Bases para el desarrollo de estrategias de manejo de moscas de la fruta (Diptera: Tephritidae)

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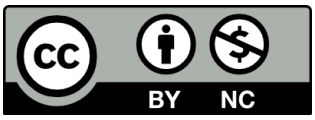

Tesis aprobada por el tribunal integrado por Dr Sergio Ovrusky, Dr Rodrigo Lasa y Dr Alfredo Abot, el (día) de (mes) de (año). Autora: Ing. Agr. M.Sc. Felicia Duarte, director Dr. Flávio Roberto Mello Garcia, codirector Dr. Enrique Morelli.

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RESUMEN

Ceratitis capitata y *Anastrepha fraterculus* (Diptera: Tephritidae) son plagas polífagas que generan pérdidas de rendimiento y calidad en frutales e incrementan costos de producción por ser cuarentenarias en algunos países destino de exportación. La técnica del insecto estéril (TIE), altamente selectiva, ha sido implementada en varios países para el control de *C. capitata*, mientras que para *A. fraterculus* su desarrollo está siendo investigando. Se realizaron: 1-análisis de la fluctuación y distribución espacial de ambas especies silvestres y la correlación entre las capturas en trampas y los niveles de daño en fruto, 2-análisis de compatibilidad sexual entre *A. fraterculus* locales y una población proveniente de Argentina y 3-análisis de dispersión de machos estériles de *C. capitata* en dos regiones. Se monitorearon 79 trampas Jackson con trimedlure y 88 trampas McPhail con torula, y se muestrearon 5.700 frutos en tres fincas frutícolas durante dos temporadas. En jaulas de campo se determinaron el índice de aislamiento sexual (ISI) y los tipos de parejas formadas de acuerdo al origen de los sexos. Además, se liberaron 20.000 machos estériles de *C. capitata* TslV8 en dos zonas y se instaló una red de 54 trampas Jackson con trimedlure formando anillos concéntricos a los puntos de liberación. Se estimó dispersión y longevidad. El coeficiente de correlación de Spearman entre capturas y porcentaje de frutos infestados fue para *C. capitata* 0,62 (P = 0,0001) con capturas en trampas McPhail, 0,34 (P = 0,02) con capturas en trampas Jackson, y para *A. fraterculus* 0,59 (P = 0,0001) con capturas en trampas McPhail. El ISI fue significativamente diferente de cero debido a un mayor desempeño de los adultos argentinos. No hubo diferencias entre la frecuencia de las parejas homotípicas uruguayas y las parejas heterotípicas, y la frecuencia de las parejas homotípicas argentinas fue mayor que el resto. Los machos estériles de *C. capitata* se dispersaron una distancia estándar de 127 m y 131 m para Salto y San José, respectivamente.

Palabras clave: *C. capitata, A. fraterculus*, compatibilidad, TIE

BASES FOR THE DEVELOPMENT OF MANAGEMENT STRATEGIES FOR FRUIT FLIES (DIPTERA: TEPHRITIDAE)

SUMMARY

Ceratitis capitata and *Anastrepha fraterculus* (Diptera: Tephritidae) are polyphagous pests that cause yield and quality losses in fruit trees and increase production costs because they are quarantine pest in some export destination countries. The sterile insect technique (SIT), highly selective, has been implemented in several countries to control *C. capitata*, while for *A. fraterculus* the technique development is being investigated. The following were carried out: 1-analysis of fluctuation and spatial distribution of both wild pest species, and the correlation between the captures in traps and the levels of fruit damage, 2-analysis of sexual compatibility between local *A. fraterculus* and a population from Argentina and 3-dispersion analysis of sterile males of *C. capitata* in two regions. Seventy-nine Jackson traps with trimedlure and 88 McPhail traps with torula were monitored, and 5,700 fruits were sampled, in three fruit farms, during two seasons. In field cages, the sexual isolation index (ISI), and the kind of pairs formed according to the origin of sexes were determined. Besides, 20,000 sterile males of *C. capitata* TslV8 were released in two areas, and a network of 54 Jackson traps with trimedlure was installed, forming five rings concentric to the release points. Dispersion and longevity were estimated. Spearman's correlation coefficient between captures and percentage of infested fruits was 0.62 (P = 0.0001) for *C. capitata* with captures in McPhail traps, 0.34 (P = 0.02) with captures in Jackson trap, and for *A. fraterculus* 0.59 (P = 0.0001) with captures in McPhail traps. The ISI was significantly different from zero due to higher performance of Argentine adults. There were no differences between the frequency of the Uruguayan homotypic couples and the heterotypic couples, while the frequency of the Argentine homotypic couples was higher than the rest. Sterile males of *C. capitata* dispersed a standard distance of 127 m and 131 m to Salto and San José, respectively.

Keywords: *C. capitata*, *A. fraterculus,* compatibility, SIT, spatial distribution

1. INTRODUCCIÓN

Existen cerca de cinco mil especies de tefrítidos en el mundo, de las cuales 70 tienen importancia económica y pertenecen a los géneros *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis* y *Toxotrypana* (Garcia, 2009). Sin embargo, en Uruguay solo *Ceratitis capitata* y *Anastrepha fraterculus* son especies de moscas de la fruta de interés económico y cuarentenario (Bentancourt y Scatoni, 2010).

La mosca del Mediterráneo *C. capitata* es nativa del norte de África y presenta actualmente una amplia distribución mundial manifestando gran adaptabilidad a condiciones climáticas variadas. Se encuentra en la mayoría de las zonas tropicales, subtropicales y templadas del mundo. Posee un elevado potencial reproductivo y es extremadamente polífaga, registrándose más de 350 especies vegetales hospedantes (Liquido et al., 1991). Se presenta como la plaga de mayor importancia económica en la citricultura mundial y, bajo determinadas condiciones agroclimáticas, como plaga primaria en durazneros, perales y manzanos. En ausencia de control puede provocar pérdidas de hasta un 100 % de la producción (Thomas et al., 2007, Ekesi et al., 2005, Herrera, 2005).

A. fraterculus es nativa de Sudamérica, localizándose desde México hasta Argentina. Es una especie polífaga que vive sobre una amplia gama de frutos silvestres y cultivados; con cerca de 100 hospedantes reportados (Norrbom, 2004), es de las especies de moscas de las frutas de mayor importancia económica en la región neotropical (Norrbom y Kim, 1988) y es de gran importancia en Uruguay, aunque su incidencia es inferior a la de *C. capitata* (FAO, 1989).

1.1. PERJUICIOS EN LA PRODUCCIÓN

1.1.1 Daños directos

Las larvas causan daños directos al alimentarse y desarrollarse en el interior de los frutos, deteriorándolos hasta un grado inaceptable tanto para el consumo fresco como para el uso agroindustrial. En muchos casos el solo hecho de que el fruto tenga el orificio de oviposición es suficiente para que pierda valor comercial. Los orificios y las galerías son vías de entrada para microorganismos, que llevan a la aparición de

pudriciones secundarias y maduración a destiempo; y si la oviposición se da en estados tempranos, los frutos no logran alcanzar un desarrollo adecuado y caen al suelo (Bentancourt y Scatoni, 2010, Porras y Lecuona, 2008, Quesada-Moraga et al., 2006, Ekesi et al., 2003).

1.1.2. Importancia cuarentenaria

La importancia económica más significativa de estas especies es su calidad de plagas cuarentenarias. Para poder acceder a ciertos mercados internacionales donde estas plagas están ausentes son necesarias medidas cuarentenarias muy estrictas tomadas en pre- y poscosecha. Esta situación conlleva a un incremento en el costo de producción, así como la necesidad de implementar técnicas y programas de manejo que demandan un compromiso de particulares y del Estado, tanto a nivel nacional como regional (Cohena et al., 2008, IAEA, 2003, Hendrich, 1996).

Desde el año 2001 la Dirección General de Servicios Agrícolas (DGSA) implementa el actual Sistema Nacional de Vigilancia de Moscas de la Fruta en cultivos citrícolas en el norte y sur del país, y más recientemente en arándanos (DGSA-MGAP, 2017), lo que paralelamente ha generado conocimientos sobre su importancia económica en estos huéspedes (Elina Zefferino, comunicación personal, diciembre de 2016). China y Estados Unidos, por estar libres de mosca del Mediterráneo en su espacio continental (Vargas et al., 2008), son muy estrictos en cuanto a la exigencia de bajos niveles poblacionales para habilitar la exportación, sin aceptar que se supere un umbral de 1 MTD (mosca/trampa/día) al momento de la cosecha (DGSA-MGAP, 2017). Asimismo, para todos los mercados de exportación que se encuentran fuera de Sudamérica, Uruguay debe garantizar la ausencia de *Anastrepha fraterculus*.

1.2. LAS MOSCAS DE LA FRUTA EN EL CONTEXTO FRUTÍCOLA URUGUAYO

1.2.1. Disponibilidad de hospedantes

La fruticultura en Uruguay presenta una diversidad de explotaciones comerciales que se distribuyen en distintas zonas agroecológicas. Los principales cultivos frutícolas son los cítricos, que ocupan casi 15.000 ha, de las cuales el 85 % está en la zona norte

(Salto, Paysandú, Rivera), donde también pueden encontrarse cultivos hortícolas. En la zona sur se encuentran cerca de 2000 ha de cítricos (San José, Canelones, Maldonado, Colonia, Montevideo) y frutales de carozo y pepita que en total ocupan en el entorno de 5400 ha (Montevideo, Canelones, San José) (DIEA/MGAP, 2017). Además, en los últimos años, se han establecido nuevos cultivos como arándano, arazá, caqui y guayabo, los que, a su vez, conviven con otros frutales no comerciales presentes en los establecimientos como el níspero, la higuera y otros hospedantes de las moscas de las frutas.

La abundancia de las poblaciones de moscas de la fruta depende, entre otros factores, de la disponibilidad de hospedantes en la zona. La polifagia registrada en *C. capitata* y *A. fraterculus* (Liquido et al., 1991, Oroño et al., 2006) y la continua presencia de frutos maduros de hospedantes cultivados (MGAP/DIEA, 2011 y 2016) y silvestres (Traversa-Tejero y Alejano-Monje, 2013) predispone a que las poblaciones puedan desarrollarse durante todo el año en algunas regiones. *C. capitata* prospera mejor en ambientes perturbados, mientras que *A. fraterculus* prefiere áreas donde existen remanentes de vegetación nativa o sitios donde predominan sus hospedantes nativos en relación a los introducidos (Ovrusky et al., 2003). No obstante, la fruta es un sustrato atractivo para la oviposición y apropiado para el desarrollo de las larvas de las moscas de la fruta en un lapso próximo a la maduración. En Uruguay, en noviembre comienzan a madurar los primeros frutos de carozo, período que se extiende hasta febrero. En enero comienza la maduración de peras y las primeras variedades de manzanas, que culmina en abril-mayo (DIEA-MGAP, 2016). La maduración de los cítricos comienza hacia fines de verano y se extiende durante el invierno. Sólo considerando estos dos grupos de hospedantes (cítricos y frutales de hoja caduca), las moscas de las frutas tienen sustrato apropiado para su desarrollo en forma ininterrumpida a lo largo de todo el año.

1.2.2. Manejo sanitario

Actualmente la principal estrategia de control consiste en aplicar insecticidas formulados como cebos, aprovechando el comportamiento de las hembras adultas que necesitan alimentarse previo a la reproducción para alcanzar la madurez sexual.

La mosca del Mediterráneo produce huevos solamente después de alimentarse de carbohidratos durante el estado adulto y la producción aumenta cuando ingieren fuentes de proteína o aminoácidos (Cangussu y Zuculoto, 1992). La atracción de estos cebos es por un período relativamente corto, por lo que las aplicaciones en el período de maduración de la fruta se hacen a intervalos de 7 días, incrementando los costos de producción. Las restricciones y niveles de tolerancia actuales al uso de plaguicidas en productos de exportación hacen que los insecticidas organofosforados registrados para este uso en Uruguay ya no puedan ser utilizados (Bryant Christie Inc., 2021), restando solo un único principio activo, el spinosad, para aplicar en estos casos, lo que podría traer aparejados problemas adicionales de resistencia. Si bien la formulación como cebo mejora mucho la selectividad del principio activo, este es capaz de atraer y matar a otros insectos con similar comportamiento. En Uruguay se requiere, por tanto, el desarrollo de estrategias de control que contemplen información del insecto, el ambiente y las exigencias de los mercados internacionales, para contribuir a un descenso sostenible de las poblaciones.

1.3. LA TÉCNICA DEL INSECTO ESTÉRIL (TIE)

La técnica del insecto estéril (TIE) ha sido empleada con éxito en diversas partes del mundo como Argentina, Chile, Perú, Méjico y EE. UU., tanto con objetivos de erradicación como de supresión de las poblaciones de moscas de la fruta (Vreysen et al., 2007, IAEA, 2003). La técnica se basa en la liberación masiva de machos estériles de la especie que se quiere controlar, con el objetivo de reducir al mínimo la descendencia.

En el caso de *C. capitata*, existen biofábricas para la cría y esterilización de machos de la especie en Estados Unidos, Méjico, Guatemala, Argentina, Brasil, Chile, Perú, España, Portugal, Israel, Australia, Sudáfrica y Austria. Estas biofábricas producen insectos en forma masiva y automatizada, cuyas pupas macho son seleccionadas y sometidas a radiación (rayos X, rayos gamma). Una vez esterilizadas se deja que completen su ciclo biológico y los machos adultos son liberados en las plantaciones donde se desea controlar la plaga. (Lance y McInnis, 2005). Sin embargo, aún no se ha logrado ajustar esta técnica para el control de *A. fraterculus* y hace tiempo se está

investigando con este objetivo (Cladera et al., 2014). Para ello se han conformado grupos de investigadores abordando diferentes temas que incluyen la mejora de dietas artificiales (Oviedo et al., 2011, FAO/IAEA, 1999, Salles, 1992), la simplificación de la cría masiva (Vera et al., 2007, Jaldo et al., 2001, Nuñez Bueno, 1998, González Bachini, 1971) el ajuste de dosis de radiación para la obtención de insectos estériles competitivos (Allinghi, 2007, Bartolucci et al., 2006, González Bachini, 1971) conocimientos básicos para el desarrollo de cepas masculinas (Segura et al., 2009), la reducción del tiempo de maduración de los machos (Peralta et al., 2014, Liendo et al., 2013), la identificación y el aislamiento de señales de comunicación química (Břízová et al., 2013, Vera et al., 2013) y estudios genéticos (Selivon et al., 2005a, 2005b, 2004 y 1999, Rocha y Selivon 2002, Lifschitz et al., 1999) y morfométricos (Hernández-Ortiz et al., 2015, 2012, 2004) para identificar y determinar la distribución de los distintos morfotipos.

