivorax* strain from the Caribbean (Jamaica-06) and one from Brazil. Mating rates between Jamaican males and Brazilian females ranged between 82 and 100%, and each male inseminated from 3.3 to 3.95 females. Sterile males, regardless of the strain, competed equally against the fertile males for Brazilian females. Jamaican sterile males and Brazilian fertile males mated randomly with fertile or sterile females. No evidence of genetic incompatibility or hybrid dysgenesis was found in the hybridization crosses. Mating barriers should not compromise the use of Jamaican sterile males for Sterile Insect Technique campaigns in Brazil.

KEY WORDS livestock pest, myiasis, insect mating, *Cochliomyia*

Brazil's livestock sector has been experiencing modernization since the 1970s. Agribusiness complexes have expanded, livestock production practices have changed and, today, Brazil has one of the largest commercial cattle herds in the world. Brazil's beef-cattle industry generates revenue of about 30 billion dollars a year, provides >7.5 million jobs, and live cattle exports have risen (≈650,000 animals were exported to Venezuela and Arab countries in 2010) (Poll 2011). However, a substantial traditional beef-cattle industry can still be found in less developed parts of Brazil, where productivity remains low and is plagued by serious management and sanitary problems.

The cumulative effects of endo and ectoparasitic diseases are major factors limiting production. The screwworm, *Cochliomyia hominivorax* (Coquerel), remains one of the most damaging parasites of livestock in Brazil, with losses estimated at 1.7 billion a year (Vargas–Teran et al. 2005). The screwworm attacks warm-blooded animals, including humans (e.g., 22 human myiasis cases by *C. hominivorax* were reported between July 2007 and May 2008 in São Gonçalo, Rio de Janeiro) (Silva et al. 2008).

The Sterile Insect Technique (SIT) is one strategy used to control *C. hominivorax* (Knipling 1955). Screwworms have been eradicated from the United States, Mexico, and Central America using SIT as part of a comprehensive approach (Wyss 2000). Panama was declared screwworm-free in 2006 and a buffer zone of 30,000 km^2 was established at the Darien Gap by the weekly release of 25–50 million sterile males (Garcia et al. 2007). Estimated annual producer benefits in the United States, Mexico and Central America exceed 1 billion dollars (Vargas–Teran et al. 2005).

In 2006, the Comisión México–Americana para la Erradicación del Gusano Barrenador del Ganado (COMEXA) presented a regional project to the Brazilian Ministry of Agriculture and a pilot-area of 466,200 ha was defined at the Brazil–Uruguay border, where losses to screwworm myiasis were estimated at 150,000 dollars a year. The project was conducted between January and May 2009. Sterile flies were imported from Mexico and released by the Uruguayan Air Force. However, after 13 wk of releasing ≈1,545 sterile flies/km^2 (≈21.6 million sterile flies per week), sterility of collected wild egg masses never exceed 21.5% (Pontes et al. 2009).

These results and some recent molecular studies suggesting significant genetic differentiation in *C. hominivorax* populations, specifically in the Caribbean and south of the Amazon basin (McDonagh et al. 2009, Fresia et al. 2011), prompted us to conduct crossing studies to evaluate mating competitiveness, mating

[1] Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Avenida Centenário 303, Caixa Postal 96, 13400-970 Piracicaba, SP, Brazil.
[2] Corresponding author, e-mail: thiagomastrangelo@gmail.com.
[3] USDA–ARS–KBUSLIRL, Screwworm Research Unit, Kerrville, TX 78028.
[4] USDA–APHIS Screwworm Eradication Program, 9100 Panama Place, Washington, DC 20521.

