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The Strategy of Organic Pest Control in Ecuador: Capture Effectiveness of Fruit Fly (*Anastrepha*) Species in Orange Tree Regulated by Volatile Alimentary Attractants

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ABSTRACT

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The fruit fly (Diptera: Tephritoidea) is a pest with high phytosanitary impacts and economic importance worldwide. In Ecuador, fruit fly causes significant losses affecting more than 260 different fruit types. This study was aimed to estimate fruit fly capture effectiveness in orange tree regulated by volatile alimentary attractants to propose an alternative biological control. The experimental unit constituted by a Multilure trap (McPhail plastic wet version) for each vegetable attractant. Five volatile alimentary attractants more one control were compared. Thirty-five fruit fly individuals were captured, corresponding to the species Anastrepha obliqua, A. fraterculus, A. striata, and A. leptozona. The most abundant species was A. obliqua with 14 individuals. The most effective treatment was guava juice covering 40 % of the total captured flies. Maximum MTD rate (flies/trap/day) was not significant, considering it as a low catch rate plague. The results indicated the possibility of using this organic control method for fruit fly. It can become one of the technologies as an environmentally friendly method and in favour of farmers' health.

INTRODUCTION

Orange is among the first staple foods for humans. It is the most representative and recognized fruit within *Citrus* genus as one of the most essential Citrus locally, regionally and globally. A part of its production is consumed as fresh fruit, and another part is processed by agroindustry, whose product depends on the variety. As a global view, more than 50 % of the production of the orange crop is planted in tropical and subtropical regions. *Citrus sinensis*

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mainly found in tropical and sub-tropical countries is the most widely used orange species for production of cultivars (Tarig, Sharif, Shah, & Khan, 2007). The leading orange producer worldwide is Asia (32.6 Mt) followed by South America (25.8 Mt) and Brazil (19.9 Mt). On the contrary, Ecuador sits at 24th in the world ranking of orange production. There are many genetic materials available, including genotypes ranging from local breeds to improved domesticated and modern varieties. Many orange varieties serve only for local consumption because their production is low, but they contribute the basic elements in the daily nutrition (Ngadze, Linnemann, Nyanga, Fogliano, & Verkerk, 2017). Orange is one of the fruits with a high content of fibre, potassium, calcium, magnesium and vitamin C.

Throughout history, the potential characteristics to improve plant production or attributes have been exploited based on local knowledge and technical criteria originating from scientific research (Segura Chávez, Coronel Espinoza, Heredia Rengifo, Landines Vera, & Muñoz Rengifo, 2017) ranging from nursery and plantation management (César Muñoz et al., 2014), different irrigation strategies, fertilization and so on. Plants that respond efficiently to adverse weather conditions avoid the increasing of the pests' population in tropical cultivars (Martínez et al., 2017). This last element (attacks of phytophagous insects) is one of the most important factors considered by entomologists and breeders in their research (Villamar, Liu-Ba, Legavre, & Viot, 2016). Fruit fly, as one of the sucking insects, has a significant presence in temperate, tropical, and sub-tropical regions. It is seen in South American countries like Brazil, Peru and Ecuador (Valarezo Beltrón, Valarezo Cely, Andrade Varela, & Valarezo Beltrón, 2015). Also, fruit fly is one of the pests of great phytosanitary and economic importance worldwide for producers and exporters of a wide variety of vegetable and fruit crops. As it directly causes the loss of fruit, it compromises production, investment and profits, generating a reduction of the crop value, gains and interest on its plantation (Carroll et al., 2002 onwards).

Since 1990, knowledge of host species of these dipterans has been deepened. Currently, it is present in the Litoral and Interandina regions and some specific sites of the Amazon and Galápagos Region. In Ecuador, more than 260 different types of fruit trees have been identified, as well as wild plants, flowers and vegetables as the host of fruit fly (Porras & Lecuona, 2008). Fifty-six vegetable species are distributed among 23 botanical families; of which, the most important are *Rutaceae*, *Myrtaceae* and *Sapotaceae* with six species each. The most important plant species are *Psidium guajava*, *Annona cherimola* and *Pouteria lucuma* hosting 7, 6 and 6 species of fruit flies, respectively (Tigrero S, 2009).

The damage caused by these flies occurs when the adult female, through her ovipositor, penetrates and deposits her eggs inside of the fruit. The larvae dig inside the fruit, exposing it to the penetration of microorganisms that deteriorate its quality. This first condition can cause an early maturation of the fruit. If the attack happens in the early stages, the fruits can be compromised, preventing adequate development (Porras & Lecuona, 2008). In such case, the fruits do not reach to the commercialisation basic parameters; they are unacceptable for direct consumption or subsequent processes and agro-industrial use, causing economic losses and guarantine restrictions.