1.3.1. Compatibilidad reproductiva

El éxito de la TIE está directamente relacionado con la compatibilidad y competitividad sexual de los insectos liberados con la población silvestre (Lance y McInnis, 2005). La compatibilidad sexual es el grado en que dos grupos de animales simpátricos tienden a aparearse al azar sin importar el grupo de origen, en vez de aparearse selectivamente con los miembros de su propio grupo, mientras que la competitividad sexual hace referencia a la aceptación de las hembras salvajes de los machos estériles (en contraposición a machos salvajes) como parejas para el apareamiento. Según Cayol (2000), no existiría aislamiento sexual entre las especies de *C. capitata*, pudiendo las líneas de insecto estériles desarrolladas en cualquier laboratorio aparearse al azar con las poblaciones silvestres a lo largo del mundo. Sin embargo, en el caso de *A. fraterculus*, los resultados obtenidos en diversos estudios sugieren la existencia de al menos ocho entidades biológicas diferentes a las que se hace referencia como los morfotipos 1, 2 y 3 brasileños, morfotipo peruano (Yamada y Selivon, 2001), morfotipo mexicano (Petit-Marty et al., 2004), morfotipo andino, morfotipo venezolano (Steck, 1991) y, más recientemente, morfotipo ecuatoriano (Hernández-Ortíz, 2015). En Argentina existiría una única población de *A.*

fraterculus compatible con las poblaciones del sur de Brasil (Rull et al., 2012). En lo referente a nuestro país, aún no se sabe si las poblaciones de *A. fraterculus* locales están aisladas reproductivamente o no y con cuáles poblaciones serían compatibles. Determinar el morfotipo presente en Uruguay es un requisito básico, ya que va a determinar la compatibilidad de apareamiento con insectos estériles que se puedan producir en la región.

1.3.2. Distribución espacial y capacidad de dispersión

Está determinado que la efectividad de las liberaciones de insectos estériles disminuye a medida que el grado de agregación de las poblaciones objetivo aumenta (Barclay 1992, Sawyer et al,, 1987). Este efecto es superado en la medida en que los insectos estériles son distribuidos o en que ellos mismos se redistribuyen en zonas de alta densidad de insectos silvestres. La distribución de las poblaciones silvestres y estériles debe entenderse para ser capaces de asignar y distribuir de manera óptima a los insectos estériles (Ito y Koyama, 2005, Lindquist et al., 1974). Los recientes avances en los sistemas de información geográfica (SIG) y bases de datos pueden facilitar la identificación, seguimiento y realización de tratamientos diferenciales de los focos poblacionales en una relativamente amplia escala espacial. Sin embargo, si las agregaciones de las poblaciones objetivo se producen en escalas espaciales más finas o no son predecibles, las tasas de liberación en general pueden tener que ajustarse hacia arriba para garantizar que las proporciones locales estéril:silvestre sean lo suficientemente altas para conseguir el nivel deseado de esterilidad (Cox y Vreysen, 2005).

En el mismo sentido, conocer la capacidad de dispersión de los machos estériles de *C. capitata* en los sistemas de producción de frutas uruguayas es información fundamental para la aplicación de esta estrategia de control. El conocimiento de la capacidad de dispersión y supervivencia de machos estériles en combinación con el conocimiento de la distribución espacial de las moscas silvestres en los diferentes hospedantes a lo largo de la temporada permite elaborar una propuesta de manejo en grandes áreas.

Existen algunos trabajos relacionados a la distribución espacio-temporal de *C. capitata* (Sciarretta y Trematerra, 2011, Papadopoulos et al., 2003); sin embargo, no se encontraron estudios sobre la variabilidad espacio-temporal de *A. fraterculus* y *C. capitata* coexistiendo en un mismo hábitat. Segura et al. (2006) estudiaron la abundancia relativa de *A. fraterculus* y *C. capitata* en diversos hospedantes y localidades de Argentina y demostraron que ambas especies conviven allí en varias áreas y exhiben requerimientos ecológicos similares. Conocer la fluctuación poblacional de *C. capitata* y *A. fraterculus* compartiendo un mismo hábitat es un factor determinante para ajustar la estrategia de control a aplicar.

1.4. RELACIÓN ENTRE LAS CAPTURAS EN TRAMPAS JACKSON Y MCPHAIL Y EL NIVEL DE DAÑO EN FRUTOS

Las capturas en trampas son el método más práctico y rápido para establecer zonas con diferente abundancia de poblaciones y determinar los momentos más apropiados para adoptar medidas de control (Lang et al., 2006, IAEA, 2003); no obstante, previamente hay que establecer cuáles son las relaciones entre dichas capturas y los daños observados en los diferentes hospedantes (Vayssières et al., 2009).

Los programas de manejo integrado de plagas se basan en el monitoreo de la población de plagas y, en los casos en que se han podido determinar, en umbrales de control preestablecidos, como componente fundamental en la toma de decisiones (Norris et al., 2003). Si bien hay algunas indicaciones sobre los umbrales de control para moscas de la fruta (Nascimento et al., 2000), se han encontrado pocos antecedentes sobre la relación entre las capturas de adultos en trampas y el daño asociado en fruto (Paiva y Parra, 2013, Grout et al., 2011, Uchôa-Fernandes et al., 2003, Agunloye, 1987).

1.5. HIPOTESIS DE TRABAJO

La abundancia relativa de las poblaciones de *A. fraterculus* y *C. capitata* en los distintos hospederos varía a lo largo del año en función de la disponibilidad de frutos hospederos maduros.

Es posible predecir los daños provocados por *C. capitata* y *A. fraterculus* previo a la cosecha a través de las capturas acumuladas de adultos en diferentes tipos de trampas. Las poblaciones de *A. fraterculus* presentes en Argentina, el sur de Brasil y Uruguay son similares y compatibles sexualmente.

La capacidad de dispersión de machos estériles de *C. capitata* no supera los 250 m.

1.6. OBJETIVO

1.6.1. Objetivo general

Generar información científica básica para el manejo de *C. capitata* y *A. fraterculus* con énfasis en el uso de la TIE para el control de tefrítidos en Uruguay.

1.6.2. Objetivos específicos

1. Analizar la fluctuación y distribución espacial de las poblaciones silvestres de *C. capitata* y *A. fraterculus*.
2. Determinar la correlación entre las capturas de adultos en trampas y los niveles de daño en fruto.
2. Analizar la compatibilidad sexual entre las *A. fraterculus* locales y una población proveniente de Argentina.
3. Determinar la dispersión y supervivencia de machos estériles de *C. capitata* en dos regiones productivas del país.

2. SPATIO-TEMPORAL DISTRIBUTION OF *ANASTREPHA FRATERCULUS* AND *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) CAPTURES AND THEIR RELATIONSHIP WITH FRUIT INFESTATION IN FARMS WITH A DIVERSITY OF HOSTS [2]*

2.1. RESUMEN

Ceratitis capitata (Wiedemann) y *Anastrepha fraterculus* (Wiedemann) (ambos Diptera: Tephritidae) causan graves pérdidas económicas en la producción de frutas; por ello, es importante conocer las fluctuaciones poblacionales de estas plagas que comparten el mismo hábitat y compiten por nichos similares, así como conocer su relación con la infestación de frutos, todos los cuales son componentes fundamentales para entender cómo manejar los riesgos de infestación en fincas con diversidad de hospedantes susceptibles. En la presente investigación se analizó la distribución espacio-temporal de *C. capitata* y *A. fraterculus* en 3 fincas frutícolas junto con la incidencia de daño en fruto en diferentes especies y cultivares hospedantes. Se monitorearon 79 trampas Jackson cebadas con trimedlure y 88 trampas McPhail cebadas con levadura torula desde septiembre de 2014 hasta junio de 2016 y se muestrearon un total de 5.700 frutos durante las 2 temporadas. Se calculó el coeficiente de correlación de Spearman entre capturas e infestación de frutos y se construyeron mapas de capturas acumuladas y distribución de infestación de frutos por sitio y temporada. Se graficaron la fluctuación de la población y la infestación de los frutos para ambas especies de moscas de la fruta, mientras que la fluctuación de la población discriminada por sexo se analizó para *C. capitata*. El coeficiente de correlación de Spearman entre las capturas de *C. capitata* en trampas McPhail durante las 2 semanas previas a la cosecha y el porcentaje de frutos infestados fue 0,62 (P = 0,0001), mientras que para las trampas Jackson fue 0,34 (P = 0,02). La correlación entre las capturas de *A. fraterculus* en trampas McPhail y la

[2] Felicia Duarte, Victoria Calvo, Soledad Delgado, Flávio R. M. Garcia, Iris Scatoni "Spatio-Temporal Distribution of Anastrepha fraterculus and Ceratitis capitata (Diptera: Tephritidae) Captures and their Relationship with Fruit Infestation in Farms with a Diversity of Hosts," Florida Entomologist, 104(4), 297-306, (17 December 2021)

infestación de frutos fue de 0,59 (P = 0,0001). Se discute la variación observada en el número de adultos e infestación de frutos de ambas especies de plagas entre sitios y grupos de especies hospedantes.
Palabras clave: Moscas del mediterráneo, Mosca Sudamericana, capturas,daño en fruto, correlación2

2.2. SUMMARY

Ceratitis capitata (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) cause severe economic losses to fruit production. Thus, it is important to know the population fluctuations of these pests that share the same habitat and compete for similar niches, as well as to know their relationship with fruit infestation, which are fundamental components for understanding how to manage the risks of infestation in farms with a diversity of susceptible hosts. In the present research, the spatio-temporal distribution of *C. capitata* and *A. fraterculus* in 3 fruit farms was analyzed together with the incidence of fruit damage in different host species and cultivars. Seventy-nine Jackson traps baited with trimedlure and 88 McPhail traps baited with torula yeast were monitored from September 2014 to June 2016, and a total of 5700 fruits were sampled during the 2 seasons. The Spearman correlation coefficient between captures and fruit infestation was calculated, and maps of accumulated captures and fruit infestation distribution were built by site and season. Population fluctuation and fruit infestation were plotted for both fruit fly species, while population fluctuation discriminated by sex was analyzed for *C. capitata*. The Spearman correlation coefficient between *C. capitata* captures in McPhail traps in the fortnight prior to harvest and the percentage of infested fruits was 0.62 (*P*= 0.0001), while for Jackson traps it was 0.34 (*P* = 0.02). The correlation between *A. fraterculus* captures in McPhail traps and fruit infestation was 0.59 (*P* = 0.0001). The variation observed in the number of adults and fruit infestation of both pest species between sites and host species groups is discussed.
Key words: Mediterranean fruit fly; South American fruit fly; captures; fruit damage; correlation

2.3. INTRODUCTION

Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) is one of the most important pest species of fruit trees in the world (Paiva y Parra, 2013), while *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) is restricted to the American continent, from northern Mexico to southern Argentina (Garcia et al., 2020). Both species are present in Uruguay, and their importance is due to the direct injury they cause to the fruit and to the restrictions on trade imposed by the countries that are free of these pests, mainly in citrus production, where 44 % is exported (DIEA-MGAP, 2020, Zefferino, 2019, Malavasi et al., 1994).

To define which control strategy to apply, it is necessary to know fruit fly population abundance; therefore, it is important to develop an efficient monitoring system that allows identifying areas with different levels of fruit fly populations. The spatial variation of fruit flies is associated with the availability of susceptible hosts throughout the year. Fruit flies are known to adjust their foraging behavior in response to changes in the spatial, temporal, and seasonal distribution of food and other resources (Hendrichs et al., 1991). There are also differences associated with sex: male and female spatial dispersal patterns appear to be linked to the need and differential use of resources, and the physiological status of its populations. Sexually mature females are expected to seek suitable laying sites, so aggregation would occur where the fruit has ripened and it is favorable for egg-laying, in addition to providing food sources. Males, however, in addition to foraging, likely concentrate in areas that provide shelter and appropriate sites to exhibit calling and lekking behavior outside of female egg-laying areas (Papadopoulos et al., 2001, Shelly, 2000).

The polyphagia of *C. capitata* and *A. fraterculus* (Liquido et al., 2019), and continuous presence of mature fruits from cultivated and wild hosts (Grové et al., 2017) could explain the abundance of fruit flies throughout the year in some regions. *Ceratitis capitata* thrives best in disturbed environments, while *A. fraterculus* prefers areas with native vegetation or sites where its native hosts predominate over exotic hosts (Ovruski et al., 2003).

Fruit is attractive for oviposition and suitable for the development of larvae of fruit flies in a period close to maturation (Aluja y Mangan, 2008, Joachim-Bravo et al.,

2001). In Uruguay, the first stone fruits (*Prunus* sp., Rosaceae) begin to ripe in November and continue until February. In January, the ripening of pears (*Pyrus communis* L., Rosaceae) and the first apple cultivars (*Malus domestica* Borkhausen, Rosaceae) begin, and they end in April-May. The ripening of citrus fruits (*Citrus* sp., Rutaceae) begins towards the end of February and extends until September (DIEA-MGAP 2017). Only considering these 2 groups of hosts, citrus and deciduous fruit trees, fruit flies have an appropriate substrate to develop uninterruptedly throughout the year.

Segura et al. (2006) studied the relative abundance of *A. fraterculus* and *C. capitata* in several hosts and localities in Argentina and demonstrated that both species coexist in several areas and exhibit similar ecological requirements. This study was carried out on a large scale through fruit sampling and at specific times. On the other hand, there are studies related to the spatio-temporal distribution of *C. capitata* at the farm level (Sciarretta y Trematerra, 2011, Papadopoulos et al., 2003), but the spatio-temporal variability of *A. fraterculus* and *C. capitata* simultaneously has not been analyzed. Furthermore, these researchers analyzed only the distribution of adults, generally as captures in *C. capitata* traps, without considering the larval stages (Sciarretta et al., 2018, Sciarretta y Trematerra, 2011, Papadopoulos et al., 2003). The objectives of this study were to analyze the population fluctuation of adults of *C. capitata* and *A. fraterculus* sharing the same habitat, and the relationship of their captures in traps with fruit infestation, and to describe the spatial distribution of both pests together with the incidence of fruit damage in different host species and cultivars.

2.4. MATERIALS AND METHODS

2.4.1. Study Location

The studies were carried out between September 2014 and June 2016 in 3 commercial farms. A farm composed exclusively of deciduous fruit trees (DFT), located in Canelones (34.6841230°S,56.3912460°W), another located in San José (34.6376560°S,6.7284920°W) with a combination of citrus and DFT, and the third one only with citrus orchards, located in Paysandú (31.5273320°S,57.9258940°W)

(Table 1). In San José and Paysandú the shelterbelts were composed of *Casuarina cunninghamiana* Miquel (Casuarinaceae), whereas in Canelones, in addition to casuarinas, there were diverse tree species, such as *Acacia caven* (Molina) and *Bauhinia forficata* Link (both Fabaceae), *Acca sellowiana* (Berg) Burret, *Eucalyptus globules* La Billardieri, *Blepharocalyx salicifolius* (Kunth) Berg, and *Eugenia uniflora* L. (all Myrtaceae), *Crataegus oxyacantha* L., *Eriobotrya japonica* (Thunberg) Lindley, and *Rubus ulmifolius* Schott (all Rosaceae), *Populus alba* L. and *Salix humboldtiana* Willdenow (both Salicaceae), as well as *Schinus longifolia* (Lindley) Spegazzini (Anacardiaceae), *Celtis tala* Gillies ex Planchon (Cannabaceae), *Melia azedarach* L. (Meliaceae), *Morus alba* L. (Moraceae), *Ligustrum lucidum* W.T. Aiton (Oleaceae), *Myrsine laetevirens* Mez (Primulaceae), *Jodina rhombifolia* (Hooker & Arnott) Reissek (Santalaceae), and *Lantana camara* L. (Verbenaceae).

