Risk for introducing *Anastrepha fraterculus* and *Ceratitis capitata* in lemons from Northwestern Argentina

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Key words: Quarantine restriction, fruit flies, risk analysis.

Chapter X

INTRODUCTION

Tucumán exports around 300,000 tn of fresh lemon (*Citrus limon* (L.) Burm. f.) fruits per year, 87% of the national exports. The harvest season for the export market is from April to the end of September (fall and winter south hemisphere). To be able to reach overseas markets, some of them very distant, and fulfil consumer demands, quality and characteristics of the lemon fruit have to be outstanding.

Based on the results obtained in the last years, detailed in he previous chapters, we will discuss the risk of introducing *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) in lemons exported from Northwestern Region of Argentina. This analysis is performed considering: the host status for both fruit flies, the natural occurrence of their adult populations in the field during the export season, and the characteristics of the lemons fruits.

BACKGROUND

Lemons have been considered a Medfly hosts by Japan and China, based on the papers by Quayle (1938), Liquido *et al.* (1990) and other. Recently, the United States incorporated lemon as a Medfly host (PPQ-APHIS, Treatment Manual, 2007) based on the interception made by APHIS on Verna lemons from Spain infested with Medflies in 2006.

Host status for fruit flies for quarantine purposes was defined by Armstrong (1986) as "any fruit or vegetable in which fruit flies can oviposit under field conditions, the eggs hatch into larvae, and the larvae adquire sufficient substance to form viable pupae from which adults eclode and are capable of reproduction".

Cowley *et al.* (1992) established the experimental procedures to determine the host status for multivoltine fruit fly species. Cage experiments in the laboratory with punctured fruits exposed to gravid females are the basis to establish the host status, being a non host if no adult flies develop, and if development is achieved, the commodity can be a fruit fly host and further research has to be done. Fruit collection (at least 1,000 fully ripe fruits per region), trapping (traps with appropiate lures each 400 m) and field cage trials (fruits hanging from the tree caged with gravid females) would define if the commodity is a host or not for quarantine purposes. Follett and Neven (2006) stated that the confidence level on which Cowley *et al.*

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(1992) based their evaluation for establishing the non host status was as low as 22.1% with 0 survivors out of 30,000.

The Asia and Pacific Plant Protection Commission (APPPC) published in 2005 the Regional Standards for Phytosanitary Measures (RSPM) No. 4, guidelines for the confirmation of non-host status of fruit and vegetables to tephritid fruit flies, which is based in the same procedures as in Cowley *et al*. (1992), using laboratory trials with punctured and unpunctured fruit and field trials with unpunctured fruit caged with gravid females. The sequence states that punctured fruit has to be tested in the laboratory, and if shows no signs of infestation is considered non-hosts, and if sign of infestation are shown, unpunctured fruits have to be tested in laboratoty and field trials, and if no infestation occurs, the commodity is considered a conditional non host, and if they become infested, they are potential host.

In 2007, Follett and Hennessey reviewed and discussed the confidence limits and sample size for determining non host status of fruits and vegetables to tephritids as a quarantine measure. They conclude that "researchers should conduct infestability studies under defined conditions and with a sufficient number of fruits and insects to convincingly determine the host status of a commodity" and that quantitative methods should be used to establish the efficacy that will give consistency to the data. They state that the confidence levels estimated for a 99.99% or a 99.9968% level (Probit 9) efficacy should be calculated using the equation provided by Couey and Chew (1996): $C = 1 - (1-p)^n$ where p is the acceptable level of survivorship and n is the number of individuals treated. According to these authors, an ideal host study would include laboratory, field cage and natural infestation experiments.

Considering that lemons in very special conditions can be a fruit fly host, we will try to establish the host status and risk of introduction of fruit flies in lemon fruits export from Northwest of Argentina.

The aim of the present work was to evaluate the risk of introduction of *A. fraterculus* and *C. capitata* in lemons from Northwestern Argentina.

Anastrepha fraterculus

Host status

In order to establish the host status and assess lemon fruit as a path way for the entry of this pest into an area, Cowley *et al.* (1992) and the RSPM Nº 4 of the APPPC (2005) guidelines were followed, and the efficacy and confidence levels of the research were analyzed.

