The Effectiveness of Fruit Bagging and Culling for Risk Mitigation of Fruit Flies Affecting Citrus in China: A Preliminary Report

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The effectiveness of fruit bagging and culling for risk mitigation of fruit flies affecting citrus in China: a preliminary report

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Abstract

Several tephritid fruit fly species that are damaging to citrus in China are world-wide quarantine pests. Two field tests were conducted in China to evaluate the effectiveness of fruit bagging (i.e., fruits were grown in bags for at least 1.5 mo until harvest) and culling for risk mitigation of these pests during the fruit harvest season of 2017. The first test was conducted in Pinghe County of Fujian Province. The purpose of this test was to assess the effectiveness of fruit bagging on risk mitigation of fruit flies affecting pomelo, *Citrus maxima* (Burm. fil.) Osbeck (Rutaceae). External inspection and internal fruit cutting of 3,000 bagged and 3,040 unbagged fruits revealed few oviposition marks and absence of living flies in the bagged fruits, compared to 129 fruit fly-infested fruits containing 634 live larvae and 4 pupae in the unbagged fruits. Later molecular and morphological identification concluded that these larvae and pupae were *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). The second test was conducted in Shimen County of Hunan Province. The purpose of this test was to assess the effectiveness of packing house culling on risk mitigation of fruit flies in Satsuma mandarin, *Citrus unshiu* (Swingle) Marcov. (Rutaceae). A total of 20,000 fruits were cut (10,000 fruits before culling, and another 10,000 after culling). In the fruits that did not receive the culling treatment, 1 infested fruit with 7 third instar larvae of *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) and 25 fruits with fruit fly oviposition marks were found. In the fruits that received the culling treatment, fruit flies were absent and 10 fruits with oviposition marks were found. These results suggest that fruit bagging and packinghouse culling could contribute to risk mitigation of fruit flies in citrus in China. This is a preliminary report, with further work necessary to develop a systems approach for risk mitigation of fruit flies in the commodities.

Key Words: phytosanitary; systems approach; *Bactrocera minax*; *Bactrocera dorsalis*; fruit cutting

Resumen

Varias especies de moscas de la fruta tefrítidas que son dañinas para los cítricos en China son plagas cuarentenarias en todo el mundo. Se realizaron dos pruebas de campo en China para evaluar la efectividad de embolsar las frutas (las frutas se cultivaron en bolsas durante al menos 1,5 meses hasta la cosecha) y seleccionadas para mitigar el riesgo de estas plagas durante la temporada de cosecha de fruta del 2017. Se realizó la primera prueba en el condado de Pinghe de la provincia de Fujian. El objetivo de esta prueba fue evaluar la efectividad de embolsar las frutas para la mitigación del riesgo de las moscas de la fruta que afectan al pomelo, *Citrus maxima* (Rutaceae). La inspección externa y el corte interno de frutas de 3.000 bolsas y 3.040 frutas sin bolsa revelaron pocas marcas de oviposición y ausencia de moscas vivas en las frutas en bolsas, en comparación con 129 frutas infestadas de moscas de la fruta que contienen 634 larvas vivas y 4 pupas en las frutas sin embolsar. Posteriormente, la identificación molecular y morfológica concluyó que estas larvas y pupas eran *Bactrocera dorsalis* (Diptera: Tephritidae). Se realizó la segunda prueba en el condado de Shimen de la provincia de Hunan. El objetivo de esta prueba fue evaluar la eficacia de las empacadoras de descartar las frutas dañadas para mitigar el riesgo de las moscas de la fruta en la mandarina Satsuma, *Citrus unshiu* (Rutaceae). Se cortaron un total de 20,000 frutas (10,000 frutas antes del descarte y otras 10,000 después del descarte). En los frutos que no recibieron el tratamiento de descarte, se encontraron 1 fruta infestada con 7 larvas de tercer estadio de *Bactrocera minax* (Diptera: Tephritidae) y 25 frutas con marcas de oviposición de la mosca de la fruta. En los frutos que recibieron el tratamiento de descarte, las moscas de la fruta estuvieron ausentes y se encontraron 10 frutas con marcas de oviposición. Estos resultados sugieren que el embolsamiento de frutas y el descarte de frutas afectadas por medio de las empacadoras podrían contribuir a mitigar el riesgo de las moscas de la fruta en los cítricos en China. Este es un informe preliminar, y es necesario seguir trabajando para desarrollar un enfoque sistémico para mitigar los riesgos de la mosca de la fruta en los productos.