2.4.2. Adult Monitoring and Fruit Sampling

A monitoring grid of Jackson traps baited with parapheromone (trimedlure, Süsbin S.A., Mendoza, Argentina) for monitoring *C. capitata* males, and McPhail traps baited with torula yeast (Süsbin S.A., Mendoza, Argentina) for monitoring *C. capitata* and *A. fraterculus*, were set up in all farms (OIEA, 2005). The monitoring materials evaluated are those used in the National surveillance system of fruit flies (Zefferino, 2019). A distance of 50 m between equal traps and of at least 30 m between Jackson and McPhail traps was maintained. The traps were monitored weekly, and McPhail traps were re-baited at each visit with 4 pellets of torula yeast and 300 mL of water, while the trimedlure was replaced every 45 days and sticky bottom whenever necessary. Fruit flies captured in McPhail traps were counted and separated by species and sex.

In Canelones and Paysandú, 60 and 50 traps were set up, on 30th October and 1st November 2014, respectively. In San José, traps were set up on 2 dates, 37 of them on 1st September 2014, and on 29th June 2015, there were added 30 Jackson traps (Table 1).

To evaluate fruit infestation during the week prior to harvest 10 plants were randomly sampled per cultivar plot, extracting 10 fruits per plant in stone fruit, and 15 fruit per plant in citrus and pome fruit, sampling a total of 5700 fruit during the 2 seasons. The fruit was taken to the laboratory to be weighed, identified, and placed individually in containers with sand as a substrate for the larvae pupation. The containers were covered with organza to allow ventilation. The fruit was kept at 25 °C and 70 % RH and it was checked weekly. The pupae were extracted from the sand and transferred to a Petri dish until the emergence of adults. The adults were classified by species and sex, maintaining the identification of the fruit from which they were collected. The fruit sampling dates are shown in table 1.

Table1. Fruit sampling dates and number of traps by species and cultivar at each farm.

Site	Species	Cultivar	Area (ha)	No. of traps		Fruit sampling date	
				Jackson	McPhail	First year	Second year
Canelones	*Prunus persica* (Peach)	June Gold	0.3	1	1	13 Nov 2014	25 Nov 2015
		Forastero	0.8	2	2	9 Dec 2014	-
		Elegant Lady	0.9	2	2	17 Dec 2014	-
		Dixieland	0.6	2	2	-	14 Jan 2016
		Pavía Canario	0.5	2	2	-	18 Feb 2016
	Prunus persica var. Nucipersica (Nectarine)	Lara	1.2	3	3	27 Nov 2014	2 Nov 2015
		Fantasía	0.3	1	1	-	5 Jan 2016
	Pyrus communis (Pear)	William´s	2.5	6	6	13 Jan 2015, 21 Jan 2015	7 Mar 2016
	Malus domestica (Apple)	Early Red One	2.1	6	6	23 Feb 2015	4 Mar 2016
		Red Chief	1.7	3	4	23 Feb 2015	4 Mar 2016
		Red Delicious	0.5	1	2	-	4 Mar 2016
		Sub total	11.3	29	31		
San José	*Citrus sinensis* (Orange)	Washington Navel	2.5	3	3	12 Jun 2015	24 May 2016
	Citrus reticulate (Mandarin)	Ellendale	4.0	3 / 8[1]	8	20 Jul 2015	26 Jun 2016
	Prunus persica (Peach)	Rich Lady	1.2	3	3	-	19 Jan 2016
		Elegant Lady	1.2	3	3	-	5 Jan 2016
		Rey del Monte	1.95	6	6	-	4 Feb 2016
		Pavía Canario	0.85	3	3	-	18 Feb 2016
	Prunuspersica var. Nucipersica (Nectarine)	Fantasía	1.2	4	5	-	19 Jan 2016
	Malus domestica (Apple)	Red Delicious	3.6	3	3	-	17 Mar 2016
		Sub total	16.5	33	34		
Paysandú	*Citrus sinensis* (Orange)	Valencia	3.2	3	4	17 Nov 2015	11 Oct 2016
		Washington Navel	3.0	5	7	16 Jun 2015	12 Jun 2016
	Citrus reticulate (Mandarin)	Satsuma	0.9	4	4	3 Mar 2015	5 May 2016
		Ortanique	1.6	3	4	19 Aug 2015	23 Aug 2016
	Citrus paradisi (Grapefruit)	Star Rubí	1.3	1	2	23 Jul 2015	27 Jul 2016
	Citrus limon (Lemon)	Lisbon	1.3	1	2	-	-
		Sub total	11.4	17	23		

1 All traps were set up in the spring of 2014 except the ones in bold that were set up on Junuary 2015

2.4.3. Data Analysis

For each species of fruit fly and season, the accumulated captures in McPhail traps were calculated. The period considered for the accumulation of captures was from the date that traps were set up in 2014 (see adult monitoring and fruit sampling) until June 2015 and for the same period from spring 2015 until June 2016. Then, the differences in captures between host species and between groups of cultivars of the same species in each study area were tested. The median was used as a measure of

central tendency and the interquartile range was used as a measure of the dispersion of the set of values.

The accumulated captures per trap and season were compared between host species and between cultivars of the same species in each study area using the non-parametric Kruskal-Wallis analysis of variance test with subsequent analysis of multiple comparisons by pairs with the Conover test. In the cases where the comparisons were between 2 groups as when compared fruit flies species, *C. capitata* males and females, difference between the 2 seasons or between 2 cultivars the Kolmogorov-Smirnov test was used (Infostat, 2018).

The Spearman correlation coefficients between captures and fruit infestation were calculated. Captures were expressed as fly/trap/day (FTD) considering for each cultivar the mean daily captures during the fortnight before harvest, analyzing separately *C. capitata* and *A. fraterculus* captured in McPhail traps, and *C. capitata* males captured in Jackson traps.

2.4.3.1. Spatial Distribution

Accumulated captures and fruit infestation distribution maps were made by site and season. The capture distribution maps were built with the GS + Version 7.0 program (Gamma Design, 2006) using the Inverse Distance Weighted (IDW) method. A layer was added using Power Point (Microsoft, 2010) with the level of fruit infestation per plot to simultaneously observe both distribution patterns.

2.4.3.2. Relationship between Captures and Fruit Infestation

Additionally, to show the relationship between the population fluctuation of both fly species in McPhail traps and the fruit infestation in different host species and sites, some representative graphs were presented for pears, peaches, mandarins, and oranges.

Distribution of males and females of C. capitata in hosts with and without fruit infestation

To visualize different distribution patterns of *C. capitata* discriminated by sex, the population fluctuation of males and females for a sample of host species by study site was plotted. In this analysis there were considered the host species with the highest captures in McPhail traps, and within these, those cultivars with the highest and lowest infestations in fruit. The records in the Jackson traps were considered as the male population indicator, and the female population was obtained from the records in the McPhail traps. The accumulated captures of males and females per cultivar were statistically compared. The same analysis was excluded for *A. fraterculus* because there were not traps for adequate monitoring of males. The captures of lemon trees were also included, assuming that it is not a host chosen by the females to oviposit under natural conditions (Staub et al., 2008, Spitler et al., 1984).

2.5. RESULTS

2.5.1. Population Fluctuation

The accumulated captures of *C. capitata* per McPhail trap were much higher than those of *A. fraterculus*. The median of captures per trap of *Ceratitis capitata* was 125, with an interquartile range of 313, while for *A. fraterculus* the median was 2 captures per trap with an interquartile range of 17 (Kolmogorov-Smirnov, n = 178, $P = 0.01$). The increases in captures of *A. fraterculus* in the 2 sites where it was found took place before the increase in captures of *C. capitata* (Figure 1). The captures of *A. fraterculus* in San José were almost zero in both seasons.

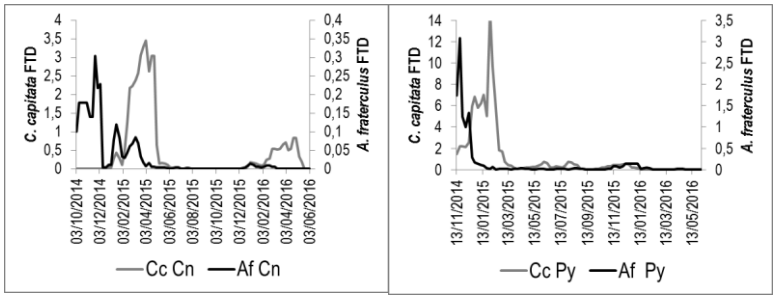


Figure. 1. Average number of fruit flies per McPhail trap per day (FTD) of *Ceratitis capitata* (Cc) and *Anastrepha fraterculus* (Af) in Paysandú (Py) and Canelones (Cn).

2.5.2. <u>Accumulated Captures per Host</u>

When we compared the fruit fly populations among host species, it was observed that in the Canelones farm, composed solely of DFT, the captures of *C. capitata* in pear (*Pyrus communis*) were higher than in apple (*Malus domestica*), peach (*Prunus persicae*) and nectarine (*P. persicae* var. *nucipersica*) trees. In the case of *A. fraterculus*, in addition to pear trees, nectarines also showed significant differences with apple and peach trees (Table 2). In San José, the citrus species had a higher population of *C. capitata* than the DFT, while the population of *A. fraterculus* was extremely low in both seasons, so no differences between hosts species were analyzed (Table 3). In Paysandú, in the farm composed only by citrus, the population of *C. capitata* was higher in mandarins (*C. reticulate*) and orange (*Citrus sinensis*) trees than in lemon (*Citrus limon*) and grapefruit (*Citrus paradisi*), while for *A. fraterculus* the grapefruit and mandarin had the highest captures (Table 4).

Table 2. Accumulated captures[1] of *Ceratitis capitata* and *Anastrepha fraterculus* by hosts in Canelones

Species	Area (ha)	N (No. of traps x 2 seasons)	*C. capitata* median	*C. capitata* interquartile range	*A. fraterculus* median	*A. fraterculus* interquartile range
Apple	4.3	23	35.0 a[3]	50	2.0 A	6
Peach	3.16	18	50.5 ab	51	3.0 A	4
Nectarine	1.6	12	79.5 b	42	26.5 B	95
Pear	2.5	12	355.0 c	766	16.0 B	20

[1]The accumulated captures per season were calculated by adding captures from 30th October 2014 until 30th June 2015 and for the same period from spring 2015 until June 2016. [2]Identical letters in the columns indicate that there is no significant difference between the variables. Kruskal-Wallis *C. capitata*, $P = 0.0015$; *A. fraterculus* $P = 0.0001$; Conover, $P < 0.05$.

Table 3. Accumulated captures[1] of *Ceratitis capitata* and *Anastrepha fraterculus* by host species in San José

Species	Area (ha)	N (No. of traps x 2 seasons)	*C. capitata* median	*C. capitata* interquartile range	*A. fraterculus* median	*A. fraterculus* interquartile range
Apple	3.6	6	7.0 a[3]	7	0 A	1
Peach	5.2	30	5.5 a	17	0 A	0
Nectarine	1.2	10	12.0 a	13	0 A	0
Mandarin	8	16	67.0 b	95	0 A	0
Orange	2.5	6	57.0 b	58	1 A	1

[1]The accumulated captures per season were calculated by adding captures from 1st November 2014 until 30th June 2015 and for the same period, from spring 2015 until June 2016. [2] Identical letters in the columns indicate that there is no significant difference between the variables. Kruskal-Wallis *C. capitata*, $P = 0.0001$; *A. fraterculus*, $P = 0.02$; Conover, $P < 0.05$.

Table 4. Accumulated captures[1] of *Ceratitis capitata* and *Anastrepha fraterculus* by host species in Paysandú

Species	Area (ha)	N (No. of traps x 2 seasons)	*C. capitata* Median	*C. capitata* interquartile range	*A. fraterculus* Median	*A. fraterculus* interquartile range
Lemon	1.4	4	11.0 a[3]	11	20.5 AB	15
Grapefruit	1.3	4	22.0 ab	13	138.0 B	32
Orange	6.2	22	98.0 bc	1013	13.0 A	30
Mandarin	2.2	16	197.5 c	525	73.0 B	136

[1]The accumulated captures per season were calculated by adding captures from 1st September 2014 until 30th June 2015 and for the same period, from spring 2015 until June 2016. [2]Identical letters in the columns indicate that there is no significant difference between the variables. Kruskal-Wallis *C. capitata*, $P = 0.0001$; *A. fraterculus*, $p = 0.06$; Conover, $P < 0.05$.