A. fraterculus, a fruit fly distributed in the Americas from the south of USA to Argentina, has not been reported attacking lemons.

Laboratory trials with punctured fruits

The first decisive test to establish host status is to infest punctured fruit in the laboratory. After performing 13 forced infestation trials in the laboratory using 910 mature lemons artificially punctured, no *A. fraterculus* development was obtained (see chapter IX). According to the guidelines established by RSPM Nº 4 APPPC, lemon is a non-host of *A. fraterculus*.

Resistance trials in the field and in the laboratory were made in order to have an additional assurance of lemons being non-hosts of *A. fraterculus*. After 33 field forced infestation trials in the field exposing 2,310 unpunctured mature lemons in the trees to 11,550 gravid *A. fraterculus* females, no live or dead larvae or pupae were found in the fruits. After 33 laboratory forced infestation trials exposing 6,880 lemons with 2, 4 and 6 storage days to 34,650 gravid *A. fraterculus* females, no live or dead larvae or pupae were found. According to the guidelines established by RSPM Nº 4 APPPC, these results strengthen the fact that lemon is a non-host of *A. fraterculus*.

According to Follett and Hennessey (2007), the efficacy data for the resistance trials was calculated:

a) if we consider the quantity of insects used in the trails, we can expect after exposing 9,190 fruits to 46,200 *A. fraterculus* females, with no survivors, following to have a 99.01% confidence that resistance is 99.99% effective, and 77.20% confidence that resistance is 99.9968% effective; and b) if we consider the quantity of eggs, considering an estimated 511,803 eggs laid by 46,200 *A. fraterculus* females in 9,190 fruits, we have 100% confidence that resistance is 99.99% effective, and 99.9999% confidence that resistance is 99.9968% effective.

Cowley *et al*. (1992) mention fruit collection as another step in the experimental path way designed to determine host status. Following this guidelines, founding no *A. fraterculus* infestation of 102,700 lemon fruits (from the ground and from the plants, both from the field, and fruit for discard from

paking houses). According Follett and Hennessey (2007) we have 99.99% confidence that resistance is 99.99% effective, and have 96.26% confidence that resistance is 99.9968% effective. Also 403,220 lemon fruits (fruit for export) from the packing houses were sampled for 3 years founding no *A. fraterculus* infestation. Following Follett and Hennessey (2007) we have 100% confidence that resistance is 99.99% effective, and have 99.99% confidence that resistance is 99.9968% effective.

CONCLUDING REMARKS

Considering that artificial and natural infestation do not occur under laboratory and field condition, and that there are no records of lemon as a fruit fly host of *A. fraterculus*, we can conclude that lemon is a non-host for *A. fraterculus*, and hence lemons are not pathway for entry of this pest.

Ceratitis capitata

Host status

In order to establish the host status and assess lemon fruit as a path way for the entry of this pest into an area, Cowley *et al*. (1992) and the APPPC RSPM Nº 4 (2005) guidelines were followed, and the efficacy and confidence levels of the research were analyzed.

Host reports

C. capitata an introduced fruit fly in the Americas, has been reported attacking lemons under very special circunstances. Lemon status as a host for *C. capitata* is an example of controversy among different phytosanitary organizations. For example, El Comité de Sanidad Vegetal del Cono Sur (COSAVE), do not consider it as a host while Japan does. Lemons fruits with larvae were reported by Quayle (1938) in packing houses in Sicily, by Liquido (1990) in fruits collected from the ground in Hawaii, and more recently the interception made in the US of infested Verna lemons from Spain (APHIS, 2006).

Laboratory trials with punctured fruits

The first decisive test to establish host status is to infest damaged fruit in the laboratory. After 13 forced infestation trials in the laboratory, using 910 mature lemons artificially punctured, 122 *C. capitata* adults developed, 115 of which developed from lemons harvested in summer (see chapter IX). According to the guidelines established by RSPM Nº 4 APPPC, lemon can be a potential host or a conditional non-host, depending on the results of the other test.