Palabras Clave: fitosanitario; enfoque de sistemas; *Bactrocera minax, Bactrocera dorsalis,* corte de fruta

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Tephritid fruit flies such as *Bactrocera dorsalis* (Hendel), *Bactrocera minax* (Enderlein), and *Bactrocera tsuneonis* (Miyake) (all Diptera: Tephritidae) are major pests of citrus in China (Cai & Peng 2008). For example, *B. minax* alone can cause up to 100% fruit loss (Anonymous 1976; Xia et al. 2018; Zhang 1984; Wang & Luo 1995; Wang & Zhang 2009). Although the distribution and the severity of destruction they cause vary substantially, fruit flies occur in almost all major citrus production regions. Pest management and risk mitigation of these pests are important to China's citrus industry, as well as to the rest of the world.

Few phytosanitary options are available for this group of pests in China. Cold treatment and irradiation are among the most commonly used measures for risk management. However, it is unlikely that a cold treatment schedule for *B. minax* or *B. tsuneonis* will be available in the foreseeable future. The 2 pests are widely regarded as the most cold-tolerant species in the genus *Bactrocera* (Luo & Chen 1987; Fan et al. 1994; Xia et al. 2018). Although scientific studies have been conducted, there is no indication that the Chinese regulatory agency soon will approve irradiation as a phytosanitary treatment for fruit flies in fresh fruits (Zhan 2013). To promote safe trade as well as to protect the citrus industry, effective phytosanitary measures should be developed.

A systems approach to phytosanitation has the potential to fill this need. Instead of relying on a single phytosanitary measure, such as cold treatment for risk mitigation, a systems approach could use 2 or more independent measures to achieve this goal (FAO 2012). In this study, we explored 2 measures for risk mitigation, i.e., fruit bagging and packinghouse culling. Fruit bagging is a commonly used pre-harvest practice in Asian orchards for pest management, fruit coloration, and other uses (Wang & Zhang 2009; Huang 2015). Fruits are grown in bags for various lengths of time before or until harvest. Culling is a common practice in packinghouse processing for citrus. Infested and damaged fruits are handpicked and discarded from the packing line. This measure often is required by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for the importation of fresh fruits (USDA-APHIS 2017a, b). However, no scientific study regarding the efficacy of these 2 measures for risk mitigation of fruit flies in citrus has ever been conducted. The goal of this study was to assess the effectiveness of these 2 measures for reducing the risk of fruit flies in citrus.

Materials and Methods

FRUIT BAGGING TEST

The fruit bagging test was conducted in Pinghe County of Fujian Province during Sep to Oct 2017. Two treatments, bagged and unbagged fruits of pomelo, *Citrus maxima* (Burm. fil.) Osbeck (Rutaceae), were used. Following standard production practices, bagged and unbagged fruits were sourced from different parts of the county. Bagged fruits were harvested from groves in the western region of the county, where almost 100% pomelo fruits were bagged, whereas unbagged fruits were harvested from the eastern region, where fruit bagging was less popular. The fruits were harvested from 4 randomly selected towns, each as a replicate in each region (Table 1). The age of groves ranged from 13 to 18 yr old. Fruit bagging was conducted by growers from 12 Jul to 10 Aug 2017 (Table 1). A total of 3,000 bagged (750 × 4), and 3,040 unbagged fruits (760 × 4) were harvested. The unbagged fruits were harvested by walking through the tree rows, harvesting 1 fruit from every 5 citrus trees. For the bagged fruits, bags were kept on the fruits until fruit cutting.