For the agroecological management of the fruit fly, attractive traps are commonly used. These serve to detect the presence of species and populations, monitor, or the massive capture of these insects (Valarezo Beltrón, Valarezo Cely, Andrade Varela, & Valarezo Beltrón, 2015). However, the success of the catches depends on the nature of the attractant and the fly species (OIEA, 2005). The most current version in traps is McPhail, it is composed of two pieces of plastic and allows working with liquid or dry attractant (ICA, 2017). The transparent upper part of this trap contrasts with the yellow base, which increases its capture capacity. In traps, an attractant element is placed with colouration, food, pheromone, para-pheromone and other possible factors of attraction. Vegetable attractants are rarely nutritious substances, are substances related to the decomposition or fermentation of food or those that produce similar responses without an apparent chemical relationship with food. They are based on the specific needs of insects at different stages of their lives. In nature, attractant products are used for insects that are chemo-statically oriented to find their food. Classically, the attack of such insects is diminished by chemical methods. Chemical methods, although effective, involve risks, such as residual toxicity in fruits, environmental contamination and reduction of the associated beneficial insect fauna (Valarezo Beltrón, Valarezo Cely, Andrade Varela,

& Valarezo Beltrón, 2015). A method very used for pest biological control for several crops are the pheromones, which are substances emitted by one individual of a species and eliciting a specific response in a second individual of the same species. They can be used for direct population suppression when the traps are deployed at a density effective in reducing mating success sufficiently to achieve control (Carde, 1976). However, its utilization does not leave to be expensive and usually difficult to acquire them from small farmers at its high cost, mainly in under-developing countries. Therefore, an alternative is the volatile alimentary attractants, being easy to acquire and elaborated on them. They allow the capture of fruit fly and identification of the species, which permit to establish control strategies with less impact on the environment. Regarding the mentioned factors, the following questions raise: Can fruit fly adults be captured in case of using volatile alimentary attractants based on natural byproducts? Could such volatile alimentary attractants exceed the number of captures than the synthetic attractant most used in controlling fruit flies? To answer these questions, this research was aimed 1) to determine the effectiveness of different volatile alimentary attractants for the capture of fruit flies; 2) to identify the fruit fly species captured with the volatile alimentary attractants and 3) to evaluate the population density of fruit flies in the study period.

MATERIALS AND METHODS

The present research was carried out during the summer of 2015 between the months from July to September, in a 6-year old orange plantations of "Valencia" variety, as one of the most cultivated in Ecuador, in the "San Cristóbal" farm, located in the Balsería Campus, belonging to the canton Quinsaloma, in the province of Los Ríos. This farm is located at the geographic coordinates 1° 12' 22.32" S and 79° 18' 52.2" W, at mean altitude of 30 m asl. The climate is a humid tropical forest. The temperature varies between 22 and 28 °C. The average annual rainfall reaches 2000 mm and the relative humidity of 60 to 90 %. The sunlight is approximately 800 hours per year. The soil is of irregular topography with low slope, loamy-clay texture, with a pH of 6.3-7.2 and regular drainage.

Constitution of Treatments

We used 5 types of volatile alimentary attractants and one control including T1) Guava

juice (*Psidium guajava*), T2) Molasses + Urea, T3) Orange juice (Citrus sinensis), T4) Carambola juice (Averrhoa carambola, T5) Araza juice (Eugenia stipitata) and T6) Hydrolyzed protein (control). The final volume was 250 ml for each preparation. Treatments 1, 4 and 5 were composed of 125 g of fresh fruit (50 % of the final volume), 123 ml of water (47 %) and 3 % of Borax (7.5 g). For treatment 3, 243 ml of orange juice and 3 % of Boric Acid (7.5 g) were used without adding water (Delmi, Morán, Núñez, & Granados, 1996). For treatment 2, the proportion was 5 % molasses (12.5 ml) + 20 % Urea (50 g) + 1 % boric acid (2.5 g) + 74 % water (185 ml) (Asaguibay, Núñez, & Gallegos, 2010). Treatment 6, Hydrolized protein was prepared with a solution consisting of 10 % hydrolyzed protein (25 ml) + 3 % boric acid (7.5 g) and 87 % water (218 ml) (Vilatuña R., Sandoval L., & Tigrero S., 2010). Borax was added 3 % of the final volume to all treatments, as a preservative to prevent decomposition of the captured flies and distortion of the protein smell (CIPF, 2006; OIEA, 2005; Ros, Wong, & Castillo, 2001.

Multilure Trap

To capture fruit fly adults, the "Multilure" trap, also called "McPhail plastic wet version", was used (Ríos, Toledo, & Mota-Sanchez, 2005). In each trap, 250 ml of each of the attractants were poured. The traps were cylindrical, the upper part was transparent and the lower yellow, at the base it had an opening through which the attracted flies entered (Fig. 1 A). The traps were placed at 3/4 of the height of the tree on a sturdy branch under shade, not surrounded by very dense foliage (Fig. 1 B).