Chemical resistance

The chemical composition of the lemon peel is a barrier to fruit fly development. Citral, linalool and cumarines are the main components of the essential oil present in the lemon peel that are responsible for the death of the fruit fly larvae and eventually of eggs (Salvatore, 2004). In coincidence, Back and Perbenton (1915) and Greany *et al*. (1985) suggested that the components in the essential oil glands were one of the mechanisms responsible for lemon resistance. These components correspond to the volatile part of the essential oil, reason why their presence in the lemon peel diminishes as time elapses after harvest (Salvatore, 2004). Back and Perbenton (1915), Greany *et al*. (1985), and Spitler *et al*. (1984) considered also that mechanical resistance is also present (encapsulation, hardening of tissues around oviposition sites).

Having in consideration these resistance mechanisms, the possibility could be achieved only if the fruit is overipe or if it has many storage days as the components responsible for the resistance as it has been proved by Salvatore (2004). The other possibility of infestation is damaged fruit, as *C. capitata* likes to puts their eggs in holes, very different from *A. fraterculus*, that needs some kind of resistance to oviposit.

Laboratory cage trials with unpunctured fruits

After 33 laboratory forced infestation trials, exposing 6,880 lemons with 2, 4 and 6 storage days to 34,650 gravid *C. capitata* females, no live or dead larvae or pupae were found. According to the guidelines established by RSPM Nº 4 APPPC these results show that lemon is a conditional non-host of *C. capitata*.

Field cage trials with unpunctured fruits

After 33 forced infestation trials were performed in the field exposing 2,310 unpunctured mature lemons in the trees to 11,550 gravid *C. capitata* females, no live or dead larvae or pupae were found in the fruits. According to the guidelines established by RSPM Nº 4 APPPC, these results show that lemon is a conditional non-host of *C. capitata*.

After exposing 9,190 fruits to 46,200 *C. capitata* females, with no survivors, following Follett and Hennessey (2007) we have 99.01% confidence that resistance is 99.99% effective, and 77.20% confidence that resistance is 99.9968% effective. Also, considering an estimated of 819,931 eggs laid by 46,200 *C. capitata* females in 9,190 fruits, we have 100% confidence that resistance is 99.99% effective, and have 100% confidence that resistance is 99.9968% effective.

Fruit sampling

After sampling 102,700 fruits from the field and from fruit for discard, no *C. capitata* infestation was found, and following Follett and Hennessey (2007) we have 99.99% confidence that resistance is 99.99% effective, and we have 96.26% confidence that resistance is 99.9968% effective.

Fruit sampling in packing houses

After sampling 403,220 fruits from the packing houses and not founding *C. capitata* infestation, we have 100% confidence that resistance is 99.99% effective, and have 99.99% confidence that resistance is 99.9968% effective following Follett and Hennessey (2007).

Considering that:

1) There are few international records of natural infestation of lemons by *C. capitata* and those only happened under very special condition.

2) Development of adults of *C. capitata* was only obtained under extreme laboratory conditions with artificially punctured fruit that do not represent the natural conditions of production.

3) The chemical components of the lemon peel offer resistance to the infestation of *C. capitata*. They start to decrease two weeks later after the lemon is harvested and the fruit become susceptibles to the infestation of the Medfly.

4) Field and laboratory forced infestation trials with unpunctured lemons of 8 storage days previous to the test do not show infestation.

5) Lemons sampling in the field and in packing houses show no infestation.

According to:

Cowley *et al*. (1992): lemons are a non-host of *C. capitata* because no infestation is achieved from fruit sampling and field cage trials.

APPPC (2005): lemons are conditional non-host because development is achieved with punctured fruits in the laboratory, but no development is achieved under field and laboratory forced infestation trials with unpunctured lemons.

Follett and Hennessey (2007): The risk is negligible as shown by the fruit sampling in the field and packing houses with an effectiveness for probit 9 level, with a confidence level of 99.95 and 99.99% respectively. The same is shown for the resistance trials, considering the number of fruit flies or the estimated number of eggs, achieving an effectiveness for a probit 9, with a confidence level of 99.01 and 100%, respectively.

CONCLUDING REMARKS

Considering all the facts presented above we can conclude that lemon fruit export from Northwestern Argentina, represent a negligible risk for introducing *C. capitata*.

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