Assessment of treatment efficacy by fruit cutting was conducted in a screened packinghouse using the following procedure:

1.) The bag was opened, inspected carefully for any insects or insect remains. This procedure was applied to bagged fruits only.
2.) Fruits were inspected for any signs of infestation, such as oviposition marks or fruit fly damage. Fruits with signs of infestation were wrapped individually and kept for 8 d before fruit cutting. This was to reduce the chance of failing to detect the eggs and first instars that are about 1 mm in size. Otherwise, fruit cutting was conducted immediately.
3.) Each fruit was cut into 10 to 16 slices of equal size.
4.) Each slice was inspected carefully with special attention paid to the joint area of pith and fruit.

CULLING TEST

The culling test was conducted in Shimen County of Hunan Province in Oct 2017. Four towns in the county, each as a replicate, were randomly selected. Six thousand fruits of Satsuma mandarin, *Citrus unshiu* (Swingle) Marcov. (Rutaceae), were collected from each town, using the method described above. Accordingly, a total of 24,000 (6,000 × 4) fruits were collected. Ten thousand (2,500 × 4) of these fruits were randomly selected for assessment of the control treatment by fruit cutting before culling. The remaining 14,000 fruits, separated by each replicate, were sent through the packinghouse line that had the following process: first culling (preliminary culling) > washing and cleaning > waxing > drying > second culling (intensive culling) > sorting > final culling and box packing.

After that, 10,000 (2,500 per rep × 4) fruits were randomly selected for assessing efficacy of culling treatment by fruit cutting.

The following steps were used for cutting the fruits of before and after culling:

1.) Fruits were inspected for any sign of infestation, such as oviposition marks, feeding activities, or damage. This was to reduce the chance of missing eggs and first instars that are about 1 mm in size.
2.) Fruit was cut into 8 slices of equal size.
3.) Each slice was inspected carefully with special attention to the joint area of pith and fruit.

Table 1. Fruit collection locations for the fruit bagging test (Fujian Province; Pomelo, *Citrus maxima*).

Treatment	No. Reps	Town	Date of bagging	Date of harvest	No. of fruits	Elevation (masl)
Bagged	1	Xiaoxi	12–17 Jul	28 Sep	750	40 – 266
Bagged	2	Wenfeng	15–20 Jul	29 Sep	750	61 – 293
Bagged	3	Shange	5–10 Aug	28, 29 Sep	750	48 – 258
Bagged	4	Banzai	25 Jul –10 Aug	30 Sep	750	118 – 441
Unbagged	1	Xiazhai	N/A	6 Oct	760	370 – 450
Unbagged	2	Qiling	N/A	7 Oct	760	420 – 650
Unbagged	3	Jiufeng	N/A	7 Oct	760	480 – 490
Unbagged	4	Luxi	N/A	8 Oct	760	460 – 610

SPECIMEN IDENTIFICATION

The larvae and pupae collected from the infested fruits were divided into 2 groups. About half of them were placed in vials with 75% ethyl alcohol for molecular identification. The work was conducted by the Chinese Academy of Inspection and Quarantine. The other group was reared to the adult stage for morphological identification, which was conducted by the Guangdong Institute of Applied Biological Resources (formerly Guangdong Entomological Institute), and the Inspection and Quarantine Technology Center of Xiamen Entry-Exit Inspection and Quarantine Bureau.

MOLECULAR IDENTIFICATION

The total genomic DNA was extracted from each larva using the TIANamp Genomic DNA kit (TIANGEN, Beijing, China) following the manufacturer's protocol for animal tissue.

The amplification reaction for mt DNA *cox1* barcode was performed in a total volume of 25 µL, including 12.5 µL 2 × Taq PCR Master Mix (TIANGEN, China), 1 µL of each primer (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'/HCO2198: 5' TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994), 1 µL of template DNA, and 9.5 µL of distilled water. PCR cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. All PCR products were visualized on 1.5% agarose gels run at 110V for 45 min and post-stained with ethidium bromide. All PCR products were sent to SinoGenoMax Co., Ltd. Beijing for bidirectional sequencing.