Table 1. Percentages of inseminated females and no. of inseminated females per male (mean ± SE) from crosses between Jamaican males (sterile and fertile) and Jamaican or Brazilian females of *Cochliomyia hominivorax*

Crosses[a] ♀ × ♂	Inseminated females (%)	No. inseminated females/male
JAM (fertile) × JAM (sterile)	96.1 ± 3.9	3.8 ± 0.15
BRA (fertile) × JAM (sterile)	94.7 ± 5.3	3.7 ± 0.21
BRA (fertile) × JAM (fertile)	84.8 ± 2.7	3.4 ± 0.12
JAM (fertile) × JAM (fertile)	94.8 ± 3.03	3.8 ± 0.12
ANOVA	$F = 1.8$ns[b]	$F = 1.82$ns
	df = 3, 11	df = 3, 11
	$P = 0.22$	$P = 0.21$
	C.V. = 7.2%	C.V. = 7.3%

[a] JAM, Jamaica-06 strain; BRA, Brazilian strain.
[b] ns, not significant; $P > 0.05$.

preference, and reproductive compatibility between two screwworm strains.

Materials and Methods

Insects. Reproductive compatibility and competitiveness tests between Brazilian and a Jamaican strains were conducted in the Screwworm Research Unit (U.S. Department of Agriculture–Agriculture Research Service) laboratory, located inside the Comisión para la Erradicación y Prevención del Gusano Barrenador del Ganado (COPEG) facility in Pacora, Panama. The Brazilian strain (BRA) was collected in Goiás state, central Brazil, and the initial colony was established with 30 ml of pupae in 2010. The Jamaica-06 strain (JAM), established from 50 screwworm egg masses collected from Jamaica in 2006, is currently being mass-reared in Panama. Flies used for crosses were standardized by size (pupal weight ≥50 mg and adult with cephalic capsule ≥3 mm). Pupae were irradiated with 65 Gy (standard dose being used in the facility) at 5.5 d postpupation in a self-contained ^{60}Co irradiator (JLF 484 R2-D) to produce sterile adults. Flies were kept in aluminum screened cages of 30 × 14 × 13 cm (mating compatibility and mating preference tests) or 24 × 28 × 18 cm (mating competitiveness and postzygotic genetic compatibility tests). Fly density was kept below 0.17 flies/cm^2 in cages (Baumhover et al. 1966). Water (wet cloth in test tubes) and diet (spray dried egg + honey at 1:4 on plates covered by rice husk) were offered ad libitum.

Mating Compatibility Between Jamaican Males and Brazilian Females. Sexual compatibility between JAM (sterile and fertile) males and BRA females was assessed using four crosses (Table 1). Males and females were separated within 24 h of emergence and held separately until 4 d old. For each cross, 20 females and 5 males were placed together for 36 h. After 36 h, the females were anesthetized by cold and their spermathecae were removed, squashed, and microscopically examined for the presence of sperm in saline solution (1% NaCl). Percentage of females inseminated and number of mated per male were determined (Adams 1979).

Mating Competitiveness Test. The mating competitiveness of sterile and fertile males from both strains for BRA fertile females was evaluated (Table 2). In crosses from 'a' to 'd,' 15 fertile females were exposed to 5 males. In crosses from 'e' to 'i,' 30 females and 5 males of each type were used. In crosses 'j' and 'k,' 30 females were exposed to 1 fertile and 9 sterile males. After 7 d, females were allowed to oviposit on a plate containing ground meat and spent larval diet in a dark room (37°C; 80% relative humidity [RH]) for 2 h (Berkebile et al. 2006). During the oviposition period, the plates with adult diet were covered to avoid feeding. Eggs were separated (Berkebile and Skoda 2002) and samples of 100–200 eggs allowed to hatch on moistened black filter paper in petri dishes at 38°C and 90% RH. Egg hatch was assessed after 16 h.

