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**Investigation note** 

## Efficiency of fumigation with 100% methyl bromide in grapefruit infested by *Anastrepha* spp.

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#### Abstract

The response of fruit fly (*Anastrepha* spp.) larvae to fumigation with 100% methyl bromide was evaluated in naturally infested grapefruits. The above was carried out at the end of the 2021 production cycle in the citrus-growing region of Nuevo León, Mexico; to this end, grapefruits (*Citrus paradisi*) infested with fruit flies were harvested. The collected fruits were divided into three batches; two were treated in a fumigation chamber with methyl bromide at 24 g m<sup>-3</sup> for a two-hour exposure and the other served as a control. After fumigation, one of the fumigated batches was refrigerated at 5 °C, while the other was kept at room temperature between 19 and 24 °C. The percentage of live larvae was evaluated 5, 24, 48 and 72 h after fumigation in 30 fruits from each batch. Eightynine to 94% of larvae stopped moving 72 h after fumigation. Using the exponential model fitted to the data, it was estimated that all larvae lose their ability to move 120-144 h after fumigation (5-6 days). Refrigeration increased the percentage of larvae with movement, but this effect was lost 48 h after fumigation. These results indicate that the effectiveness of fumigation on the host fruit of *Anastrepha* spp. with methyl bromide can be assessed with certainty 5-6 days after treatment.

#### **Kevwords:**

Anastrepha spp., fumigation, grapefruit, methyl bromide.



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#### Introduction

In Mexico, the fruit fly of the genus *Anastrepha* is a quarantine pest with a high economic and phytosanitary impact, as it affects various fruit crops, including citrus fruits. It represents a major problem in production, postharvest management, marketing, and export due to phytosanitary restrictions imposed at the national and international levels (Cesave, 2015; Senasica, 2016).

Methyl bromide is considered an ozone-depleting substance, which is why its use is restricted under the guidelines established in the 1987 Montreal Protocol. However, exemptions have been granted for specific phytosanitary applications, particularly in quarantine and pre-shipment treatments, where viable alternatives with equal efficiency do not exist (Heather and Hallman, 2008).

Postharvest fumigation with methyl bromide is one of the most widely used phytosanitary strategies for the quarantine treatment of fruits with the presence of *Anastrepha* spp., such as citrus fruits, mangoes and other fruits. This management is essential to ensure the transportation of these products from controlled areas to free or low-prevalence areas (APHIS, 2010; NOM-075-FITO-1997). This treatment reduced the risk of spreading quarantine pests from controlled areas to free areas, in accordance with international biosafety guidelines (UNEP, 2010).

In addition, its application is widely recognized in the export processes of fruit and vegetable products, as it is regulated by bilateral work plans signed between Mexico and importing countries, as well as by the phytosanitary requirements established by the health authorities of these countries (Senasica, 2020). In Mexico, reports have been made of live larvae being detected in fruit shipments that were fumigated with methyl bromide, applied according to the concentrations and exposure times specified in NOM-075-FITO-1997 or in accordance with import requirements. This situation compromises the efficiency of the treatment, generates rejections of the fruit, and implies sanctions (temporary suspension) for the entities responsible for the phytosanitary certification of previously treated shipments.

Usually, the treated fruit is immediately transferred to refrigerated transport units (refrigerated trailer boxes), which allows its arrival at the destination point within a period of hours to a maximum of 1 to 2 days after fumigation. The immediate transport of the fumigated fruit favors the preservation of its organoleptic and commercial quality; this practice has implications for the biological efficiency of the treatment. The decrease in temperature in the trailer boxes can induce a reduction in the insect's metabolism and therefore delay or even inhibit the lethal action of the fumigant. Consequently, the expected effect of fumigation (100% death of the pest) may not be achieved before phytosanitary inspection at entry points to free or low-prevalence areas.

The objective was to develop a model to evaluate the efficiency of methyl bromide application at a concentration of 24 g m<sup>-3</sup> on the control of fruit fly larvae and its effect under refrigeration conditions.

#### Materials and methods

#### **Experiment location**

The treatment was carried out in the fumigation chamber of the company Distribuidora de Naranjas Tamez, SA de CV, located in the municipality of Allende, Nuevo León, Mexico, at coordinates 25° 16' 56" north latitude and 100° 01' 44" west longitude.