Field Experiment Setup and Statistical Analysis

Trees were planted at 6 m x 7 m in an area of 5000 m². A density of 50 traps per ha was used. Attractants of the traps were changed every 7-days. Individuals collected in the traps were placed in containers with 70 % alcohol, which were labelled and sent to the AGROCALIDAD Entomology Laboratory of the Ministry of Agriculture and Livestock of Ecuador (MAG). A Completely Random Design (CRD) experiment was considered with three repetitions, where a Multilure trap constituted the experimental unit for each attractor. To fulfil normality and homogeneity requested for the analysis of variance (ANOVA), one transformation (ANOVA assumptions) of the data for the variable number of captured fruit flies was performed. The means were separated using Duncan's multiple range test. Mean differences were considered significant at values P < 0.05 level. Confidence limit was 95 %. Statistical software Infostat was used (Di Rienzo et al., 2011).





Fig. 1. McPhail trap (A) and location on the branches on the top of orange tree (B)

Number of Flies Per Trap Per Day (MTD)

The number of fruit flies captured in each of the traps was recorded. A taxonomic diagnosis was made at family, gender and/or species level, counting the number of each species (Faria et al., 2014). Calculation of MTD value was performed through division of the total number of caught flies (M) for the product obtained between from the multiplication of the total number of used traps (T) by the number of days that the traps were exposed (D) (Delmi, Morán, Núñez, & Granados, 1996; OIEA, 2005) as following formula:

RESULTS AND DISCUSSION

Number of Captured Fruit Flies

The greater attraction effect of fruit flies was observed with the natural attractant Guava Juice, concerning the other attractants (P < 0.05, Table 1). With the natural attractant orange juice and the commercial attractant hydrolyzed protein a lower percentage of fruit flies was captured (20 % and 40 % respectively, Table 1). Our results are in concordance with what described by others (Cuevas Salgado, Romero Nápoles, & Carrillo Cruz, 2011). They observed that a natural attractant made from ripe guava fruits showed effectiveness similarity with a commercial attractant (hydrolyzed protein) for the attraction of a Tephritidae species (A. ludens). Similar results to that were observed by Delmi, Morán, Núñez, & Granados (1996). They evaluated different attractants (Urea, orange juice, ammonium sulfate, hydrolyzed protein and MOX (protein, salt, sodium tetraborate, socenose) and observed a higher percentage (62.4 %) of capture of fruit flies by orange juice. This superiority of fruit fly captured by guava juice is a food-type attraction due to the aromatic substances and sweetness of the attractant. It is in accordance with the observation by Díaz-Fleischer & Castrejón-Gómez (2012) who reported that certain insects perceive part of their environment by volatile and non-volatile chemical substances emitted by their hosts (fruits), which acts as the signals for the location of resources (couple, hosts, food). Coincidingly, it was described by Escudero Colomar (2016) who released 54,000 flies to test the dispersive capacity with the Jackson and McPhail traps, Trimedlure attractant and Torula protein and managed to recapture only 2.2 % of the

released flies. The authors explained this low rate recapturing phenomenon as the effect of wind, the surrounding vegetation, land topography and the presence of fruits those determine the population dynamics. In this regard, Nishida, Shelly, Whittier, & Kaneshiro (2000) pointed out that the attraction of males of Ceratitis capitata (Diptera: Tephritidae) to orange, mango and guava fruits occurs due to α -copaene, a minor component of essential oils. This same substance is identified by Vera et al. (2013), as the major mating promoter of A. fraterculus after exposure to guava and lemon pulp (Citrus aurantifolia)

Table 1. Fruit flies captured (average) by differentalimentary volatile attractants

| Treatments | Averages of flies captured | SD |
|------------------|-------------------------------|----|
| T1 | 5.0 | а |
| Т3 | 4.0 | b |
| Т6 | 3.0 | с |
| T4 | 1.0 | d |
| Т5 | 0.0 | d |
| T2 | 0.0 | d |
| Mean | 1.9 | |
| C. variation (%) | 21.5 | |

Remarks : T1= Guava juice (*Psidium guajava*); T2= Molasses + Urea; T3= Orange juice (*Citrus sinensis*); T4= starfruit (*Averrhoa carambola*); T5 = Araza pulp (*Eugenia stipitata*); T6 = Hydrolyzed protein (control). Different letters show statistical significance p < 0.05; equal letters have no statistical significance p > 0.05 (ns), according to the Duncan test at 95 % probability

