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HOST STATUS OF PURPLE PASSIONFRUIT FOR THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a key pest of a wide range of fruit crops and the focus of rigid quarantine restrictions and eradication measures in several countries. In Colombia, the susceptibility of purple passionfruit (*Pasiflora edulis* f. *edulis* Sims; Violales: Passifloraceae) to *C. capitata* is uncertain. Field collections of fruit were made to evaluate natural infestation. Forced infestation studies were conducted in the laboratory with punctured and intact fruit to determine the acceptability of fruit at different stages of maturity and physiological suitability of fruit to development. No *C. capitata* larvae were found and no adults emerged from a total of 976 hand-picked fruit and 623 fallen fruit. In the meantime, trap data indicated that *C. capitata* is not present in the principal passionfruit production regions. For intact fruit, *C. capitata* females oviposited exclusively in fruit of maturity level zero, with 41.67% of fruit accepted for oviposition and an average of 183.1 ± 33.8 eggs per fruit. No oviposition was recorded in fruit of maturity levels 2 and 4. For punctured fruit, *C. capitata* oviposited a total of 84,410 and 84,250 eggs into fruit of maturity levels 0 and 2, respectively, but no *C. capitata* adults emerged from fruit at either maturity level. Laboratory tests suggest that purple passionfruit is a non-host for *C. capitata*.

Key Words: quarantine pest, *Ceratitis capitata*, host status, risk analysis, fruit fly

RESUMEN

La mosca del Mediterráneo *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) es una plaga clave de una amplia gama de frutales y es el foco de estrictas restricciones cuarentenarias y medidas de erradicación en varios países. En Colombia, la susceptibilidad del maracuyá morado (*Pasiflora edulis* f. *edulis* Sims; Violales: Passifloraceae) a *C. capitata* es incierta. Se hicieron colectas de frutos en campo para evaluar el nivel de infestación. En el laboratorio se desarrollaron estudios de infestación forzada con frutos perforados e intactos para determinar la aceptabilidad del fruto en los diferentes estados de maduración e idoneidad fisiológica del desarrollo de los frutos. No se encontraron larvas de *C. capitata* ni adultos emergidos en un total de 976 frutos recogidos manualmente y 623 frutos caídos. Mientras tanto, los datos de captura indicaron que *C. capitata* no está presente en las principales regiones de producción del maracuyá. Para frutos intactos, las hembras de *C. capitata* ovipositaron exclusivamente frutos de nivel de maduración cero, con 41.67% de aceptación de frutos para oviposición y en un rango de 183.1 ± 33.8 huevos por fruto. No se registró oviposición en frutos con niveles de maduración 2 y 4. Para frutos perforados, *C. capitata* ovipositó un total de 84,410 y 84,250 huevos dentro de frutos con nivel de maduración 0 y 2 respectivamente, pero no emergieron adultos de *C. capitata* de los frutos en ningún nivel de maduración. Las pruebas de laboratorio sugieren que el maracuyá morado no es hospedero para *C. capitata*.

Translation provided by the authors.

Tephritid fruit flies are key pests of a wide variety of fruit species, affecting crop yield, quality of harvested produce, and (international) market access (e.g., Robinson & Hooper 1989; Aluja & Mangan 2008). Given the polyphagous nature of many fruit fly species, quarantine restrictions are in place to avoid their introduction in certain countries or geographical regions. A key quarantine pest for the continental United States is the Mediterranean fruit fly, *Ceratitis capitata* (Wiedeman), a destructive pest of multiple fruit crops worldwide (Liquido et al. 1991). In assessing risk of *C. capitata* arrival in the U.S. and developing associated quarantine protocols, supreme precaution is taken to avoid entry of potential host fruits of this pest. Listings of the status of particular fruits as hosts of *C. capitata* are the cornerstone of quarantine restrictions (Liquido et al. 1991). However, current restrictions include fruit species for which there is poor information

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regarding *C. capitata* host status. Hence, research is needed to revise and update *C. capitata* host information and thereby improve quarantine decision making (Aluja et al. 2004; Peña et al. 2006; Jenkins & Goenaga 2008; Staub et al. 2008; De Graaf 2009; Follett et al. 2009).