## Obtaining the biological material

At the end of the 2021 grapefruit production cycle, in the municipality of General Terán, 360 grapefruits infested by fruit flies were collected in two farms with conventional management. The farms are located between coordinates 25° 14′ 19″ north latitude and 99° 44′ 58″ west longitude. The harvested fruits were placed in plastic boxes and transferred to the fumigation chamber. The farms and the fumigation chamber belong to the same phytosanitary status (low prevalence zone).



## Methyl bromide fumigation

A total of 240 grapefruit fruits were fumigated following the procedure established in the Manual of Phytosanitary Treatments (Senasica, 2016). The dose of methyl bromide applied to the fumigation chamber was 24 g m<sup>-3</sup>, with an exposure time of 2 h. After application, the chamber was ventilated for 30 min. A batch of 120 unfumigated fruits served as a control in the trial (between 19 and 24 °C).

## Evaluation of the effectiveness of 100% methyl bromide

The fumigated fruit was divided into 2 batches, each containing 120 grapefruits. One of the fumigated batches was refrigerated at 5 °C, and the other two (one treated and the control) were kept at room temperature (between 19 and 24 °C). Subsequently, 30 fruits were taken from each batch (2 fumigated and the control) at 5, 24, 48 and 72 h after fumigation. The fruit was examined in accordance with the procedure established in NOM-075-FITO-1997. Each grapefruit was sliced into thin parts 1 cm thick, all larvae were extracted, and live and dead were counted, considering those that showed movement to be alive (movement was observed for one minute).

The percentage of live larvae was obtained with the following equation:

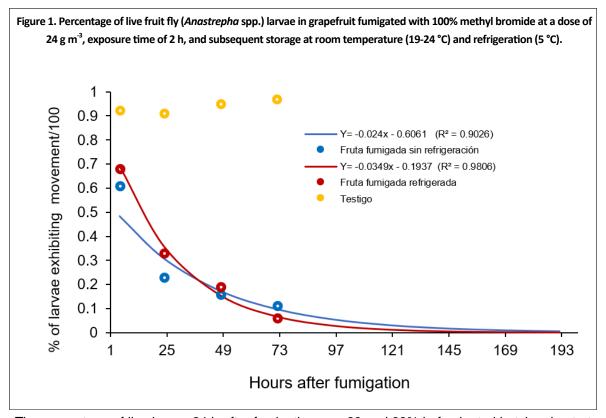
$$\% Larvae = \frac{Larvae*100}{Total number of larvae in the batch}$$

The data on the percentage of live larvae (all stages) in treated grapefruit, stored at refrigeration and at room temperature, were plotted against days hours of fumigation and statistically processed to select the model that best described the relationship between these two variables. The models of the percentage of larvae with movement evaluated were the Exponential, Power Law and Berger (Campbell and Madden, 1990).

### **Results and discussion**

The percentage of live larvae removed from fumigated grapefruits and kept in refrigeration or at room temperature decreased with increasing time after treatment application. In the unfumigated fruit (control), the percentage of live larvae remained between 92 and 98% (Figure 1).





The percentage of live larvae 24 h after fumigation was 23 and 33% in fumigated batches kept at room temperature and refrigerated, respectively. In the case of fruits with 72 h after fumigation, 6 and 11% of live larvae were found when the fruit was kept refrigerated and at room temperature, respectively (Figure 1). Refrigeration of the fumigated fruit increases the percentage of live larvae during the first 48 h; however, this effect does not persist 72 h after treatment.

The model that best described the relationship between the percentage of movement and the time after fumigation was the Exponential one.  $R^2$  of 0.9 and 0.98 for the case of the fruit batch at room temperature and refrigerated, respectively. With this model, it was projected that the time required to kill 99% of larvae is achieved 120 and 144 h after fumigation, depending on the storage temperature after fumigation (Figure 1), results similar to those of Hallman and Thomas (2011), who reported that, when the fruit was kept at room temperature, movement was observed in 0.23% ( $\pm$ 0.12%) of larvae, four days after fumigation.

The effect of fumigation on fruit fly larvae is gradual and is not complete at the time of opening the fumigation chamber, but it takes days to inactivate 100% of the larvae. Larvae exposed to 100% methyl bromide at the dose and exposure times specified in NOM-075-FITO-1997 may show movement 3 or 4 days after fumigation, even when the fumigation was done in accordance with the treatment manual.

#### **Conclusions**

The phytosanitary measures established in NOM-075-FITO-1997, such as rejecting shipments of fumigated fruit due to the detection of larvae exhibiting movement, should be adjusted considering that both this trial and other studies have shown that mobility is gradually lost.

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