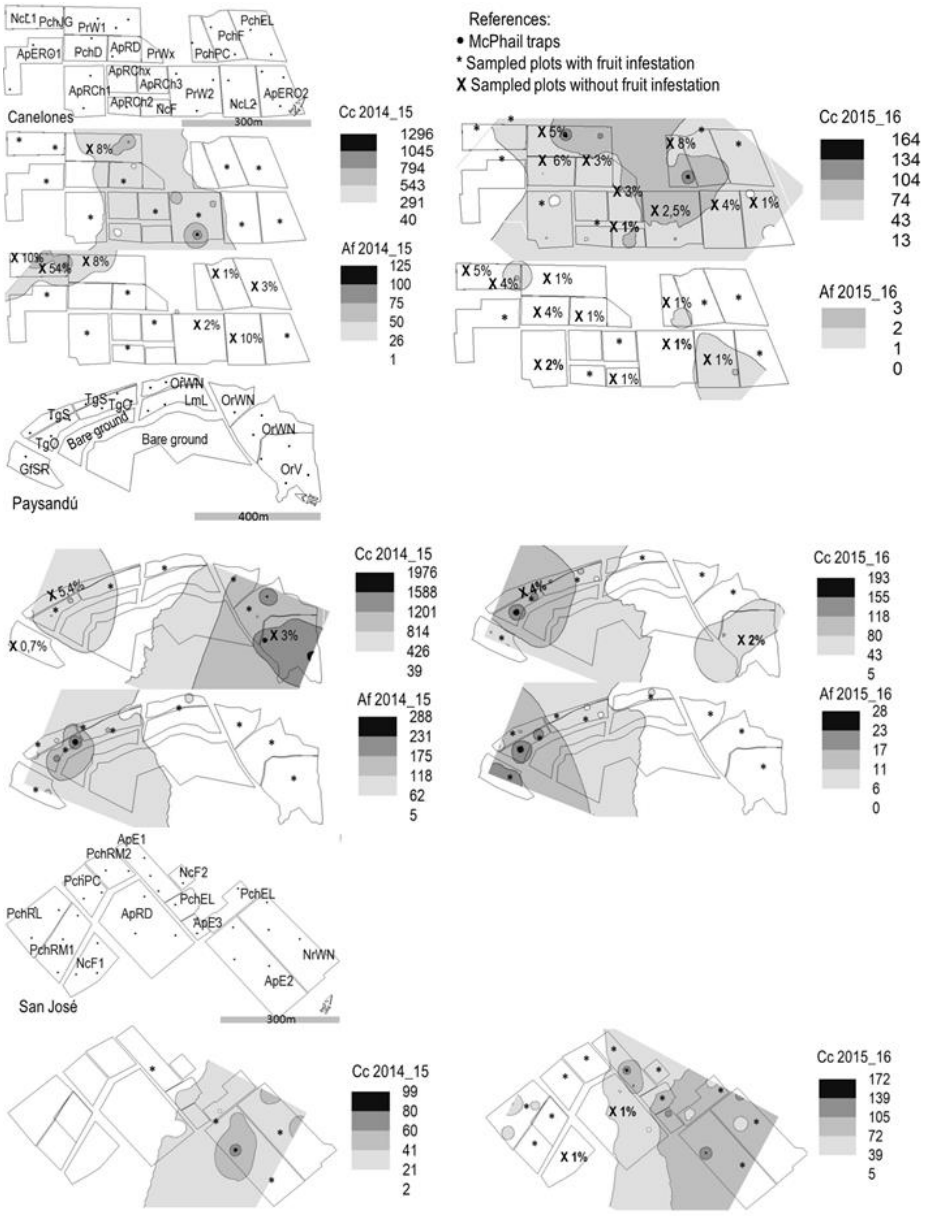
In the comparison of *C. capitata* captures between cultivars of the same species, in Paysandú in the orange trees, no significant differences were observed between Valencia (me = 712.5, IQR = 1393) and Washington Navel (me = 70.5, IQR = 326), nor in mandarins between Ortanique (median = 197.5, IQR = 623) and Satsuma (median = 240.0, IQR = 51). In Canelones, no significant differences were found between the apple cultivars Red Chief (median = 47, IQR = 150), Early Red One (median = 20, IQR = 37) and Red Delicious (median = 40, IQR = 139). On the other hand, in San José, significant differences were found between peaches, with *C. capitata* captures being lower in Pavía Canario (median = 4, IQR = 1) and Rey del Monte (median = 4.5, IQR = 5), intermediate in Rich Lady (median = 35.5, IQR = 65) and higher in Elegant Lady (median = 35.5, IQR = 24), which did not have significant differences with Rich Lady but with the other 2 cultivars (Kruskal-Wallis, H = 10.3, $P = 0.01$; Conover, $P < 0.05$). For the captures of *A. fraterculus* in Ortanique mandarin (median = 58 and IQR = 135) a tendency was observed to be higher than in Satsuma (median = 7, IQR = 7) (Kolmogorov-Smirnov, P = 0.1), while no significant differences were detected between cultivars of orange, apple or peach trees, which had low captures in both seasons (Table 2, 3 and 4).

2.5.3. Spatial Distribution

In the maps (Figure 2) it was observed that there is a pattern of captures distribution that is repeated in both seasons and in both fruit fly species. In Canelones, the foci of *C. capitata* are observed in the pear orchard and its surroundings, in the central area of the farm, while the foci of *A. fraterculus* appear associated with the nectarine cv. Lara in 2 orchards located in opposite corners of the farm. Regarding the fruit infestation distribution, the season in which the highest captures of *C. capitata* occurred (Figure 2), fruit infestation was recorded in a single orchard in pear, while in the 2015-2016 season when the captures were significantly lower (Kolmogorov-Smirnov, $P < 0.05$), fruit infestation was recorded in almost all the orchards around the focus of captures. In relation to the fruit damage caused by *A. fraterculus*, a more erratic behavior was observed, since although the fruit infestation levels were higher around the capture foci, fruit damage was recorded in orchards with a low number of captures in both seasons.

In Paysandú it was observed that the foci of *C. capitata* were located in Valencia orange and Ortanique mandarin, while *A. fraterculus* is located in Ortanique mandarin and Star Rubí grapefruit. In this case, the incidence of damage by *C. capitata* showed a pattern quite similar to the distribution of the captures, while no fruit infestation caused by *A. fraterculus* was recorded despite of being detected in the traps.

In San José, the foci of *C. capitata* captures were associated with citrus, Elendalle and Washington Navel, although neither of the 2 cultivars had fruit damage, but damage was recorded in the cultivars Red Delicious and Rich Lady where the record of captures was relatively low. On the other hand, the presence of *A. fraterculus* was near zero and there was no incidence on fruit in either of the 2 seasons.



Ap = Apple: ERO = Early Red One, RCh = Red Chief, RD = Red Delicious; Pch = Peach: JG = June Gold, EL= Elegant Lady, RM= Rey del Monte, PC= Pavía Canario, F= Forastero; Nc= Nectarine: L= Lara, F= Fantasía; Pr= Pear: W= William's; Mn= Mandarin: E= Elenadalle, O= Ortanique, S= Satsuma; Or= Orange: V= Valencia, WN= Washington Navel; Gf= Grapefruit: SR= Star Rubí. Cc= *C. capitata*, Af= *A. fraterculus*. The accumulated captures per season were calculated by adding captures from the date that traps were installed (1st Sep, 30 October, and 1st November 2014 in San José, Canelones, and Paysandú, respectively) until 30 June 2015, and for the same period from spring 2015 until June 2016.

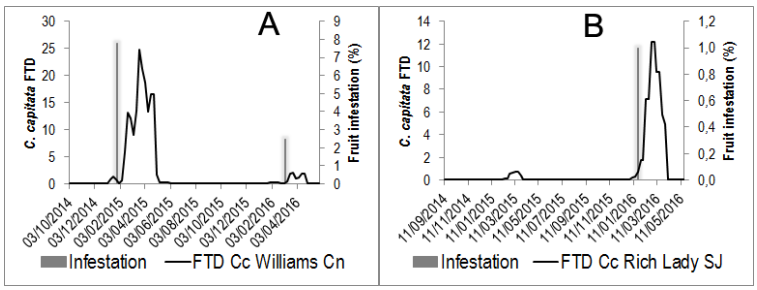
Figure 2. Spatial Distribution of Accumulated Captures and Fruit Infestation of *Ceratitis capitata* and *Anastrepha fraterculus*, during the 2014-2015 and 2015-2016 seasons.

2.5.4. Relationship between Captures and Fruit Infestation

The Spearman correlation coefficient between *C. capitata* captures in McPhail traps in the fortnight prior to harvest and the percentage of infested fruits was 0.62 (N = 37, *P* = 0.0001), while for Jackson traps it was 0.34 (N = 37, *P* = 0.02). The correlation between *A. fraterculus* captures and fruit infestation was 0.59 (N = 37, *P* = 0.0001).

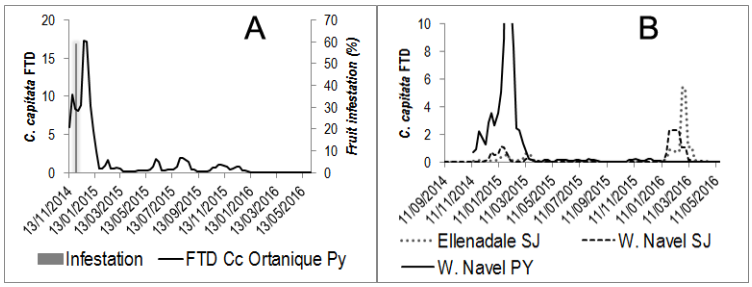
Regarding the relationship between captures over time and fruit infestation, in the DFT it was observed that the highest increases of *C. capitata* always occurred after harvest when there was no fruit left on the plants in most fruit orchards (Figure. 3A and B), while in citrus, greater variation between species and cultivars was observed. In Ortanique mandarin, the population increase was during and after harvest (Figure. 4A). In Washington Navel, orange and Ellendale mandarin fruit infestation was not recorded in either of the 2 seasons while captures were recorded in McPhail traps, with the largest population increase registered at least 3 months before the beginning of harvest (Figure. 4B).

In the case of *A. fraterculus* the population fluctuation was more erratic between host species and cultivars, sometimes detected before and near harvests and sometimes detected long after harvest (Figure. 5A, B).



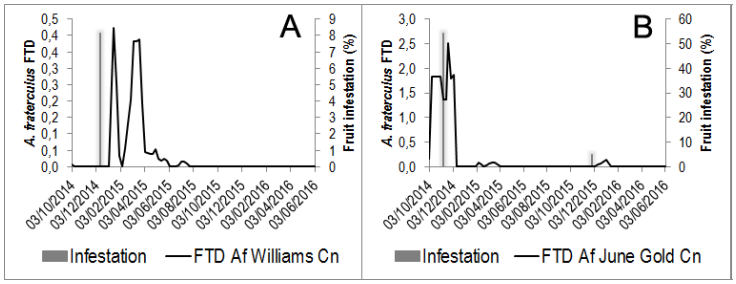
Cn: Canelones, SJ: San José; FTD: flies per trap per day, Cc: *Ceratitis capitata*

Figure. 3. Fruit infestation and population fluctuation of *Ceratitis capitata* registered in McPhail Traps in (A) pears and (B) peaches. Cn = Canelones, SJ = San José; FTD = flies per trap per d; Cc = *Ceratitis capitata*.



Py: Paysandú, SJ: San José, W.: Washington, FTD: flies per trap per day, Cc: *Ceratitis capitata*

Figure. 4. Population fluctuation of *Ceratitis capitata* registered in McPhail traps in mandarins (A) with fruit infestation and (B) without fruit infestation. Py = Paysandú, SJ = San José, W = Washington; FTD: flies per trap per d; Cc = *Ceratitis capitata*.



Cn: Canelones, Py: Paysandú, FTD: flies per trap per day, Af: Anastrepha fraterculus.

Figure. 5. Fruit infestation and population fluctuation of *Anastrepha fraterculus* in (A) pears, (B) peaches, and (C) mandarins. Cn = Canelones, Py = Paysandú; FTD = flies per trap per d; Af = *Anastrepha fraterculus*.

2.5.4.1. Distribution of Males and Females of *C. capitata* in Hosts with and without Fruit Infestation

Regarding the distribution of *C. capitata*, it was observed that in all cultivars with fruit infestation (Figure6A, B and C) the population of females (median = 157) was higher than males (median = 72.5) (Kolmogorov-Smirnov, $P = 0.02$) and that the increase in female population in some cases occurred earlier. On the other hand, in grapefruit and lemon trees in Paysandú (Figure6E and F), as well as in citrus hosts of San José where no fruit infestation was recorded (Figure6G and H), the proportion of males (median = 228.5) was significantly higher than females (median = 45) (Kolmogorov-Smirnov, $P = 0.01$).

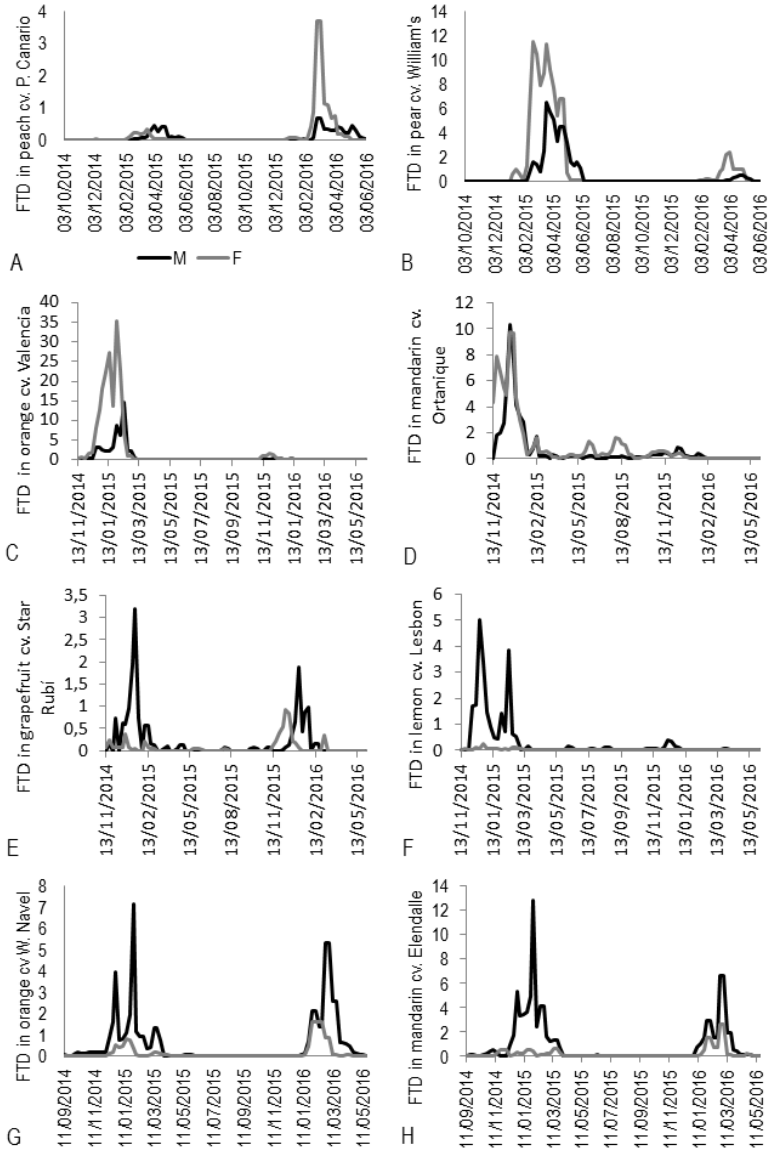


Figure6. Population fluctuation of *Ceratitis capitata* males (M) in Jackson traps and females (F) in McPhail traps. A, B, C, D = cultivars where fruit infestation was recorded (A, B = Canelones; C, D = Paysandú); E, F, G, H = cultivars where no fruit infestation was recorded (E, F = Paysandú; G, H = San José).

2.6. DISCUSSION

Great variation was found in the number of adults and fruit infestation of both fruit fly species among localities and the compared groups of host species. The population increases of *A. fraterculus* observed before the increase of *C. capitata* could be due

to the preference of *A. fraterculus* to oviposit on unripe fruit (Malavasi et al., 1983), while *C. capitata* prefers ripe fruit (Joachim-Bravo et al., 2001), favoring its earlier detection in susceptible hosts. Likewise, the lower competitive capacity of *A. fraterculus* concerning *C. capitata* (Duyck et al., 2004) might diminish *A. fraterculus* presence in the orchards, in certain situations.

2.6.1. Relationship between *A. fraterculus* Captures and Fruit Infestation in Different Hosts

Anastrepha fraterculus populations exhibited greater variation over time and space being almost absent in San José, with adults captured in traps but without fruit infestation in Paysandú, and with the presence of adults and fruit infestation in Canelones. Although there was a significant correlation between captures and fruit infestation, it was not very strong, and in the spatial distribution maps, a well-defined pattern was not observed. This weak relationship in Canelones could be due to females already mated coming from neighboring wild vegetation to oviposit on the crop. In Rio Grande do Sul, early peach cultivars are more affected by *A. fraterculus* than mid and late-cycle cultivars and this is attributed to migration from other wild plants or citrus hosts (Branco et al., 2000). In apple orchards, in southern Brazil, Kovaleski et al. (1997) suggest that *A. fraterculus* populations are not established in orchards but rather the infestation source is the native host of the surroundings, especially those that belong to the Myrtaceae family (Selivon, 2000). This approach supports the hypothesis that in Canelones the shelterbelts composed of several wild species, some of them belonging to Myrtaceae family, could serve as potential hosts. Besides, this farm is in the influence area of the Wetlands of Santa Lucía, which is part of the National System of Protected Areas (MITUR, 2020), with abundance of native species that favor the maintenance of *A. fraterculus* in the area. On the other hand, in Paysandú the absence of fruit infestation could be associated with the absence of susceptible citrus fruit during the periods with the highest population of fruit flies. Some authors attribute the absence of damage in early harvest cultivars to the escape of fruits at at the time of highest incidence of flies (Branco et al., 2000).