Purple passionfruit (*Passiflora edulis* f. *edulis* Sims) is one of several tropical fruits that is well-positioned in local markets and gradually becoming popular internationally (Ocampo 2007; Wyckhuys et al. in press). In Colombia, purple passionfruit is mainly grown by small-scale, resource-poor farmers on a total area of 100-400 ha. It is a profitable crop and fresh fruit is increasingly being exported to northern Europe and Canada (Wyckhuys, unpublished data). Entry of fresh fruit into the continental U.S. is not permitted currently, based upon its presumed suitability as a host for *Anastrepha* spp. and *C. capitata*.

Liquido et al. (1991) list *C. capitata* as a potential pest of *P. edulis*, but provide no evidence of adult fly emergence from field-collected fruit. Other reports indicate *C. capitata* is an occasional pest of *Passiflora* sp., without specifying the exact crop species, botanical form or variety (Thomas et al. 2001). Yellow passionfruit (*P. edulis* f. *flavicarpa* Degener) is reported as a possible host of *C. capitata* in Hawaii (Akamine et al. 1954), while many tephritids attack certain *Passiflora* species in Brazil (Aguiar-Menezes et al. 2002). In Colombia, national pest survey records for *C. capitata* maintained since 1986 have not detected this pest in the principal production regions of purple passionfruit (ICA, 2009). As a note of caution, it is important to indicate that climate change could cause altitudinal range shifts of pest tephritids and may eventually bring *C. capitata* into those production regions in the future (Hill et al. 2011).

Considering a lack of scientific information regarding purple passionfruit host status for *C. capitata* and the importance of its production as source of income for rural smallholders, we attempted to determine the host suitability of Colombia-grown purple passionfruit for *C. capitata* using standard methods (Cowley et al. 1992). This information can be used to re-evaluate the quarantine status of this fruit for market access to the United States.

Materials and Methods

All methodologies for host status screening were adopted from Cowley et al. (1992), taking into account parameters set by RSPM No. 30 (NAPPO 2008) and APPPC RSPM No. 4 (FAO 2005; Follett & Hennessey 2007).

Field Collections

Between Sep 2008 and May 2010, sampling was done during 4 distinct events in the principal purple passionfruit production regions, located in the departments of Boyacá, Cundinamarca, Tolima, and Huila (Colombia). During each sampling event, 9-16 different purple passionfruit orchards were visited and fruit was collected from each orchard. Fruit samples consisted of hand-picked fruit of different maturity levels (i.e., fruit harvested from vines) and fallen fruit, collected from the ground. Fruit was sampled in a random fashion, and the number of fruit collected from each orchard depended upon phenological stage of the crop. We collected a total of 405, 285, 183, and 113 hand-picked fruit from Boyaca, Cundinamarca, Huila, and Tolima, respectively. Respective numbers of fallen fruit collected from each department were 345, 124, 96, and 58.

Fruit samples were counted, weighed and taken to the Horticulture Research Center CIAA (Chia, Colombia) in ventilated plastic containers (70 × 50 × 50 cm) for further laboratory processing. In the laboratory, fruit samples were kept at 22.0 ± 2.0°C, 65% RH and 12:12 L:D. Within 1 week following the collection, containers were screened for presence of fruit fly puparia, and fruit were dissected to assess presence of tephritid larvae. Larvae were subsequently transferred to ventilated plastic Petri dishes with moistened vermiculite. Petri dishes were checked daily for adult emergence. We recorded the number of tephritid larvae and *C. capitata* adults for each sampling event and production region.

Simultaneous with field collections, McPhail traps (baited with protein hydrolysate; Cebofrut, AgroBiologicos SAFER, Medellin, Colombia) were deployed in orchards in each production region and visited bi-weekly to record the number of *C. capitata* adults. A total of 6 traps were deployed per orchard, of which 5 were placed within the orchard itself and a sixth trap was placed outside the orchard in the dominant surrounding habitat type. To check trap attractiveness, we recorded captures of other tephritids.