Chromatograms were checked using BioEdit v7.0.9 (Ibis Therapeutics, Carlsbad, California, USA) software and sequence assembly was performed with DNAMAN software (Demonstration Version) to produce completed sequences for *cox1* barcode. Each of the derived sequences was queried in BOLD (Barcode of Life Data System, http://www.boldsystems.org/) for species confirmation.

STATISTICAL ANALYSIS

The numbers of fruit with damage and insect count data were analyzed to understand the effectiveness of bagging and culling treatments for mitigating the risk of fruit fly infestation. The data were fitted to the Bayesian generalized linear model with binomial distribution with logit link function and t-prior distribution for the coefficients in R (package: arm) (R Core Team 2017).

Results

EFFECTIVENESS OF BAGGING FOR RISK MITIGATION OF FRUIT FLIES OF CITRUS

A total of 3,000 bagged fruits were inspected and cut. Fruit inspection before cutting found 9 fruit fly oviposition marks on the skin. These marks appeared to occur before fruit bagging. Fruit cutting revealed no tephritid fruit flies (Table 2). Twenty-seven larvae and 11 pupae of *Zaprionus* sp. (Diptera: Drosophilidae), a decaying fruit-feeder, were found in a badly decaying fruit. Ants and other predatory insects often were found inside bags.

A total of 3,040 unbagged fruits were inspected and cut. Fruit skin inspection revealed 222 fruits with fruit fly oviposition marks (Table 2). Fruit cutting produced 48 fruits with a total of 634 tephritid larvae, and 4 pupae. Molecular and morphological identifications confirmed that they were *B. dorsalis*. Additionally, 81 fruits (out of the 222 suspected fruits) had dead tephritid eggs and larvae.

There were statistically significant differences in the number of oviposition marks ($z = 9.707$; $P < 0.001$), the number of fruits with dead fruits flies ($z = 3.449$; $P < 0.001$), the number of fruits with live fruit flies ($z = 3.214$; $P < 0.01$), and the number of larvae inside fruits ($z = 4.216$; $P < 0.001$) between bagged and unbagged fruits.

EFFECTIVENESS OF CULLING FOR RISK MITIGATION OF FRUIT FLIES OF CITRUS

The efficacy of the control treatment (fruits that were not culled) was assessed by inspecting and cutting 10,000 fruits (Table 3). Twenty-five fruits had fruit fly oviposition marks, and 1 fruit was infested with 7 fruit fly larvae. Later, molecular identification confirmed these larvae were *B. minax*, the most destructive fruit fly of citrus in China.

The efficacy of the culling treatment also was assessed by inspecting and cutting 10,000 fruits. Ten fruits had fruit fly oviposition marks. No fruit fly-infested fruit were found.

Table 2. Oviposition and fruit infestation rates in the bagging test (Fujian Province; Pomelo, *Citrus maxima*)[1]

			Internal inspection by fruit cutting				
Treatment	Replicate	Oviposition marks[2]	Fruits w/ dead flies[3]	Fruits w/ live flies[4]	Larvae[5]	Pupae	Ants inside of bags
Bagged	1	6	0	0	0	0	Yes
Bagged	2	0	0	0	0	0	Yes
Bagged	3	0	0	0	0	0	Yes
Bagged	4	3	0	0	0	0	Yes
	total	9*	0*	0*	0*	0	
Unbagged	1	2	2	0	0	0	
Unbagged	2	2	0	0	0	0	
Unbagged	3	214	79	48	634	4	
Unbagged	4	4	0	0	0	0	
	total	222*	81*	48*	634*	4	

[1]Asterisk after the totals in each column indicate statistically significant differences between bagged and unbagged fruits in that column only; see [2,3,4,5] below, respectively.
[2]Significant difference in the total number of oviposition marks between bagged and unbagged fruits ($z = 9.707$; $P < 0.001$).
[3]Significant difference in the total number of fruits with dead fruit flies between bagged and unbagged fruits ($z = 3.449$; $P < 0.001$).
[4]Significant difference in the total number of fruits with live fruit flies between bagged and unbagged fruits ($z = 3.214$; $P < 0.01$).
[5]Significant difference in the total number of fruit fly larvae inside fruit between bagged and unbagged fruits ($z = 4.216$; $P < 0.001$).