2.6.2. Relationships between *C. capitata* Captures and Fruit Infestation in Different Hosts

In the case of *C. capitata,* the Pearson correlation coefficient showed a significant relationship between *C. capitata* captures in McPhail traps and fruit infestation. Comparing different cultivars, this relationship seemed stronger in cultivars harvested during spring, summer, and autumn, as the case of DFT, but less strong in those cultivars harvested in winter, such as some citrus cultivars, where captures but not fruit infestations were recorded. Despite being considered one of the main pests in citrus, some authors affirm that fruit flies are not perfectly adapted to development in these fruits. Among the most critical parameters that determine the difference in performance in different cultivars are the resistance of the skin and the presence of essential oils in the flavedo, which have lethal effects on neonatal larvae (Papachristos y Papadopoulus, 2009, Branco et al., 2000). Dias et al. (2017) determined that *C. capitata* laid eggs deeper in orange and mandarin compared to *A. fraterculus,* which they associate with greater survival by not exposing the eggs to the essential oil glands (Back y Pemberton, 1915). Besides, in some cultivars, the presence of susceptible fruit only during the winter period when the population decrease could also reduce the risk of infestation. Within the DFT group, pears were the host with the highest presence of *C. capitata* adults and fruit infestation. However, in the 2014-2015 season, when fly populations were higher, fruit injury was only recorded in one pear plot. An explanation is that the harvest in this plot was carried out a week later than in the orchard where fruit infestation was not observed. Aluja y Mangan (2008) mention that there is an inverse relationship between the resistance of the fruit and its degree of maturity. The extra week of exposure of fruit to fly attack when the populations were increasing and the fruit was susceptible, was possibly a determining factor in the infestation level. Something similar happened on the farm located in Canelones during the second study season. The fruit ripening in most cultivars occurred up to 20 days earlier than in the 2015-2016 season, and the peaches and nectarines were harvested when the fruits were still unripe. It is possible that reducing the exposure time of susceptible fruits favored the low infestation level, despite the high captures that were recorded on the farm

throughout the season. In the present research it was found that the populations of *C. capitata* in the DFT tend to increase in late summer and autumn, regardless of the harvest period, which suggests a marked seasonal influence.

2.6.2.1. Distribution of Males and Females of *C. capitata*

The low correlation between captures of *C. capitata* in Jackson traps and fruit infestation is probably because the presence of males in the orchard is not always directly related to the presence and oviposition of females, as observed in Ellendale and Washington Navel cultivars. The spatial and temporal variation of males and females in the orchards suggests that citrus trees would be suitable sites of refuge for adult males, while the proportion of females varies probably depending on the susceptibility of host. Sciarretta et al. (2018) observed that when fruit was available in several cultivars, males and females of *C. capitata* had a similar distribution, but when few late cultivars remained, the distribution of males and unfertilized females tended to diverge from that of fertilized females, adding males in non-fruiting cultivars.

According to these results, the captures of *C. capitata* and *A. fraterculus* in McPhail traps baited with torula and captures of *C. capitata* in Jackson traps baited with trimedlure are not always associated with fruit infestation, therefore, to base the management decisions only on the number of fruit flies collected in traps can occasionally lead to control failures. Early season fruit infestation could be a good measure of fly activity to complement traps captures to guide management decisions when the commercial value of the crop justifies it. Another aspect to consider is the large increase in captures observed post harvest, so concentrating monitoring and control measures only in the commercial value of the crop in its susceptible state involves the risk of entry of these pests from these areas. When planning a monitoring and control strategy, including already harvested plots could help anticipate this risk and reduce damage.

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2.8. ANEXO

https://journals.flvc.org/flaent/article/view/127649/132944

3. MATING COMPATIBILITY BETWEEN TWO POPULATIONS OF *ANASTREPHA FRATERCULUS* (WIEDEMANN) (DIPTERA: TEPHRITIDAE) FROM ARGENTINA AND URUGUAY[3]

3.1. RESUMEN

Antecedentes: se ha informado que *Anastrepha fraterculus* (Wiedemann) muestra una gran variación morfológica a lo largo de su distribución geográfica y actualmente se reconoce como un complejo de especies crípticas compuesto por al menos 8 morfotipos diferentes. El morfotipo Brazilian-1 incluye las poblaciones de Argentina y del sur de Brasil. Para contribuir con información básica sobre la distribución de los morfotipos de *A. fraterculus*, se evaluó la compatibilidad sexual entre una población uruguaya y otra argentina.

Métodos: se evaluó la compatibilidad de apareamiento en jaulas de campo en condiciones seminaturales. La población argentina se obtuvo de una colonia del laboratorio del Instituto de Genética "E. A. Favret" (INTA Castelar), Buenos Aires, establecida en 2007. La población uruguaya provenía de frutos infestados de *Acca sellowiana* (Berg. 1855) Burret 1941 (Myrtaceae). En el momento de las pruebas, las moscas argentinas tenían entre 11 y 17 días y las moscas uruguayas tenían entre 16 y 26 días. La compatibilidad sexual se estableció utilizando el índice de aislamiento sexual (ISI), los índices de rendimiento relativo de machos y hembras (MRPI, FRPI) y un análisis de varianza unidireccional de Kruskal-Wallis con pruebas de comparación por pares posteriores de los cuatro tipos de parejas formadas de acuerdo con el origen de machos y hembras. La latencia, la duración del apareamiento y la ubicación de las parejas también se registraron.

Resultados: el valor del ISI fue significativamente diferente de cero debido a un mayor desempeño de los adultos argentinos. No hubo diferencias significativas entre la frecuencia de las parejas homotípicas uruguayas y las parejas heterotípicas,

[2] Trabajo publicado como capítulo de libro. Duarte F, Calvo MV, Delgado S, Vera MT, Garcia FM, Scatoni IB. 2019. Mating Compatibility between Two Populations of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) from Argentina and Uruguay. *In*: Area-Wide Management of Fruit Fly Pests, Crc Press-Taylor & Francis Group; pp 45-53. ISBN: 9780429355738

mientras que la frecuencia de las parejas homotípicas argentinas fue significativamente mayor que el resto. No se encontraron diferencias significativas para los otros parámetros evaluados.
Conclusiones: los resultados sugieren que las poblaciones uruguayas pertenecen al morfotipo Brazilian-1 y que el mayor rendimiento de las moscas argentinas se debe probablemente a tasas de maduración sexual más rápidas y a una mayor propensión inherente al apareamiento en lugar de al aislamiento reproductivo.

Palabras clave: moscas de la fruta, apareamiento, aislamiento sexual, jaula de campo, índice de rendimiento relativo

3.2. SUMMARY

Background: Anastrepha fraterculus (Wiedemann) has been reported to show extensive morphological variation along its geographic distribution and is currently recognized as a complex of cryptic species composed of at least 8 different morphotypes. The Brazilian-1 morphotype includes the Argentinean and southern Brazilian populations. In order to contribute with basic information on the distribution of *A. fraterculus* morphotypes, the sexual compatibility between a Uruguayan and an Argentinean population was evaluated.
Methods: Mating compatibility was evaluated in field cages under semi-natural conditions. The Argentinean population was obtained from a colony of the laboratory of the Instituto de Genética "E. A. Favret" (INTA Castelar), Buenos Aires, established in 2007. The Uruguayan population came from infested fruits of *Acca sellowiana* (Berg. 1855) Burret 1941 (Myrtaceae). At the moment of the trials, Argentinean flies were between 11 and 17 days old and Uruguayan flies were between 16 and 26 days old. Sexual compatibility was established using the sexual isolation index (ISI), the male and female relative performance indeces (MRPI, FRPI), and a Kruskal-Wallis one-way analysis of variance with subsequent pairwise comparison tests of the four types of couples formed according to male and female origin. Latency, mating duration, and location of the couples were also recorded.

Results: The ISI value was significantly different from zero due to a greater performance of the Argentinean adults. There were no significant differences between the frequency of homotypic Uruguayan couples and heterotypic couples, whereas the frequency of Argentinean homotypic couples was significantly higher than the rest. No significant differences were found for the other evaluated parameters.
Conclusions: Results suggest that Uruguayan populations belong to the Brazilian-1 morphotype and that the greater performance of Argentinean flies is probably due to faster sexual maturation rates and an inherent greater mating propensity rather than to reproductive isolation.

Keywords: fruit flies, mating, sexual isolation, field cage, relative performance index

3.3. INTRODUCTION

Anastrepha fraterculus (Wiedemann), the South American fruit fly, is widely distributed from the Rio Grande Valley in northern Mexico to central Argentina (Malavasi et al., 1999). With over 100 plants reported as hosts (Norrbom, 2004), it is a species of major economic importance in many countries in South America, not only because of its destructive potential, but also because of quarantine restrictions imposed on fruit export (Steck, 1999).

The South American fruit fly has long been reported to show extensive morphological variation along its geographic distribution (Lima 1934, Stone 1942, Steck 1999, Hernández-Ortíz et al., 2004, 2015). Many studies confirm that this morphological variation is associated with differences in host use, the presence and degree of reproductive isolation, karyotypic differences, isozyme divergence, and DNA sequence divergence. The existence of this morphological variation has been revealed by multivariate morphometric analyses (Hernandéz-Ortiz et al., 2012). An extensive list of bibliography reviewing this research can be consulted in Rull et al. (2013), Cladera et al. (2014), Hernández Ortíz et al. (2015), and Manni et al. (2015). In consequence, *A. fraterculus* is currently recognized as a complex of cryptic species composed of at least 8 different morphotypes clustered into three

phenotypic lineages. The Mesoamerican-Caribbean lineage consists of Mexican and Venezuelan morphotypes. The Andean lineage consists of the Andean, the Peruvian, and the Ecuadorian morphotypes. Finally, the Brazilian linage is composed of three Brazilian morphotypes: Brazilian-1, Brazilian-2, and Brazilian-3 (Hernández-Ortiz et al., 2015).

In the Brazilian lineage, the Brazilian-1 morphotype includes the Argentinean populations (Alberti et al., 2002, Petit-Marty et al., 2004, Vera et al., 2006) and southern Brazilian populations (Dias et al., 2016, Vaníčková et al., 2015, Rull et al., 2013, Hernández-Ortíz et al., 2004, Basso et al., 2003, Alberti et al., 2002, Smith-Caldas et al., 2001). In the state of Sao Paulo, Brazilian-1 overlaps with Brazilian-2, where both morphotypes maintain their genetic integrity despite sympatry and partial reproductive compatibility (Selivon et al., 2005). The Brazilian-3 morphotype was also found in sympatry with Brazilian-1 and Brazilian-2 in the coastal areas of the state of Sao Paulo and in the inland plateau of southeastern and southern Brazil (Selivon et al., 2004).

The main practical reason that makes the delimitation of the *A. fraterculus* morphotypes essential is that it is a basic requirement for the implementation of the sterile insect technique (SIT). The SIT is a method of pest control that consists of inundative releases of sterile insects into a wide area to reduce reproduction in a field population of the same species (FAO/IPPC, 2016). A great research effort is being made to gather basic knowledge to enable the adjustment of this technique for the control of *A. fraterculus* (Cladera et al., 2014). In addition to the control of the pest, the key benefits of SIT derive mostly from a reduction in pesticide use, minimizing environmental and health costs. Furthermore, SIT is an environmentally friendly strategy for use in area wide pest management (AWPM) because it can be applied not only in commercial orchards but also in backyards and urban areas not usually protected by insecticides (FAO/IAEA, 2005, Dias and Garcia, 2014).

Although there is a great deal of research attempting to elucidate how the complex of cryptic species under the name of *A. fraterculus* is composed, none of it includes populations from Uruguay. Rull et al. (2013) suggest a geographical range of the Brazilian-1 morphotype from Monte Alegre do Sul in southeastern Brazil to Buenos

Aires, Argentina; the southern limit of this morphotype has been determined up to the present day. In order to contribute with basic information on the distribution of *A. fraterculus* morphotypes and to prepare for an eventual application of the SIT in Uruguay, a study on the sexual compatibility between a population from Uruguay and a population from Argentina (Brazilian-1 morphotype) of *A. fraterculus* was carried out.

3.4. MATERIALS AND METHODS

3.4.1. Biological Material

The Argentinean population of *A. fraterculus* was obtained from a laboratory colony. This population was derived from an experimental colony kept at the Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Tucumán, Argentina, which was originally established with pupae recovered from infested guavas (*Psidium guajava* L., Myrtaceae) at the vicinity of Tafí Viejo (Tucumán) in 1997 (Jaldo et al., 2001).

The Uruguayan population was obtained directly from infested fruits of *Acca sellowiana* (Berg. 1855) Burret 1941 (Myrtaceae) collected from a commercial orchard located in Montevideo (34° 44'S; 56° 16' W) (Figure 1). Fruits were placed in sandboxes covered with voile and checked daily to separate the pupae.

Pupae and adult flies from both origins were maintained under the same controlled conditions (T: 23 ºC, RH: 60-70 %, photoperiod: 10L-14D) until the day of the trials. Pupae were first placed separately in emergence cages. After emergence, flies were separated and placed in one-liter containers with 25 adult flies each and sorted according to date, sex and origin. In each container, adult flies were supplied with water and a diet composed of bee honey, hydrolyzed yeast (in a 3:1 ratio) and food dye (Laboratorio Fleibor S.R.L., Buenos Aires, Argentina). The Argentinean population was colored red and the Uruguayan population was colored blue.

3.4.2. Mating Compatibility Test

The test took place in the campus of the Facultad de Agronomía, Universidad de la República, Uruguay. Three field cages made of screen fabric, measuring 3 meters in

diameter x 2 meters in height, were set inside a greenhouse to obtain suitable temperature conditions at the moment of release. Six potted plants of *Citrus limon* (Rutaceae) var. "Limón criollo" were placed inside each cage to provide perching places.

Between the 9th and 22nd of June of 2018, eight replicates of the trial were carried out: three on June 9th, one on June 13th, three on June 20th, and one on June 22nd. The dates of the replications depended on the availability of sexually mature adults. At the moment of the trials, Argentinean flies were between 11 and 17 days old and Uruguayan flies were between 16 and 26 days old.

Twenty-five males and 25 females of each origin were released inside of each field cage. As mating occurs mainly at sunrise (Malavasi et al., 1983, Vera et al., 2006) and given that the experiments were carried out in winter (where the average temperature is 10.4 ºC), the photoperiod in the rearing room was adjusted so the lights were turned on at 10 am in order to allow the greenhouse to reach temperatures higher than 18 ºC at the moment when the flies were released. The maximum temperature recorded during the tests was 32 °C and the mean RH was of 75 %.