Laboratory Experiments

Insect material was collected from coffee fruit (*Coffea arabica* L.) in commercial orchards in Fredonia (Antioquia, Colombia), at 1,400 m altitude, and Medellín (Antioquia), at an altitude of 1,493 m. Upon field collection, fruits were transferred to the ICA Entomology Laboratory in Bello (Antioquia). Each fruit was dissected and any tephritid larvae were allowed to pupariate in vermiculite. Puparia of *C. capitata* were subsequently taken to the Quarantine Treatment Laboratory of the Colombian Institute for Agriculture and Lifestock ICA in Mosquera (Cundinamarca) for further experimenting. Adults from field collected puparia were exposed to mango (*Mangifera indica* L.), a preferred host of *C. capitata* (NAPPO 2008).

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Adult flies were maintained within mesh cages (25x25x25 cm), allowed ample access to water and fed *ad libitum* with torula yeast and sugar. All insect developmental stages were maintained within climate-controlled rearing chambers at 25 ± 1ºC, 65 ± 5% RH and 12:12 L:D. Second generation *C. capitata* adults were then used for host status trials. Laboratory experiments were carried out between Oct 2008 and May 2009. Voucher specimens of study insects were kept at the ICA laboratory.

All fruits used in the experiment were selected and harvested in several purple passionfruit orchards in Venecia (Cundinamarca) or mango orchards in La Mesa (Cundinamarca). Fruit of different maturity levels were selected based on commonly-used color tables for either mango or purple passionfruit (ICONTEC 1999; Pinzón et al. 2007). Prior to use in experiments, fruit was disinfected by immersion in a 0.05% sodium hypochlorite solution for 10 min. Subsequently, each fruit was dried and stored in plastic containers to use in host status trials. Fruit was used for experimenting within 72 h of harvest.

Oviposition Preference Assay

A total of 120 *C. capitata* pairs, aged 14 d, were placed within a mesh cage (70 × 50 × 50 cm) (Vidal et al. 2005) and allowed access to water and *ad libitum* torula yeast and sugar. Within each cage, we placed 8 purple passionfruit of each of 3 maturity levels (i.e., maturity 0, 2, and 4; see Pinzon et al. 2007). Purple passionfruit are approximately 5 cm in diameter. After 24 h, fruit was removed from the cages and dissected to determine the total number of *C. capitata* eggs. Over the course of 3 d, fruit were placed within each cage and subject to the same ovipositing *C. capitata* females. The experiment was carried out with 3 replicates, thus screening 72 fruit per maturity level. The number of eggs within fruit of differing maturity level was compared by one-way analysis of variance (ANOVA). For all analyses, the statistical package SAS was used.

Host Status Trials

Based upon results of the previous assay, further trials were conducted to determine purple passionfruit host status to *C. capitata*. To stimulate fly oviposition, fruit was punctured with standard dissection pins (10 pinholes 1-2 mm into the fruit) before placing them within experiment cages (FAO 2005; NAPPO 2008). Purple passionfruit of maturity levels 0 and 2 were included in trials, while mango fruit (maturity degree 2 or 3) was used as a positive control. We placed 11 fruit per cage (70 × 50 × 50 cm) with 120 *C. capitata* pairs, aged 14-19 d and provided with water and *ad libitum* torula yeast and sugar. There were 3 replicates of each fruit type and maturity level, and simultaneous trials were conducted. In total, 990 purple passionfruit and 495 mango fruits were subjected to an infestation pressure of 10.9 *C. capitata* females per fruit.

Over the course of 15 d, fruits within each cage were replaced on a daily basis, and subsequently kept within ventilated plastic containers. In a random fashion, a subsample of 45 fruits of either species or maturity degree was dissected upon removal from experimental cages to assess the number of *C. capitata* eggs. Remaining fruits were kept at 25 ± 1ºC, 65 ± 5% RH and 12:12 L:D and were checked daily for larval emergence, puparia formation, or adult eclosion. After 15 d, all fruits were dissected and *C. capitata* larvae (per fruit) were counted and placed within vermiculite to allow pupariation.