Mating Preference Test. Two groups of crosses were conducted to examine the preference of JAM sterile males and BRA fertile males for sterile or fertile females (Figs. 1 and 2). For each cross, 10 females of each strain were placed with 5 males at a male:female ratio of 1:4. Females were marked individually to distinguish the strains by applying a small dot of water-based paint on the dorsal surface of the thorax. Flies were kept together for 36 h, after which females were

Table 2. Competitiveness indices (mean ± SE) of sterile males from Jamaican and Brazilian strains of *Cochliomyia hominivorax*

Crosses[a] ♀ × ♂	Egg hatch (%)	Competitiveness index	ANOVA
a. BRA × BRA (fertile)	92.3 ± 2.3	N/A	N/A
b. BRA × BRA (sterile)	0.7 ± 0.1	N/A	
c. BRA × JAM (fertile)	86.6 ± 4.2	N/A	
d. BRA × JAM (sterile)	1.6 ± 0.6	N/A	
e. BRA × BRA (fertile) + JAM (fertile) (1:1)	87.8 ± 1.2	N/A	
f. BRA × BRA (fertile) + BRA (sterile) (1:1)	51.1 ± 3.8	0.84 ± 0.13[b]	$F = 0.47$ns[c]
g. BRA × JAM (fertile) + JAM (sterile) (1:1)	45.6 ± 4.7	0.93 ± 0.33	df = 3, 11
h. BRA × BRA (sterile) + JAM (fertile) (1:1)	53.4 ± 4.1	0.71 ± 0.15	$P = 0.71$
i. BRA × BRA (fertile) + JAM (sterile) (1:1)	44.9 ± 1.3	1 ± 0.06	C.V. = 17.8%
j. BRA × BRA (fertile) + JAM (sterile) (1:9)	11.1 ± 1.6	0.95 ± 0.21	$F = 0.05$ns
			df = 1, 5
k. BRA × BRA (sterile) + JAM (fertile) (9:1)	12.8 ± 3.9	1 ± 0.53	$P = 0.84$
			C.V. = 23.1%

[a] JAM, Jamaica-06 strain; BRA, Brazilian strain. Ratio fertile:sterile inside parenthesis.
[b] Original means in this table. The competitiveness indices were transformed by "$(x + k)$" before the analyses.
[c] ns, not significant; $P > 0.05$.

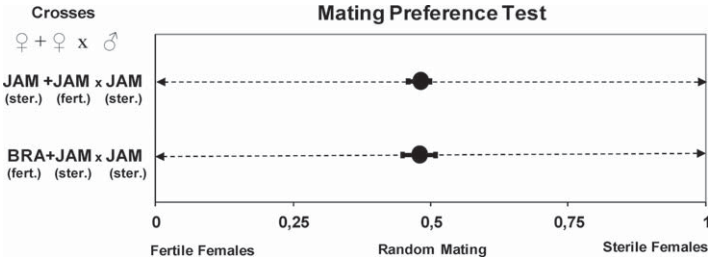

Fig. 1. Mating preference indices for Jamaica-06 sterile males (ster., sterile; fert., fertile).

anesthetized and their spermathecae examined as previously described to determine the number of inseminated females. The mating preference index was estimated as: number of sterile inseminated females per total number of inseminated females. The mating preference index may range from zero (males mate only with fertile females) to one (males mate only with sterile females).

Assessment of Postzygotic Genetic Compatibility. Two sets of crosses were conducted to assess the postzygotic compatibility between JAM and BRA strains. The first set (parental crosses) compared intrastrain and reciprocal interstrain crosses ('a' to 'd') and the second set (F_1 crosses; 'e' to 'i') compared four backcrosses to BRA and a BRA intrastrain cross (Table 3). For each cross, 80 couples were placed together for 7 d, after which the females were allowed to oviposit (Berkebile et al. 2006). Eggs were separated according to Berkebile and Skoda (2002). To assess larval viability, 100 mg of eggs were used for 1.5 liters of cellulose fiber-based diet (Chaudhury and Skoda 2007). Parameters recorded were egg hatch (%), larval viability (%), adult emergence (%), and sex ratio. Larval viability was calculated as: (number of pupae per initial number of larvae) × 100. The adult emergence was assessed by placing samples of 100 pupae in petri dishes. Sex ratio was obtained from the same petri dishes used for adult emergence and estimated as: number of females/(number of females + males). Comparisons between the two sets of crosses were not performed because they were not conducted simultaneously.