Table 3. Oviposition and fruit infestation rates in the culling experiment (Hunan Province; Satsuma mandarin, *Citrus unshiu*)

	Control (no culling)		Culling	
Replicate/town	Fruits w/ ovip. marks[1]	Fruits w/ fruit flies (# larvae)[2]	Fruits w/ ovip. marks[1]	Fruits w/ fruit flies[2]
1/Mata	7	0	2	0
2/Guihuacun	5	1 (7)	2	0
3/Xiajiagang	12	0	4	0
4/Sidoupingcun	1	0	2	0
Total	25 A	1 (7) C	10 B	0 D

[1]Significant difference in the total number of oviposition marks between fruits before culling and fruits after culling (z = 2.42; *P* = 0.0155).
[2]Significant difference in the total number of fruit fly larvae between fruits before culling and fruits after culling (z = 1.966; *P* = 0.0459).

Statistical analysis reveals significant differences in the numbers of oviposition marks (z = 2.42; *P* = 0.0155) and numbers of larvae inside fruits (z = 1.966; *P* = 0.0459) between the control and culling treatments.

Discussion

Systems approaches have been developed successfully for risk mitigation of fruit flies in various fresh commodities when a single phytosanitary measure was not available (Jang & Moffitt 1994; Jang 1996; Jang et al. 2006; FAO 2012). Results of this study suggest that fruit bagging and packinghouse culling, commonly used in China, can be effective components of a systems approach for risk mitigation of fruit flies in citrus exports from China. No fruit flies were found either in bagged fruits or fruits after culling. As a comparison, 638 larvae and pupae of *B. dorsalis*, as well as 7 larvae of *B. minax*, were found in unbagged fruits before going through packinghouse culling.

However, precaution is needed in interpreting the outcome of this study. The effectiveness of using fruit cutting for detecting fruit flies inside fruits varies substantially, and is impacted by a number of factors, such as the size of the target insect stage, fruit type, etc. It is especially time consuming and challenging to detect eggs and first instars of tephritid fruit flies in large fruits such as pomelo. According to Gould (1995), the probability of detecting the larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), by fruit cutting ranged from 1 to 36%, whereas the probability of detecting infested fruits was from 17.0 to 83.5%. To improve the probability of detection in the bagging test of this study, suspected pomelo fruits were placed in a warm packinghouse (31 to 48 °C) for 8 d before cutting. However, there was no wait time before fruit cutting in the culling test. Finally, the number of fruits used in this study enabled a detection level of fruit infestation at 0.001 in the bagging test, and 0.0003 in the culling test, respectively. The required detection level by importing countries may differ substantially.

It was not surprising that fruit flies were not detected in the bagged fruits. Other studies had demonstrated that fruit bagging can provide 100% fruit pest control (Graaf 2010; Estradea 2004). This measure is particularly practical as a phytosanitary option for pomelo. The fruits are large in size and each tree bears few fruits, so bagging is more economical compared to smaller fruits. This is why bagging is already widely adopted in pomelo production in China (Chen 2015; Huang 2015). Substantial numbers of fruit fly-infested fruits were found in the unbagged fruits collected from the elevated groves. This suggests that fruit bagging also should be used in these groves.