Results

Field Collections

From 2008 up to 2010, a total of 976 purple passionfruit were hand-picked and 623 fallen fruit were collected. No *C. capitata* adults emerged from any fruit. Diptera larvae were found within (immature) fruit; all of which successfully developed into lonchaeid adults. No *C. capitata* adults were caught in McPhail traps deployed in or near orchards in any of the production regions. Trap effectiveness was confirmed through capture of Lonchaeidae (Diptera: Tephritoidea) at all locations.

Laboratory Experiments: Oviposition Preference Assay

The number of *C. capitata* eggs significantly differed between fruit of distinct maturity degrees ($F = 18.84$, $df = 2$, $P < 0.0001$). The highest number of eggs per fruit (183.7 ± 33.8; mean ± SE) was oviposited in purple passionfruit of maturity level 0, while no eggs were laid in maturity levels 2 and 4.

Host Status Trials

Ceratitis capitata successfully completed its development on the preferred host mango, but no adults emerged from punctured fruit of maturity levels 0 and 2 (Table 1). Few *C. capitata* larvae developed in passionfruit, with larval weights ranging from 2.5 to 3.2 mg. In mango, the weight of third instars ranged from 9.7 to 10.3 mg. Of the 194 *C. capitata* that were obtained from passionfruit (maturity 0), <10% successfully pupariated. Puparial weights of individuals developing on passionfruit ranged from 2.3 to 3.1 mg, compared to *C. capitata* puparia from mango that weighed between 9.3 and 10.2 mg. Also, most *C. capitata* puparia that developed from passionfruit were

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TABLE 1. OVIPOSITION AND SUBSEQUENT DEVELOPMENT OF *C. CAPITATA* ON MANGO AND PURPLE PASSIONFRUIT (PPF) OF 2 MATURITY LEVELS UNDER LABORATORY CONDITIONS. DATA REPRESENT CONSOLIDATED NUMBER OF INDIVIDUALS WITHIN EACH *C. CAPITATA* DEVELOPMENT STAGE ON A TOTAL OF 990 PPF FRUIT OR 495 MANGO FRUIT.

Tested commodity	*C. capitata* development stages			
	Eggs	Larvae	Puparia	Adults
Mango	139,410*	64,990	53,854	46,920
PPF - maturity degree 0	84,410	194	18	0
PPF - maturity degree 2	84,250	0	0	0

*The total number of eggs was determined by counting the number of *C. capitata* eggs on 10% of (dissected) fruits, and extrapolating this for all tested fruits.

malformed. No adults eclosed from purple passionfruit puparia, whereas 46,920 adults emerged from infested mangos.

DISCUSSION

Fruit fly host status determination lies at the basis of trade and can help connect small-scale fruit producers in the developing world to lucrative export markets. To aid developing nations in the process of assessing whether a given fruit is a host to a particular fruit fly species, well-defined protocols and experimental guidelines have been defined (FAO 2005; Hennessey 2007; Aluja & Mangan 2008; NAPPO 2008). Natural field infestation trials and a set of screen-house or laboratory experiments all help determine whether a given fruit crop is natural host, non-host or conditional host (e.g., Jenkins & Goenaga 2008; De Graaf 2009). These protocols have been adopted for a wide range of fruit crops, such as mamey sapote (*Pouteria sapota* (Jacq.)), litchi (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* L.), avocado 'Hass' (*Persea americana* (Mill.) 'Hass'), highbush blueberry (*Vaccinium corymbosum* L.), green mango (*Mangifera indica* L. 'Tommy Atkins' and 'Keitt'), and others.