Statistical Analyses. All crosses were replicated three times and all tests were done as completely random designs. The means of percentage of inseminated females and the number of inseminated females per male from the mating compatibility test and the competitiveness indices from the mating competitiveness test were compared by analysis of variance (ANOVA) (ANOVA; $P \leq 0.05$). The mating preference indices from the two set of crosses were compared separately by the Student's t-test ($\alpha = 5\%$). The estimated means for the parameters recorded in the hybridization test (i.e., egg hatch, larval viability, adult emergence, and sex ratio) were compared by ANOVA (ANOVA; $P \leq 0.01$). All analyses were performed by the statistical program SAS 9.1. The assumptions of homoscedasticity (Hovtest; PROC GLM) and normality (Shapiro–Wilk test; PROC UNIVARIATE) were assured (SAS Institute 2003).

Results

Mating Compatibility Between JAM Males and BRA Females. The percentages of females mated with sterile or fertile males ranged between 82 and 100% and did not differ significantly ($F = 1.8$; df = 3, 11; $P = 0.22$). The number of inseminated females per male ranged between 3.3 and 3.95 females per male and did not differ significantly (Table 1).

Mating Competitiveness Test. Egg hatch ranged between 82 and 95% in the crosses between fertile flies from both strains (Table 2; crosses 'a', 'c,' and 'e'). The observed egg hatch varied between 0.6 and 2.2% in crosses between only sterile males (JAM or BRA) and BRA fertile females (Table 2; crosses 'b' and 'd'). These values were used to estimate the competitiveness indices for the sterile males from both strains in the other crosses (Fried 1971).

The mean egg hatch values in the four crosses with males at the ratio one fertile: one sterile (crosses 'f–i') ranged between 40 and 58%. The competitiveness indices from these crosses did not differ significantly (Table 2), indicating that the sterile males, regardless

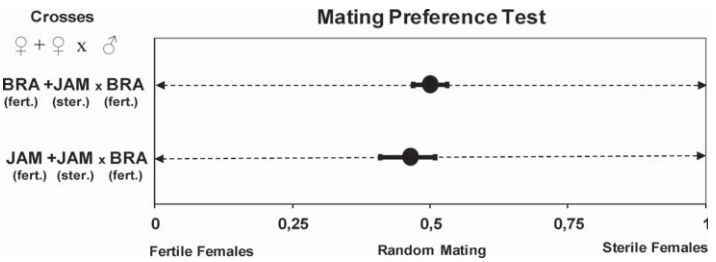

Fig. 2. Mating preference indices for Brazilian fertile males (ster., sterile; fert., fertile).

Table 3. Biological parameters (means ± SEs) from the assessment of the postzygotic genetic compatibility between the Jamaica-06 strain and a Brazilian strain of *Cochliomyia hominivorax*

Crosses[a] ♀ × ♂	Egg hatch (%)	Larval viability (%)	Adult emergence (%)	Sex ratio[b]
a. JAM × JAM	85.9 ± 5.7	89.9 ± 5.2	97.7 ± 1.5	0.6 ± 0.07
b. BRA × BRA	89.3 ± 0.4	91.2 ± 4.5	97.3 ± 1.3	0.5 ± 0.06
c. BRA × JAM	89.8 ± 4.9	89.9 ± 7.5	95 ± 2.9	0.5 ± 0.05
d. JAM × BRA	96.2 ± 1.3	89.6 ± 5.7	98.3 ± 1.7	0.5 ± 0.03
ANOVA	$F = 1.3$ns[c]	$F = 0.01$ns	$F = 0.5$ns	$F = 1.9$ns
	df = 3, 11	df = 3, 11	df = 3, 11	df = 3, 11
	$P = 0.35$	$P = 0.99$	$P = 0.65$	$P = 0.21$
	C.V. = 7.3%	C.V. = 11.2%	C.V. = 3.5%	C.V. = 17.2%
e. BRA × BRA (control)	90.1 ± 0.7	87.8 ± 6.1	93.3 ± 3.3	0.5 ± 0.06
f. (BRA × JAM) × BRA	87.9 ± 2.3	83.1 ± 11.6	98.3 ± 1.7	0.5 ± 0.08
g. (JAM × BRA) x BRA	91.8 ± 2.7	87.8 ± 1.5	95 ± 0.6	0.6 ± 0.08
h. BRA × (BRA × JAM)	95.7 ± 1.7	91.1 ± 4.6	96.7 ± 3.3	0.5 ± 0.03
i. BRA × (JAM × BRA)	95.5 ± 0.9	84.9 ± 9.9	98.3 ± 1.7	0.4 ± 0.03
ANOVA	$F = 3.5$ns	$F = 0.16$ns	$F = 0.84$ns	$F = 0.88$ns
	df = 4, 14	df = 4, 14	df = 4, 14	df = 4, 14
	$P = 0.05$	$P = 0.95$	$P = 0.53$	$P = 0.51$
	C.V. = 3.4%	C.V. = 15.2%	C.V. = 4.3%	C.V. = 20.2%