Most releases started at 9:45 am with males and finished at 10:00 am with females. Only on June 13th, due to prevailing low temperatures, fruit flies were kept in the dark and males and females were released at 10:45 am and 11:00 am, respectively. One observer remained inside each cage until the end of the trials, until two hours after male callings, and until sexual activity ceased. Each observed mating pair was collected in a 50 ml vial and placed in the shade until the couple disengaged. Male and female color, time of start and end of copulation, and location were recorded for each mating couple. Location was recorded as either net, ground, stem, abaxial-adaxial side of leaf, height on the tree, and cardinal point (FAO/IAEA/USDA 2014).

3.4.3. Data Analysis

Percentage of mating inside each cage was calculated to corroborate that all replicates had reached at least 20 % of potential couples on the plants (FAO/IAEA/USDA 2014).

Mating compatibility was assessed by means of the Index of Sexual Isolation (ISI). An ISI value of 1 indicates complete assortative mating, an ISI value of -1 indicates complete outbreeding, and an ISI estimate of zero indicates random mating. Mating competitiveness was assessed by means of the Male and Female Relative Performance Indices (MRPI and FRPI). MRPI varies from 1, when males of one of the tested populations engage in all the copulations, to -1, when males from the other population are present in all copulations. The range for FRPI is the same as that of MRPI. Values close to zero indicate similar participation from males (MRPI) or females (FRPI), independent of the origin of the population (Cayol et al., 1999). To verify random mating, confidence intervals at 95 % were estimated to assess departures from zero (Rull et al., 2013). Differences among mating combination frequencies were tested using a Kruskall-Wallis one-way analysis of variance by ranks, followed by pairwise comparisons (Conover 1999). Statistical analyses were performed with InfoStat/Libre (Infostat 2018).

Data recorded for latency to first mating and mating duration were log transformed and compared between mating combinations by means of a one-way ANOVA followed by Tukey multiple comparison tests. Differences between homotypic couples in location of mating on plant height and cardinal point were tested using a Kruskall-Wallis one-way ANOVA by ranks and pairwise comparisons.

3.5. RESULTS

The percentage of mating exceeded 20 % in all cages, with a mean value of 28.8 ± 2.59 %, indicating that the environment was suitable for mating. The indices of sexual isolation and relative performance of males and females were statistically different from zero (Figure 1). The sexual isolation index was 0.29 ± 0.13, a positive value that indicates that there were more homotypic than heterotypic mating couples. The mating performance index was 0.23 ± 0.07 for males and 0.18 ± 0.12 for females, which implies that males and females from the Argentinean population were engaged in most of the copulations, which indicates a greater activity of the Argentinean population in both sexes (Figure 1). In addition, when we compared the number of couples per type of mating combination, there were no statistically significant

differences among the heterotypic mating combinations and the Uruguayan homotypic combination, whereas the frequency of the homotypic combination of the Argentinean population was about twice the frequency of the homotypic combination of the Uruguayan population (Figure 2).

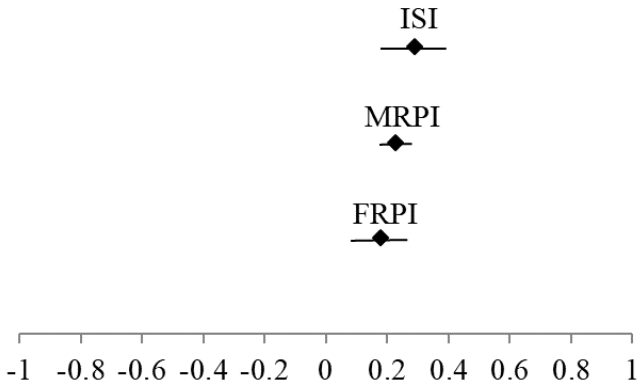

Figure 1. Index of sexual isolation (ISI) and relative performance indices for males (MRPI) and females (FRPI) with associated 95 Cis.

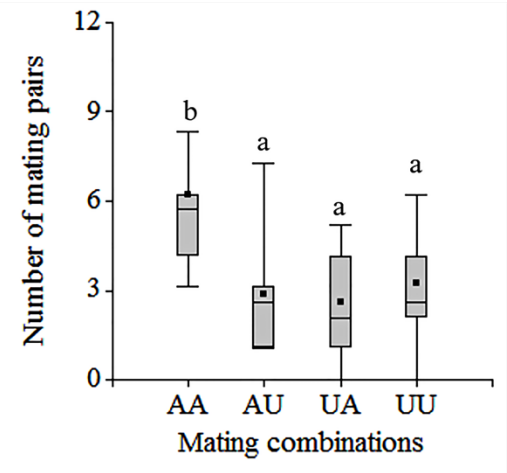

Figure 2. Mean, median, and quartiles of each mating combination. Capital letters indicate the origin of fruit flies: A refers to fruit flies from Argentina and U refers to fruit flies from Uruguay. The first letter indicates the origin of males and the second letter the origin of females. Data with the same lowercase letter are not significantly different according to the Kruskal-Wallis test ($p < 0.02$) and pairwise comparisons tests ($p < 0.05$) (Conover, 1999).

There were no statistically significant differences (p = 0.13) in location on plant height, and just one out the 113 couples that mated on the leaves did so on the adaxial side. Matings outside the tree involved 17.2 % of those recorded, with 9 % taking place on the floor of the cage and 8.2 % on the net walls and the celling. Matings on the ceiling were all homotypic mating couples from Uruguay, whereas matings on the floor were all homotypic mating couples from Argentina.

Mating duration and latency were no statistically different among mating combinations. (Table 1).

Table 1. Mean ± SE mating duration and latency in minutes.

Mating combination	**Mating duration**	**Latency**
AA	59.5 ± 6.8	45.9 ± 5.4
AU	55.6 ± 6.5	34.5 ± 6.7
UA	45.1 ± 8.5	51.0 ± 14.4
UU	60.5 ± 6.8	43.6 ± 7.7

n=8; Data were log transformed prior to the ANOVA and no statistical differences were found for neither mating duration (p = 0.23) nor latency (p = 0.4). Capital letters indicate the origin of fruit flies: A refers to fruit flies from Argentina and U refers to fruit flies from Uruguay. The first letter indicates the origin of males and the second letter the origin of females.

All copulations occurred mostly during the first hour after flies were released (Figure 3) and no sexual activity was observed after three hours.

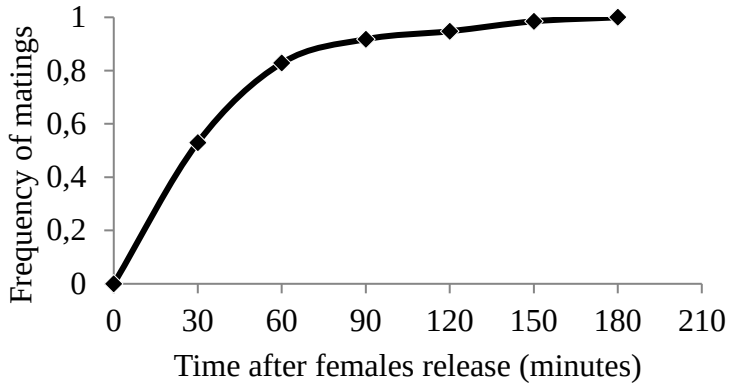

Figure 3. Accumulated mating frequency as a function of time.

3.6. DISCUSSION

In previous studies where *A. fraterculus* populations from southern South America showed full sexual compatibility, ISI values ranged between -0.01 and -0.14. Pairwise comparisons included populations from Argentina (Petit-Marty et al., 2004), southern Brazil (Dias et al., 2016), and Argentina and southern Brazil (Rull et al., 2012). In comparisons among populations with strong sexual incompatibility, such as the Mexican, Peruvian, and Brazilian-1 linages, ISI values varied between 0.74 and 0.92 (Rull et al., 2012, Cáceres et al., 2009, Vera et al., 2006); whereas populations from southeastern Brazil mating with one Argentinean population and one Peruvian population showed partial incompatibility, with ISI values of 0.43 and 0.55, respectively (Dias et al., 2016, Vera et al., 2006).

The ISI value obtained during this study (0.29) indicates a tendency towards random mating, being closer to 0 than to 1 or -1. Comparisons among different mating combinations showed similar frequencies for the Uruguayan homotypic combination and both heterotypic combinations, thus the slight deviance from 0 (random mating) could be attributed to a greater propensity to mate of the Argentinean laboratory population. Liedo et al. (2002) observed that laboratory reared females of *C. capitata* were more prone to mate than wild females, and that laboratory flies, both females and males, matured much earlier than wild flies. Calkins (1984) stated that it takes a few generations for insects to thrive under artificial rearing conditions and that the rearing regime probably selects for simpler courtship and mating behavior, and also

for earlier reproduction than in wild populations. That is considering that long and intense courtships have a risk of being interrupted by other males adapted to crowded conditions. In addition, because under artificial conditions all females become sexually mature at about the same time and earlier than wild females, an intense competition between females for fit males is promoted, favoring females that require fewer criteria for male selection (Calcagno et al., 1999, Calkins, 1984).

Argentinean males had a better mating performance than Uruguayan males. Although it is expected that males from artificial rearing conditions are less competitive than wild males when they are competing for wild females (Liedo et al., 2002), the better performance of Argentinean males was due to their mating with females coming from the same artificial rearing conditions. In addition, the evaluated laboratory colony of *A. fraterculus* has shown good competitiveness in previous studies, particularly when given the same adult diet as wild males (Rull et al., 2013). If we consider only Uruguayan females, Uruguayan and Argentinean males showed a similar performance. It is possible that the Argentinean population had a greater proportion of sexually mature individuals due to artificial selection during rearing and, in some way, it favored a greater mating performance in this population.

Regarding the location on the plant and cardinal point, we did not find significant differences. However, it is important to consider that in order to interpret results on the location on the plant, we have to take into account that the plants were relatively small, although they provided enough foliage for flies to perch on, and it was not possible to clearly delimit the top, middle and bottom. A similar situation occurred with the cardinal points.

Most of the copulations occurred during the first two hours after artificial sunrise, which agrees with the results of Petit-Marty el al. (2004). Also, there were no differences in latency, indicating that there is no temporal isolation between these populations.

Different studies agree with the fact that the morphotype present in Argentina and Southern Brazil is the Brazilian-1 morphotype (Hernández-Ortíz, 2012). Petit-Marty et al. (2004) evaluated the mating compatibility among four populations from Argentina, including a population from Tucumán, the same region where the

Argentinean flies used in the present study came from, and confirmed full compatibility among populations. Rull et al. (2013) evaluated the reproductive compatibility among two strains from southern Brazil (Pelotas and Vacaria) and one strain from Argentina (Tucumán) and also found that they were fully compatible. These results were later confirmed by a study with a multidisciplinary approach that included wild flies of four populations from southern Brazil (Bento Gonçalvez, Pelotas, Vacaria and São Joaquim) (Dias et al., 2016) and by a recent courtship behavior study including different populations of *A. fraterculus* (Roriz et al., 2019).

The distribution suggested by Rull et al. (2013) puts Montevideo, Uruguay, in the distribution limits of the Brazilian-1 morphotype. The relatively small size and geographical location of Uruguay, with relatively homogeneous climate conditions and a geographical landscape with a topography without great variation in elevation (INIA, 2018), allows to suggest that Brazilian-1, reported as the only morphotype currently present in Argentina and also present in the south of Brazil, would be the morphotype that is present in Uruguay. Although our results are not entirely conclusive, if we consider only the ISI value, the fact that the mating combination of flies that deviated from the expected behavior were those coming exclusively from artificial breeding could be indicating that the observed behavioral differences were mainly due to breeding conditions and origin rather than to inter population differences. In any case, it is important to consider that the three morphotypes of the Brazilian lineage are reported to coexist in Vale do Paraiba in the state of Sao Paulo (Selivon et al., 2005, 2004) and, hence, it is still not clear whether the distribution of the Brazilian morphotypes is related to lower and higher altitudes or to a north/south differentiation (Vanickova et al., 2015, Selivon et al., 2005).

This is the first study intending to begin to clarify the taxonomic situation of *A. fraterculus* in Uruguay. Further studies using molecular and morphometric approaches need to be carried out before a specific morphotype can be conclusively assigned. Comparing populations of wild origin from Argentina, Uruguay and Brazil would be useful to resolve this issue.

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3.8. ANEXO

https://www.taylorfrancis.com/chapters/oa-edit/10.1201/9780429355738-5/mating-compatibility-two-populations-anastrepha-fraterculus-wiedemann-diptera-tephritidae-argentina-uruguay-felicia-duarte-mar%C3%ADa-calvo-soledad-delgado-mar%C3%ADa-teresa-vera-fl%C3%A1vio-garc%C3%ADa-iris-scatoni

4. RELEASE-RECAPTURE TEST OF DISPERSAL AND SURVIVAL OF STERILE MALES OF *Ceratitis capitata* (DIPTERA: TEPHRITIDAE) [4]

4.1. RESUMEN

La técnica de insectos estériles se utiliza en todo el mundo para suprimir o erradicar las poblaciones de *Ceratitis capitata* (Wiedemann) con resultados exitosos. Consiste en liberaciones inundativas de insectos estériles en un área amplia para reducir la reproducción en una población de campo de la misma especie. Es necesario conocer la dispersión de los machos estériles en el campo para definir la distancia máxima entre los puntos de liberación que asegure la distribución de las moscas estériles en toda el área objetivo. Los métodos de liberación pueden variar según el área a cubrir y los recursos disponibles. La liberación manual desde el suelo requiere menos tecnología. El objetivo de esta investigación fue estimar la capacidad de los machos estériles para sobrevivir y dispersarse en el campo, en las dos áreas principales de producción de cítricos en Uruguay. Se realizó una liberación de 20.000 machos estériles de *C. capitata* TslV8 (-inv D53) en el punto central de cada área definida para los ensayos. Alrededor de estos puntos se instaló una red de 54 trampas Jackson cebadas con trimedlure formando cinco anillos concéntricos, que se colocaron los días 1, 3, 5 y 7 después de la liberación y se retiraron a las 24 horas en todos los casos. Se estimó la tasa de emergencia, la capacidad de vuelo, la dispersión y la longevidad. La distancia estándar obtenida por los modelos de regresión fue 127 m y 131 m para Salto y San José, respectivamente. En Salto las trampas tuvieron capturas hasta el octavo día y en San José no hubo capturas después del sexto día.