Although data from natural field infestation trials provide the most accurate assessment of host status of a given fruit (NAPPO 2008), a key limitation of these trials is that one cannot control variability in fruit fly abundance. In our experiments, no *C. capitata* adults were reared from field-collected passionfruit in the principal production regions of Colombia. However, McPhail trapping in orchards and surrounding habitats also did not encounter any wild *C. capitata* populations in any of these zones. Purple passionfruit crops are located at 2016.1 ± 250.9 m (mean ± SD) above sea level (Wyckhuys et al. in press), while *C. capitata* has not been reported above 1,600 m (ICA 2009). Thus, under the current altitudinal and geographic distribution of *C. capitata* in Colombia it is very unlikely that this species affects purple passionfruit orchards. Climate change could eventually bring *C. capitata* into actual cropping regions and equally shift current passionfruit production zones to higher altitudes (Hill et al. 2011). At present however, natural field infestation data remain inconclusive with respect to passionfruit host status.

Forced infestation trials under laboratory conditions proved critical in delineating purple passionfruit host status to *C. capitata*. Even though *C. capitata* females oviposited in intact fruit (maturity degree 0) as in punctured fruit of different maturity degrees, larval development was very poor and no adults emerged. No adult emergence from fruit under laboratory conditions is either indicative of its character as non-host under experimental conditions (NAPPO 2008) or as non-host overall (FAO 2005). Nevertheless, we need to indicate that adult development from purple passionfruit could have been affected by dissecting infested fruit 15 d after oviposition. On less suitable hosts, *C. capitata* likely develop slow and take longer to complete larval development. However, fruit was dissected according to its deterioration status (see FAO 2005; NAPPO 2008), while taking into account an upper *C. capitata* egg-larval development time of 15 d (EPPO 2010). In conclusion, even though early dissection of purple passionfruit may have affected pupation and adult eclosion, the poor larval development and lack of emergence of adults from 18 ºC *capitata* puparia clearly indicate the poor suitability of this fruit.

For intact fruit, maturity level 0 was preferred, while fruit of more advanced maturity were not accepted for oviposition by *C. capitata*. Fruit maturity state can greatly affect its acceptability as an oviposition substrate by certain fly species (Armstrong 2001; Willink & Villagran 2007). Certain physical stimuli determined by fruit maturity level (e.g., color) influence *C. capitata* acceptance or rejection of fruit of particular maturity levels (Prokopy et al. 1984; Suarez et al. 2007). Also, fruit maturity level can affect physical resistance to oviposition and interfere with successful *C. capitata* oviposition (Gould & Hallman 2001). To circumvent such, *C. capitata* tend to oviposit in existing oviposition holes, bird pecks or crevices

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(Aluja & Mangan 2008). This could further explain high degrees of oviposition in punctured fruits and low acceptability of intact fruit, more so at advanced maturity degrees at which purple passionfruit has an exceptionally firm epicarp.

Fruit fly oviposition in hosts that are inadequate for larval development is commonly observed (Joachim-Bravo et al. 2001). Especially for highly polyphagous species such as *C. capitata*, behavioral adaptations cause oviposition in a wide range of fruit crops (Aluja & Mangan 2008). Additionally, under highly artificial conditions, time-limited gravid females may accept a broad range of substrates for oviposition (see Robacker & Fraser 2002). A high level of acceptance for oviposition of intact and punctured fruit does not necessarily imply suitability of the infested fruit for further larval development or adult emergence. Increased mortality, poor larval development and reduced puparia size or weight all are indicative of antibiosis and biochemical defenses (Greany et al. 1983) that cannot be detected by ovipositing females. *Passiflora* species are cyanogenic and liberate hydrogen cyanide in fruits or leaves when under (insect) attack (Spencer & Seigler 1983). Possibly, these compounds disrupt larval development in passionfruit.

As presence of low numbers of larvae in fruit is not indicative that it is an acceptable host (Gould & Hallman 2001; Jenkins & Goenaga 2007; Willink & Villagran 2007), we can conclude that Colombia-grown purple passionfruit is a non-host under the experimental conditions used in these tests and may be a non-host in the field. Since *C. capitata* is currently not established in the principal growing areas in Colombia, it is very unlikely that this pest will infest purple passionfruit under natural conditions. There may therefore be significant potential for the establishment of pest free areas to allow exports to the United States or a systems approach based upon low *C. capitata* prevalence and poor host status.

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