[a] JAM, Jamaica-06 strain; BRA, Brazilian strain.
[b] Sex ratio, ♀/♂ + ♀.
[c] ns, not significant; $P > 0.01$.

of the strain, competed equally against the fertile males for BRA females.

In the two crosses with males at the ratio one fertile: nine sterile, mean egg hatch values were 11.1 ± 1.6% for the cross with BRA fertile male and 12.8 ± 3.9% for the cross with JAM fertile male and were not significantly different.

Mating Preference Test. The mating preference indices varied between 0.41 and 0.53 (Figs. 1 and 2), and no significant difference was found among crosses ($F = 0.01$; df = 1, 5; $P = 0.9$). These values indicate that males mated randomly when sterile and fertile females were exposed simultaneously in the cages.

Assessment of the Postzygotic Genetic Compatibility. Biological parameters assessed in the parental crosses were high and did not differ significantly among crosses (Table 3). In backcrosses, the mean values for egg hatch, larval viability, and adult emergence were all >70%, sex ratio was ≈0.5, and no significant differences were observed among treatments (Table 3).

Discussion

The existence of genetic differences among populations or even reproductive incompatibility in cryptic species is a critical factor for Area-Wide Integrated Pest Management programs that integrate the SIT (Krafsur 2005). In the early 1970s, the screwworm campaign experienced many difficulties in the southwestern United States despite releases of thousands of sterile flies per square kilometer. Richardson et al. (1982) then proposed that *C. hominivorax* was a complex of reproductively isolated populations in North America. However, subsequent studies failed to find any evidence of speciation (LaChance et al. 1982, McInnis 1983, McInnis et al. 1983, Dev et al. 1986, Taylor et al. 1991, Krafsur and Whitten 1993, Taylor and Peterson 1994, Torres et al. 2007).

Krafsur and Hightower (1979) did not find significant differences in mating frequencies between sterile flies of two strains (Tex-Mex and FF8) and wild populations from Mexican coastal areas. After the infestation of Libya in 1988, Taylor et al. (1991) crossed the strain mass-reared in Mexico (OW-87, originated from Belize) with flies originated from egg masses collected near Tripoli and observed no reproductive incompatibility. All the results of this work indicated that the Brazilian *C. hominivorax* population is reproductively compatible and competitive with the Jamaica-06 strain. The Brazilian females did not reject the fertile and sterile Jamaican males.

Alley and Hightower (1966) observed 2.6 matings per male in strains from Mexico and Florida after 1 h of exposure (3 males placed with 30 females). Crystal (1967) reported 4.8 matings/male after 1 h (1 male with 15 females) and 6.4 matings/male after 2 h. Adams (1979) verified that males between 4 and 16 d old inseminated 3 females after 4 h (1 male with 5 females). Taylor et al. (1991) reported that males of the OW-87 strain inseminated an average of 5 females after 7 d together (1 male placed with 10 females). In the current work, males mated with between 3.3 and 3.95 females after 36 h, what is consistent with the previous reports.