Fruit Fly Species

Four species of fruit fly were captured, all belonging to the genus Anastrepha. The specimens of highest independently capture of the attractant correspond to A. obliqua by 40 % (14, Table 2). Specimens of A. fraterculus and A. striata were collected at a lower rate (22.9 % and 20 %) than A. obliqua. Our results reflect a low presence of fruit fly species concerning the 11 Anastrepha species recorded in another study (Manuel et al., 2012) carried out seven years before between the same months were our investigation was performed (July and September) in South America. Presence of hosts and the climatic conditions of the area may generate

more or less presence of these populations during the year, especially in regions with more defined seasons (Aluja, 1994). On the other hand, Nolasco & lannacone (2008) reported 3506 captured flies distributed in 7 different species using McPhail traps with attractant hydrolyzed protein, borax and water. Interestingly, among the species captured in our study are Anstrepha fraterculus and A. striata. These flies have a high economic and quarantine importance in Ecuador, due to the damage they cause and the significant presence in horticultural crops located from sea level to 2500 m (Tigrero S, 2009).

Table 2. Species and their pertinent numbercaptured in the study

| Species captured | Averages for each treatment |
|------------------------|--------------------------------|
| Anastrepha obliqua | 14 |
| Anastrepha fraterculus | 8 |
| Anastrepha. striata | 7 |
| Anastrepha leptozona | 6 |
| Total | 35 |

Species Captured by Volatile Alimentary Attractants

Four species of fruit fly were captured, all Attractants based on guava and orange juice appealed more species (4 Spp.; Fig. 2), followed by hydrolyzed protein and starfruit juice (3 and 1 Spp.; respectively, Fig. 2). In the others, no catches were observed. Our results are coherent with those described by Delmi, Morán, Núñez, & Granados (1996), who observed a higher rate of flies trapped with orange juice. This could be because of the colour of the attractant and the characteristic smell, stimulating food for these diptera. These advances ratify the importance of natural attractants. This number of species captured by attractant with a wide range of hosts suggests that the capture of their specimens has no direct relationship with the tree where the traps were placed, but it may be mediated by the volatiles emitted from the attractors under study. In this regard, Díaz-Fleischer & Castrejón-Gómez (2012) pointed out that the volatile components of fruits generate a greater response in polyphagous insects than in oligophagous insects.



Fig. 2. The number of species captured in the different natural food attractants



Fig. 3. Variation of the population density of fruit flies (Anastrepha spp.) considering the MTD during the study period

Number of Flies Per Trap Per Day (MTD) Rate

During the first four weeks, the MTD rate had the same behaviour in numerical terms (0.032 MTD). After the fourth week, an increase of 42.9 % was registered, reaching the maximum values in week five and six (0.056, Fig. 3). In the coming weeks, MTD remained below 0.024. It is in agreement with that described by Tucuch-Cauich, Chi-que, & Orona-Castro (2008), who observed the maximum values of the MTD index (0.050) for orange at the early summer and its reduction in the coming weeks. In parallel, De Los Santos-Ramos, Bello-Rivera, Hernández-Pérez, & Leal-García (2012) observed ranges of the MTD Index from 0.71 to 4.71 using food attractant (MS2-Cera Trap). Delmi, Morán, Núñez, & Granados (1996) reported that by applying orange juice, the MTD index reached 20.4. According to Toledo, Paxtián, Oropeza, Flores, & Liedo (2005) the fermentation of the attractant has a relationship with the population dynamics. According to the results of Ríos, Toledo, & Mota-Sanchez (2005), among the tested attractants the fermented treatment of pineapple peel on the second day caused the highest number of catches with a total of 136 flies per trap. In this regard according to Bateman (1972) and Tucuch-Cauich, Chi-que, &

Orona-Castro (2008) the environmental conditions could be related to fruit fly dynamics. However, according to what was described by Vanoye-Eligio, Pérez-Castañeda, Gaona-García, Lara-Villalón, & Barrientos Lozano (2015) in approximately three years there was no relationship between the population dynamics of fruit flies and environmental conditions. Regarding the MTD index levels, León M. (2007) mentioned that the population of the pest does not cause higher than 5 % of damaged fruits while MTD index was 0.080 or less. Additionally, Cruz Fernández (2003) stated that phytosanitary control measures must be applied when the MTD index is 0.080 or higher. Taking these criteria as a reference, it would not require quarantine or application of any chemical control for fruit flies on orange, because the attack of fruit flies presents low MTD levels.

CONCLUSION

Treatment T1 (volatile attractant based on guava), was the most effective in capturing fruit flies. The predominant species of fruit fly was *A. obliqua*. With the attractant juices of guava and orange, the highest capture of the four species of fruit flies was achieved. The hydrolyzed protein showed low efficacy than natural attractants. Although MTD index was low, concerning to the rate needed to control fruit flies, our outcomes permit us to suggest the possibility of using this organic mechanism for fruit fly control in an orange tree, avoiding synthetic pesticides as a hazard for farmers and environment.

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