Palabras clave: TIE, mosca de la fruta, mosca del mediterráneo, manejo de plagas

[4] Artículo publicado. Duarte F, Calvo MV, Delgado S, Bartolucci A, Asfenato A, Borges A, Scatoni I, Garcia FM. 2020. Release-Recapture Test of Dispersal and Survival of Sterile Males of *Ceratitis capitata* (Diptera: Tephritidae). Neotropical Entomology 49, 893–900. https://doi.org/10.1007/s13744-020-00801-x

4.2. SUMMARY

The sterile insect technique is used around the world to suppress or eradicate populations of *Ceratitis capitata* (Wiedemann) with successful results. It consists of inundative releases of sterile insects into a wide area to reduce reproduction in a field population of the same species. It is necessary to know the dispersion of the sterile males in the field in order to define the maximum distance between the release points that ensures the distribution of the sterile flies in the entire target area. The release methods may vary depending on the area to be covered and the resources available. Manual ground release requires less technology. The aim of this research was to estimate the ability of sterile males to survive and disperse in the field, in the two main areas of citrus production in Uruguay. A release of 20,000 sterile males of *C. capitata* TslV8 (-inv D53) was performed at the central point of each area defined for the trials. Around these points a network of 54 Jackson traps baited with trimedlure was installed forming five concentric rings, which were placed on days 1, 3, 5 and 7 after the release and were removed at 24 hours in all cases. The emergence rate, flight ability, dispersion, and longevity were estimated. The standard distance obtained by the regression models was 127 m and 131 m for Salto and San José respectively. In Salto the traps had catches until the eighth day and in San José there were no catches after the sixth day.

Keywords: SIT, fruit fly, medfly, pest management

4.3. INTRODUCTION

Ceratitis capitata (Wiedemann) is a major pest of worldwide fruit and vegetable production with a host range that exceeds 350 species (Liquido et al., 1991). In addition to the direct injury it causes to fruits, the quarantine pest status of this species in several export destination markets imposes serious economic losses to fruit growers.

The sterile insect technique (SIT) is widely used around the world to suppress or eradicate invasive populations of *C. capitata* with successful results. The SIT is a method of pest control that consists of inundative releases of sterile insects into a

wide area to reduce reproduction in a field population of the same species (FAO/IPPC, 2016). Eggs resulting from the mating of a sterile male with a wild female do not hatch, which leads to a reduced population growth rate of the pest. Additionally to the pest control, the key benefits of SIT derive mostly from a reduction in pesticide use, minimizing environmental and health costs. Furthermore, SIT is an environmentally friendly strategy for use in area wide pest management (AWPM) because it can be applied not only in commercial orchards but also in backyards and urban areas not usually protected by insecticides (Dias y Garcia, 2014, FAO/IAEA, 2005).

To the success of SIT programs the sterile male sexual competitiveness is a primordial condition that refers to how readily wild females accept sterile males as mates in the presence of wild males. Once this condition has been overcome, the ability of sterile flies to survive in the field and to move from the point of release to feeding, mating and resting sites is critical (FAO/IAEA/USDA, 2014).

For the correct implementation of the technique, it is necessary to know the dispersal ability of the sterile males in the field in order to define the maximum distance between the release points that ensures a uniform distribution of the sterile flies in the entire target area. Little dispersal could affect result in uneven coverage or even no coverage of some patches (Meats et al., 2006). In the same way, it is necessary to know the permanence of sterile insects in the field to identify the homogeneity of the distribution of individuals over time. There are several research works of dispersal of *C. capitata* involving release-recapture of marked flies (Gavriel et al., 2012, Meats y Smallridge, 2007, Barry et al., 2002, Baker y Chan, 1991, Plant y Cunningham, 1991, Wong et al., 1982, Wakid y Shoukry 1976) as a consequence of a constant effort to improve SIT efficiency, design surveillance trapping programs and to know the ability of the pest to establish itself in new places (Meats y Smallridge, 2007). An important thing to consider is that most of the research studies were carried out in areas with dry summers, such us southern California (Andress et al., 2013), the central coast of Israel (Gavriel et al., 2012), northeast Brazil (Paranhos et al., 2010), and southern Australia (Meats y Smallridge, 2007). Besides, research studies differ in the kind of traps used, the intervals between traps and the release methods.

The release methods used (automated ground release, manual ground release and aerial releases) are decisive in the distribution of flies (Andress et al., 2013, Salvato et al., 2003) and may vary according to the surface to be covered and to the resources available to perform the release. Manual ground release is probably the one that requires the least technology. Most of the dispersal studies were carried out using land releases, although recapture values are presented at different distances, a specific value for the distance between sterile fly release points is not concluded. The aim of this research was to estimate the maximum distance between sterile male release points as well as the time intervals between releases that allow for homogeneous coverage in a SIT Program in a humid temperate zone. Some quality parameters of the sterile males were also evaluated.

4.4. MATERIALS AND METHODS

Sterile insects of the strain of genetically enhanced *C. capitata* TslV8 (-inv D53) were provided by the Institute of Health and Agricultural Quality in Mendoza, ISCAMEN. Males were sterilized using gamma radiation from a Cobalt 60 source with an irradiator IMCO20 (Aplicated Investigation-INVAP, San Carlos de Bariloche, Río Negro). The pupae were first coated with fluorescent dye Red Fire at a dose of 2 g/l pupae, packed in plastic bags, and irradiated under hypoxia at a target dose of 110 Gy at the Santa Rosa irradiation facility 48 hours before the expected adult emergence. Application of dye allows differentiation between released and wild males when they are observed under ultraviolet light. Following irradiation, bags with pupae were packed along with coolant and kept at 15 °C in an insulated shipping box and transported via commercial airlines to the international airport of Carrasco, where it was collected and transported to the Laboratory of Entomology of the Facultad de Agronomía in Montevideo. The time spent under hypoxia during handling and shipping pupae was 40 h.

4.4.1. Emergence Rate and Flight Ability

Emergence and flight ability tests were carried out simultaneously in the ISCAMEN and in the Laboratory of Entomology of the Facultad de Agronomía in Montevideo,

in order to evaluate the effect of handling and shipping on the quality of the pupae. The following parameters were evaluated:

Emergence rate. One hundred pupae were placed in three emergence grids, considering each grid as a repetition, and the percentage of pupae emerged at 25 ºC was measured (FAO/IAEA/USDA, 2014).

Flight ability. The test consisted of placing 300 pupae in 3 black Plexiglas tubes of approximately 9 cm in diameter and 10 cm high, 100 pupae per tube, considering each tube as a repetition. Talc was placed on the inside walls to prevent flies from walking on the tubes. At the base of each tube was placed a petri dish with a black background and a paper ring inside which pupae were deposited. The tubes were placed inside a plexiglass box at 25 ºC and 65 % ± 15 R.H. The flies were extracted as they left the tube and the total number of flies extracted was counted until 5 days after emergence (FAO/IAEA/USDA, 2014).

In addition, an average of the total flies that left two of the bio containers (see release recapture) was obtained using the following formula. F = T - (NE + NC) F = T - (NE + MM + NC, being: F: flies out of the bio containers; T: total number of pupae, NE: flies that did not emerge, and NC: emerged flies that did not come out of the containers. Malformed flies were not discriminated and were considered as part of the group of NC.

Differences among pre shipping, post shipping and prior to release emergence rate, and flight ability were tested. Pre shipping sample was evaluated in Santa Rosa labs, post shipping sample was evaluated in Facultad de Agronomía labs from a sample taken before placing all the pupae in the bio-containers and prior to release sample was evaluated by counting two complete bio containers, one from Salto and one from San José, immediately after the release. Statistical differences were tested using a Kruskall-Wallis test or one-way analysis of variance on ranks, followed by pairwise comparisons (Conover 1999). Statistical analyses were performed with InfoStat/Libre (Infostat 2018).

4.4.2. Release Recaptures Experiment

The study was carried out in two plots, covering an area of 20 ha of citrus orchard each, in the southern citrus zone in San José (34°35'44.84"S; 56°55'57.61"O) and in the northern citrus zone in Salto (30°57'24.86"S; 57°46'44.31"O) approximately 500 km apart from each other. The average annual temperature varies from 19.4 °C in Salto to 16.8 °C in San José (Castaño et al., 2011). The date of the trial was defined according to the phenology of the pest considering that early December (late spring) populations are incipient.

Upon arrival at the Entomology laboratory of the Facultad de Agronomía, the pupae were removed from the shipping box and placed into eight bio-containers (25 x 20 x 15 cm), approximately 5000 pupae each one, with a density of 0,66 flies per cm^3. The density of flies in the biocontainers was lower than that currently used in the Argentinean Program of Suppression and Eradication of the Mediterranean fruit fly-PROCEM that is about 1,5 flies/cm^3 (not published data). It was defined according to the size of the available biocontainers considering that lowering the density has not negative effects on flies (Carey, 1993). The flies were supplied with water and sugar spread on a sheet of paper, according to the recommendations based on the experience of specialists from ISCAMEN. Prior to the release, all flies were maintained in the laboratory at 25 ± 1 °C, 65 ± 10 % R.H. The emergence was 3 days after arrival, and flies were released 3 days later. The ones to be released in Salto were put in a van with air condition and carried by land the previous day, this shipment lasted about 6 hours.

A single release of 20,000 sterile males of *C. capitata* marked with fluorescent powder was performed at the central point of each area defined for the trials. Around these points a trapping network was installed forming five concentric rings. In the first ring, six traps were set up and there were 12 traps in each of the four remaining rings, distanced from the release point 50, 100, 150, 200 and 250 m, respectively. The network consisted of 54 Jackson traps baited with trimedlure, which were placed on days 1, 3, 5 and 7 after the release and were removed at 24 hours in all cases. The same lures were used throughout the trial.

The flies captured in the traps were counted under ultraviolet light to see if flies fluoresce and thus identify if they are wild or sterile males.

The comparison between the numbers of catches over the successive days after the release provides estimates of survival in the field in that period.

To estimate the dispersion capability the standard distance was calculated (Thomas and Loera, 1998, Carey, 1993, Freeman, 1977), that is mathematically the same to the standard deviation of the mean, where the average distance is zero at the point of release and the distance of each capture is the deviation. The standard distance can be calculated as a linear regression of the number of recaptures (Y) against the distance (X), based on the Gompertz equation: Ln Y = a + b log X (Thomas, 2010). Dispersion was modeled by least squares regression and goodness of fit was measured by the coefficient of determination (R^2) and residual mean square of the ANOVA test for regression.

4.4.3. Post Release Survival

Simultaneous to the dispersion trial, in both releasing plots 450 sterile males were taken from the bio containers to evaluate survival in the field. A voile cage of approximately 0.5 m^2 containing 9 jars of 1l capacity with 50 sterile males each was hung under a tree. Three of the jars were supplied with water and sugar spread on a sheet of paper, in other three just a water source was placed and in the remaining three, no supply was provided. The total number of live flies at 48 hours post release was counted to estimate the survival rate. Statistical differences were tested using a Kruskall-Wallis one-way analysis of variance by ranks, followed by pairwise comparisons (Conover, 1999).

Temperature and rainfall records during the trial period presented below (Figure 1) were obtained from the INIA agroclimatic stations located in Las Brujas (34°40'15.85"S; 56°20'28.67"W), and Salto (31°16'17.74"S; 57°53'27.70"W) to contribute to the discussion.

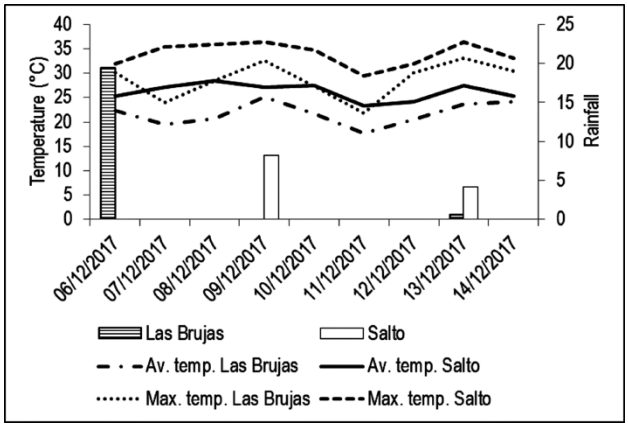

Figure 1 Rainfall, average and maximum temperature post release.

4.5. RESULTS

4.5.1. Emergence Rate and Flight Ability

There was a significant decrease in the emergence rate (p-value = 0.0107) and flight ability (p-value = 0.0250) of the flies after release (Table 1 and 2); however, when comparing flies pre and post shipping, neither the emergence rate, nor the flight ability were statistically different.

Table 1. Emergence rate of sterile insects

	Emergence rate (%)
Pre-shipping sample	88.0 ± 2.0a (n = 100, r = 3)
Post-shipping sample	78.0 ± 1.0ab (n = 100, r = 3)
Prior to release sample	66.8 ± 7.1b (n ≈ 10000, r = 2)

Table 2. The percent flight ability of sterile insects

	% of fliers [(total of fliers /total of emerged flies) *100]
Pre-shipping sample	94.0 ± 0.6a (n = 100, r = 3)
Post-shipping sample	92.0 ± 0.6ab (n = 100, r = 3)
Prior to release sample	80.3 ± 7.8b (n ≈ 10000, r = 2)

4.5.2. Post Release Survival

The post release survival was different depending on the supplies (p-value = 0.0214); there was a slight increase in the rate of survival with the addition of water although not significant, which was even higher with the addition of water and food, being statistically different to not supply treatment (Table 3).

Table 3. Post release survival in a voile cage in the field

Treatment	**Survival 48 hrs. post release (%)**
Without water and food	82.7 ± 2.3b
Just water	86.7 ± 4.2ab
With water and food	94.0 ± 2.0a

4.5.3. Release Recapture

There was a rapid decrease of catches post release, which was greater in San José than in Salto. While in Salto the traps had catches until the eighth day, in San José there were no catches after the sixth day (Figure 2)

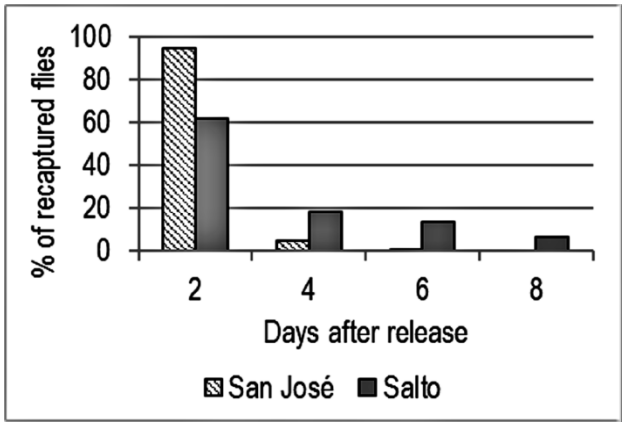

Figure 2 Percentage of recaptured flies according to the days after release in two different zones (San José and Salto).

According to the emergence rate and the percentage of fliers post release (Table 1 and 2), from the 20,000 released flies at each site, 10,728 had the ability to fly. A total of 272 (2.5 %) flies were recaptured in San José and 210 (1.9 %) in Salto.

The standard distance obtained by the regression models (Figure 3) was 127 m, and 131 m for Salto and San José, respectively. Both equations are very similar confirming a similar dispersion in both sites. Figure 4 shows that approximately half of the catches are in the first 50 m, and 75 to 80 % are in a 100 m radius.