Crystal (1979) reported competitiveness values for sterile males ranging from 0.39 to 0.91, with an average of 0.75. Pitti et al. (2011) evaluated the competitiveness of sterile males of different sizes and observed relative competitiveness indices between 0.32 and 1.55. They also reported that the competitiveness of the smaller sterile males was only 0.51 ± 0.14. The competitiveness of the sterile males was around 100% in this work. Even the lowest observed competitiveness index, the value of 0.71 for BRA sterile males, indicated that 1.4 sterile males 'equals' 1 fertile male to produce an expected sterility of 50%.

Studies to verify the preference of *C. hominivorax* males for females from different strains or reproductive status (sterile or fertile) are very rare. García (2002) performed mating studies in small laboratory cages using a mass-reared strain (Panama-95) and wild Jamaican and Cuban flies and reported high preference (96–100%) of the wild males for wild females, providing some evidence of asymmetric mating. However, this was probably related to differences in the profile of the cuticular sex pheromones between the strains (Hammack 1987, Carlson et al. 2009). All the mating preference indices in this work were around 0.5, indicating that males mated randomly when sterile and fertile females were exposed simultaneously in the cages.

No evidences of genetic incompatibility or hybrid dysgenesis were found from the interstrain crosses in the current work. All the hybrids demonstrated normal fertility in backcrosses to the Brazilian strain.

Genetic diversity patterns of *C. hominivorax* populations have been extensively investigated through nuclear and mitochondrial markers, restriction fragment length polymorphism (RFLP) analyses and sequence data (Roehrdanz 1989; Taylor et al. 1996a,b,c; Lyra et al. 2005, 2009; Torres et al. 2007; McDonagh et al. 2009; Torres and Azeredo–Espin 2009; Fresia et al. 2011). The majority of genetic studies have suggested low differentiation among *C. hominivorax* populations.

Studies using *mt*DNA and microsatellites as molecular markers revealed low differentiation among populations from Uruguay and Brazil (Lyra et al. 2005; International Atomic Energy Agency [IAEA] 2006; Torres et al. 2007). McDonagh et al. (2009) revealed a separation of two Texan samples from the South American and Caribbean samples, besides grouping the Cuban populations together (although a Brazilian sample from Minas Gerais appeared strongly associated with the Cuban populations). According to Fresia et al. (2011), the general distribution pattern of genetic variability is structured into four regional groups: Cuba, the Dominican Republic, the North and South Amazon regions. The authors suggested that the Caribbean area and the North and South Amazon regions should be considered as independent units for forthcoming SIT campaigns.

Although both nuclear and mitochondrial DNA sequences have been used to investigate population genetic variability in *C. hominivorax*, most of the molecular studies have focused more on analysis of mitochondrial markers. In fact, most of *mt*DNA-based population studies in insects have often used only single protein coding genes from a limited range of those potentially available, with many *mt*DNA genes being largely ignored and others receiving only sporadic attention (Caterino et al. 2000, Shao and Barker 2007).

Genetic studies can be useful for the identification of genetic relationships among *C. hominivorax* populations, but they say very little about the compatibility between the populations in terms of mating. For SIT effectiveness, to assure the mating between wild females and the released sterile insects is much more critical than only knowing the genotype of the insects (Robinson et al. 2009). So far, there is not sufficient, unequivocal evidence through genetic analyses to contest the hypothesis that *C. hominivorax* is a single, panmitic species throughout both its historical and existing ranges.

In conclusion, no reproductive barrier would compromise the use of Jamaican *C. hominivorax* flies for SIT campaigns in Brazil, once the quality of the sterile flies is assured. Ultimately, the suppression of the *C. hominivorax* from the countries of the Mercado Comun del Sur (MERCOSUR) would contribute greatly to the alleviation of rural poverty and the promotion of better livestock production systems.

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