The low fruit infestation in the culling test was unexpected. Our previous work in the county suggested that a typical average fruit infestation rate was 0.1 to 0.5% (Y. X., unpublished data). In other words, we expected at least 5 out of every 10,000 fruits to be infested before culling. Three reasons might explain the observed low infestations. First, the intensive cullings before and inside the packinghouse might significantly eliminate more infested fruits than we expected. A total of 5 cullings, i.e., 2 cullings outside of the packinghouse, plus 3 cullings in the packing line, were conducted (Fig. 1). The first culling occurred at fruit harvest in the field. The packinghouse, also the largest citrus exporter in China, enforced the harvesting of only healthy fruits by growers. The harvested fruits were then shipped to a local purchase station where 3 to 4 packinghouse graders went through fruits individually, handpicking the suspected infested and low-grade fruits (Fig. 2). This was the second culling. Three more intensive cullings were carried out in the packing line (Fig. 1). The final culling might be particularly effective in finding the infested fruits, because each fruit was inspected before being placed into the packing box (Fig. 3). Due to time constraints in the culling test, fruit cutting was conducted immediately in the first few days after harvest. This might result in less detection of infested fruits and fruit flies inside fruits, especially in those newly infested fruits. Finally, because the citrus industry is vital to the local economy in areas where this test was conducted, area-wide pest management, mainly by pesticide spraying and collecting the fallen fruits, has been carried out in the past several years. This results in a sustained reduction in the field population of fruit flies.

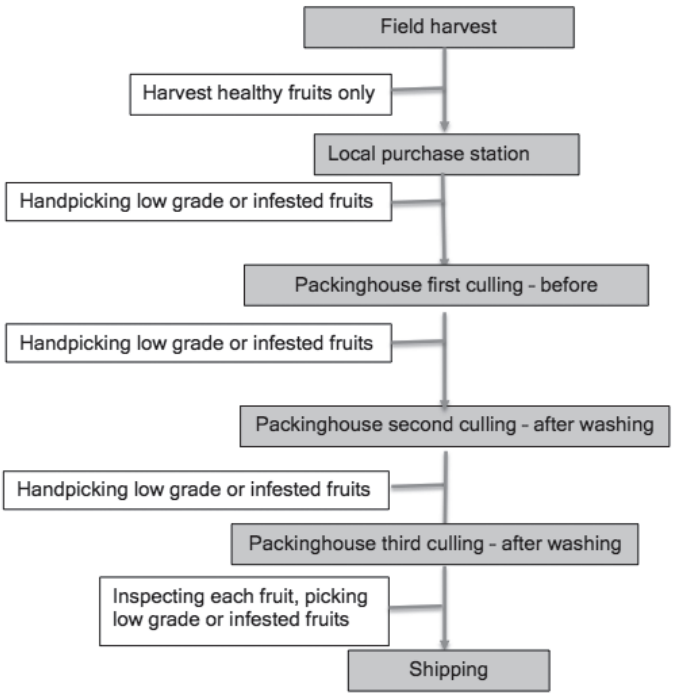

Fig. 1. Five culling procedures for quality control and risk mitigation of fruit flies in the county where this test was conducted.

Fig. 2. Culling at local purchase station.

Fig. 3. Final packinghouse culling.

This reported work is just a beginning attempt to explore a systems approach for risk mitigation of fruit flies for citrus exports from China. A systems approach requires the use of 2 or more independent measures to achieve the goal of mitigation (FAO 2012). However, the 2 measures studied here, i.e., packinghouse culling and field fruit bagging, were effective for 2 different commodities, and under different production systems. To develop a systems approach for each of the commodities, more quantitative information regarding the efficacies of other risk mitigation measures along the pathway are needed. For Satsuma mandarins or other mandarin fruits, for example, what are the field practices that constitute a good pest management program? And what is the efficacy of this program, as well as the efficacies of other measures before the packinghouse in reducing the risk? For pomelos, what is the efficacy of shrink-wrapping for risk mitigation of fruit flies? Shrink-wrapping has been demonstrated as an effective measure for risk mitigation of fruit flies in other fresh fruits (Gould & Sharp 1990). Almost all pomelo fruits were shrink-wrapped in packinghouses in China. A combination of this packinghouse measure with the pre-harvest measure of fruit bagging can be a practical and economical systems approach for the pomelo trade.

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