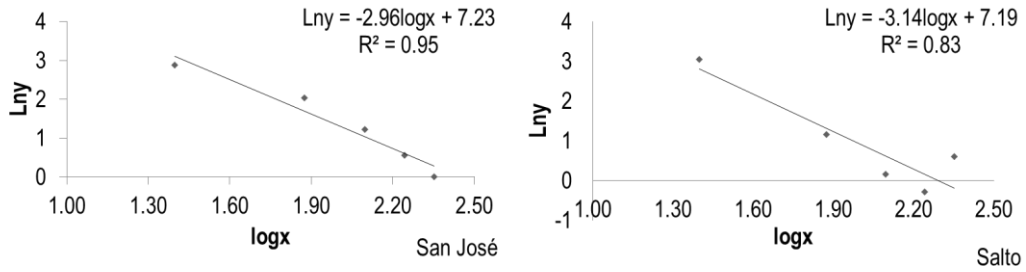

Figure 3 Linear regression model of the neperian logarithm of the recaptures (y), as a function of the base 10 logarithm of the recapture distance from the release point (x), for two different zones (San José and Salto).

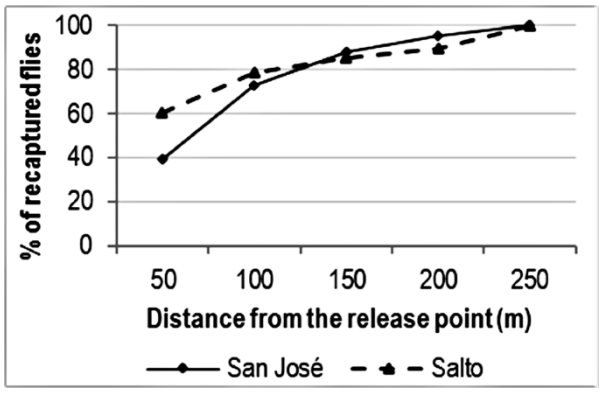

Figure 4 Percentage of recaptured flies according to distance from the release point, for two different zones (San José and Salto).

Figure 5 shows the total dispersion area at the end of the trial. The recaptured population remained in symmetric dispersal pattern around the releasing point. InSan José, recaptures were influenced by the direction of the prevailing winds during the evaluation period (SW).

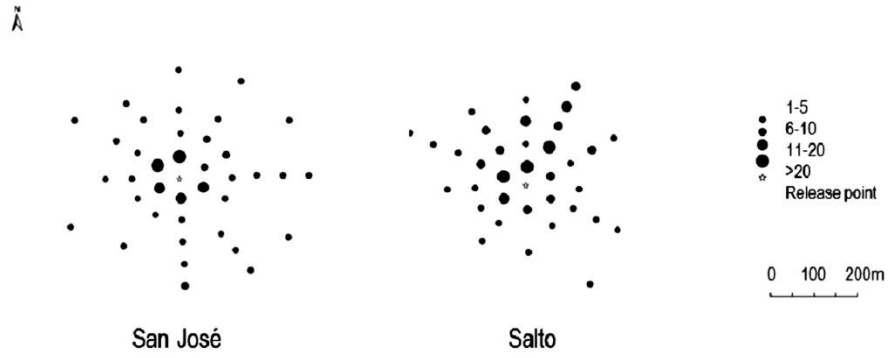

Figure 5 Distribution of the accumulated catches during the eight days post release, for two different zones (San José and Salto).

Although in San Jose the flies died earlier and had less time to disperse than in Salto, the flies in San José advanced more the first two days, eventually reaching approximately the same average distance. In Salto the flies disperse in smaller numbers but for a longer time (Figure 6).

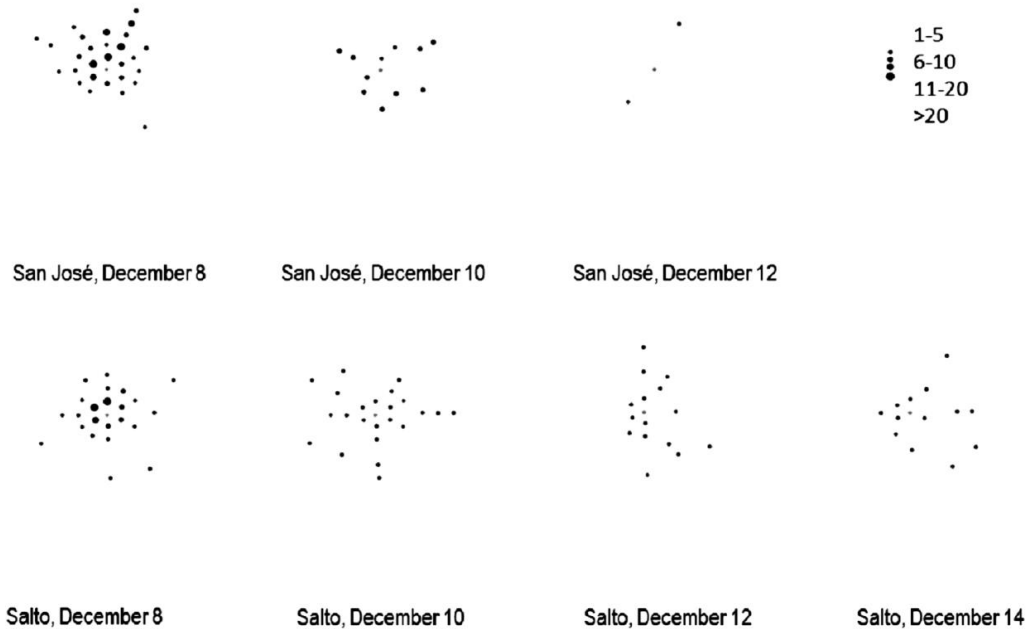

Figure 6 Distribution of catches according to monitoring date. Above: San José department, below: Salto department.

4.6. DISCUSSION

The decrease in the emergence rate from pre shipping to post release sample might be due in part to the hypoxia conditions during the shipping.

The decrease in the percentage of fliers in the post release sample can also be attributed to the crowded conditions in which flies were kept in the bio containers during the sexual maturation period (0.66 flies per cm^3). Fruit flies are widely spaced in nature; it is expected that when individuals are confined in a reduced space stress is increased. The crowded conditions could cause physical injury, due to accidents and because flies can have an aggressive behavior (Briceño et al., 1999). Although the mortality level of *C. capitata* heightens with increased density of flies, density effect due to confinement cannot be removed because it is an integral component of the experiment (Carey et al., 1995). The flies had good physical reserves at the time of release, considering the high survival rate even in the absence of water and food. This is determinant for the success of the flies in the field because it gives them time

to disperse and find sources of water and food and, what is the most important, to find the wild females.

The post release survival test in voile cages showed, as it was expected, that flies with food and water supplies had a better chance of surviving in the field and reaching sexual maturity. In any case, the survival without supplies was 82.7 % of the flies, which proves that, in the worst situation, flies have a rather high percentage of survival.

The rapid decrease of catches in the study area could be attributed to emigration and/or more probably to the death of the flies. From the comparison of the catches in the course of the days, the survival as a function of time can be estimated. The strong fall of the flies catches 4 days after release coincides with that observed for Gavriel et al (2012) and with the results of Andress et al (2013), who had 66 % of the catches in the first 3 days. The time that the sterile males remain alive in the field is also important because sterile flies must reach sexual maturation to mate wild females and this sexual maturation is reached approximately 5 days post emergence (FAO/IAEA/USDA, 2014). If sterile flies die before reaching sexual maturation, they will not be able to compete with wild males for mating.

The recovery rates are within the ranges described by others researches (Andress et al., 2013, Gavriel et al., 2012, Thomas and Loera-Gallardo, 1998).

In the same way, other authors had similar results for the dispersion distance, where most of recovered flies were within a 100 m radius from the release point (Gavriel et al., 2012, Paranhos et al., 2010, Barry et al., 2002) and the average distance flown was lower than 300 m (Thomas and Loera-Gallardo, 1998, Plant and Cunningham, 1991).

The distance that an insect moves is influenced by external factors (Thomas 2010). *Ceratitis capitata* disperse just the distance necessary to find vital requirements such as nutrients, water, refuge and host fruit (Hendrichs et al., 1991). However, the sexes respond differently to the spatial and temporal environmental variability found in the orchard. While females spatial aggregation is more related to the phenology of the host tree and to the sequential availability of ripe or semiripe fruits in the orchard, males can have a more random dispersion pattern (Papadopoulos et al., 2003).

Some factors that affect the distribution and survival in the field are temperature and rainfall. In San José rained on the afternoon of the release day and this could have negatively affected the survival in the field. Dispersal patterns, trap catches and detection chances are positively correlated with temperature (Slawomir, 2018). Considering that after 4 days post release the number of catches in Salto were always between 1 to 5 and that, according to Slawomir (2018), higher temperatures favored the detection ability of the traps, it could be possible that a similar population density in San José that in Salto was not detected due to the lower temperature in San José.

For the success of SIT programs, the ability of sterile flies to move from the point of release to mating sites with the wild population is critical; therefore, it is necessary to define a release protocol that allows sterile flies to disperse all over the target surface.

This research provides us with indispensable base information to define the minimum distance required between sterile fly release points under a SIT program, as well as a first indication of the necessary frequency of releases. According to our results, the minimum distance recommendable between release points is between 254 and 262 m, and the release frequency should be between 3 and 4 days.

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4.8. ANEXO

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5. RESULTADOS Y DISCUSIÓN

En la secuencia de trabajos presentados se estudió, en primera instancia, la relación entre la distribución espacial, la fluctuación poblacional y los niveles de infestación en fruto de las dos especies de tefrítidos de importancia económica presentes en Uruguay. En este estudio se determinó que existe una baja correlación entre el nivel de capturas en las trampas y los niveles de infestación en fruta, resultados visibles en los mapas de distribución de capturas y daño y en los gráficos de fluctuación poblacional e infestación. En los mapas se observa que para ambas especies existen sitios donde hay capturas y no hay daño, y, en el caso de *A. fraterculus*, sitios donde ocurre daño y no se registran capturas. Por otra parte, en los gráficos de fluctuación poblacional se observa que las capturas de *C. capitata* en frutales de hoja caduca se incrementan luego de la cosecha. Además, en ciertos hospedantes que no mostraron infestación en fruto, las capturas de machos de *C. capitata* fueron superiores a las de hembras. Esto se explicó en el caso de *C. capitata* por el hecho de que muchas veces las moscas se encuentran en cultivos sin sustrato susceptible para la oviposición, en busca de alimento, refugio o sitios donde aparearse (Hendrichs y Hendrichs, 1990, Papadopulos et al., 2000, Sciarretta et al., 2018), por lo que la presencia de adultos no estaría necesariamente relacionada con la presencia de daño en ese cultivo. También es probable que existan reservorios de moscas en hospedantes silvestres (Grové et al., 2017, Ovrusky et al., 2003) y que haya movimiento de hembras fecundadas desde los hospedantes silvestres a los cultivos para oviponer (Branca et al., 2000). La gran polifagia de *C. capitata* y *A. fraterculus* (Norrbom, 2004, Liquido, 1991) y las diferencias observadas entre patrones de dispersión espacial de machos y hembras vinculadas a la necesidad y utilización diferencial de los recursos hacen necesario un enfoque de manejo regional de estas plagas, ya que existen diversos sitios que pueden ser reservorios de plaga fuera del período de susceptibilidad de los cultivos.

Cuando se piensa en un esquema de manejo regional para moscas de la fruta la técnica del insecto estéril aparece como una de las herramientas con atributos más deseables para ser aplicada masivamente por ser una técnica selectiva e inocua para el ambiente y la salud, que puede ser aplicada incluso sobre zonas pobladas donde el

uso de insecticidas está restringido (FAO / IAEA, 2005, Dias y Garcia, 2014). Una de las condiciones necesarias para el correcto funcionamiento de la TIE es la compatibilidad sexual. Se ha informado durante mucho tiempo que la mosca de la fruta sudamericana muestra una amplia variación morfológica a lo largo de su distribución geográfica (Hernández-Ortíz et al., 2015, 2004, Steck, 1999, Stone, 1942, Lima, 1934). Diferentes estudios concuerdan con el hecho de que el morfotipo presente en Argentina y el sur de Brasil es el morfotipo Brasileño-1 (Hernández-Ortíz, 2012). La distribución sugerida por Rull et al. (2013) coloca a Montevideo, Uruguay, en la zona límite de distribución para este morfotipo. Los resultados de las pruebas de compatibilidad reproductiva realizados en este trabajo sustentan la hipótesis de que el morfotipo Brasileño-1 es el que se encuentra presente en Uruguay, aunque se deben realizar más estudios que utilicen enfoques moleculares y morfométricos antes de que pueda asignarse de manera concluyente a un morfotipo específico.

En el caso de *C. capitata*, no existiría aislamiento sexual entre las poblaciones (Cayol, 2000), por lo que podrían utilizarse para la aplicación de la TIE insectos estériles de diferentes orígenes. Esto permite la posibilidad de importar insectos estériles de biofábricas presentes en la región, siendo la biofábrica Santa Rosa (Mendoza, Argentina) la más cercana. Para el éxito de los programas TIE, la capacidad de las moscas estériles para moverse desde el punto de liberación a los sitios de apareamiento con la población silvestre es crítica; por lo tanto, es necesario definir un protocolo de liberación que permita que las moscas estériles se dispersen por toda la superficie objetivo. Esta investigación nos proporcionó información básica indispensable para definir la distancia mínima requerida entre los puntos de liberación de moscas estériles bajo un programa con TIE, así como una primera indicación de la frecuencia necesaria de liberaciones. Según nuestros resultados, la distancia mínima recomendada entre los puntos de liberación es de entre 254 y 262 m, y la frecuencia de liberación debe ser de entre 3 y 4 días, resultados similares a los encontrados en otros trabajos (Gavriel et al., 2012, Paranhos et al., 2010, Barry et al., 2002, Thomas and Loera-Gallardo, 1998, Plant and Cunningham, 1991).

6. CONCLUSIONES

Las capturas de *C. capitata* y *A. fraterculus* en trampas McPhail cebadas con torula y las capturas de *C. capitata* en trampas Jackson cebadas con trimedlure no siempre están asociadas con la infestación de frutos; por lo tanto, basar las decisiones de manejo solo en la la cantidad de moscas de la fruta recolectadas en trampas ocasionalmente puede conducir a fallas en el control. La infestación de frutas al inicio de la temporada podría ser una buena medida de la actividad de las moscas para complementar las capturas en trampas y guiar las decisiones de manejo cuando el valor comercial del cultivo lo justifique. Otro aspecto a considerar es el gran aumento de capturas observado poscosecha, por lo que concentrar las medidas de monitoreo y control sólo cuando está el fruto con valor comercial presente y susceptible, implica el riesgo de ingreso de estas plagas desde los montes cosechados. Al planificar una estrategia de seguimiento y control, incluir estas parcelas ya cosechadas podría ayudar a anticipar este riesgo y reducir los daños.

Quedaron determinadas las distancias máximas requeridas entre puntos de liberación para definir un esquema de aplicación de la TIE para manejo de *C. capitata* en Uruguay. Por otra parte, ante un eventual desarrollo de la TIE para control de *A. fraterculus* se debe considerar que el origen de la población estéril coincida con el morfotipo *Brasilian 1*, que todo indicaría que es el que se encuentra presente en Uruguay.

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