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Non-target Insects Captured in McPhail Traps Baited with Proteinaceous and Salts in Citrus Crop

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Authors' contributions

This work was carried out in collaboration between both authors. Author LTG provided the collections and the laboratory activities. Author AR helped the field collections, designed the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Traps baited with food attractants are commonly used for monitoring or mass trapping of fruit flies in many crops. The use of attractive food has a limitation in terms of catching non-target organisms. Attractives used alone or in combination may reduce the capture of non-target insects. Therefore, the mass trapping system in fruit crops can potentially impact negatively on the environment.

Materials and Methods: A test was conducted in an orange orchard situated in the municipality of Mogi Mirim, state of São Paulo, Brazil. The following fruit fly lures were disposed into McPhail traps exposed to non-target insects for 55 days: 1) the liquid protein Cera trap®; 2) Cera trap® + Acetone; 3) Cera trap® + Ammonium phosphate dibasic (APD); 4) Cera trap® + Brazilian orange oilphase essence (BOE); 5) Cera trap® + Trimetylamine (TMA); 6) TMA; and 7) APD + BOE. Except Cera trap®, the other attractants were replaced weekly. Each treatment comprises five replications and each plot contained 35 orange trees. We compared effects of attractants and insects using 2-way ANOVA.

Results: A large number of *Diptera* specimens were obtained in all attractants, corresponding to

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98.12% of the total capture. The majority of flies belonged to the families Drosophilidae, Muscidae and Calliphoridae. The numbers of Hymenoptera, Hemiptera, Lepidoptera, Neuroptera and Coleoptera insects were similar for each attractant.

Conclusion: The findings of the present work showed no selectivity of Cera trap® even in combination with synthetic lures.

Keywords: Insecta; capture; trap; attractant; citrus.

1. INTRODUCTION

Tephritidae is amongst the largest and economically most important family of *Diptera*; it includes about 4,400 species, of which 200 are considered pests [1,2]. Fruit flies cause heavy annual losses of produce around the world and their occurrence impose quarantine restrictions in areas where fruit is growing for export.

Anastrepha is endemic to the New World and restricted to tropical and subtropical environments [3]. Almost 300 species of *Anastrepha* Schiner are known [4], of which 120 occur in Brazil [5]. The medfly *Ceratitis capitata* (Wied.) was detected in Brazil early in the 20th century [6] and is currently established all over the country.

Tephritid fruit flies are trapped for a variety of reasons – surveillance, suppression, and research among others [7]. The need to reduce pesticide use has led to the development of alternative control strategies such as mass trapping [8]. Such control tatics were effective in reducing the density of *Anastrepha* fruit flies in Mexican mango orchards, and their performance is not affected by the rainy season [9].

Protein acquisition by adults of *Tephritidae* is necessary to reach sexual maturation and influences related behaviour [9]. For almost a century, McPhail traps baited with an aqueous mixture of torula yeast or others proteins have been used for monitoring *Anastrepha* and *Ceratitis* genera [10]. Food lures are of conventional use for monitoring *Anastrepha* species, for which pheromone-based methods are not available commercially [11], and they are effective on both females and males.

Liquid proteins are more effective than sugar cane syrup and fruit juice and they are largely used for capturing *Anastrepha* spp. and *C. capitata* (Wied.) in McPhail traps in Brazil [12,13, 14,15]. Hydrolysed protein degrades rapidly and usually requires replacement at intervals of one or two weeks. This lure changes colour quickly and becomes putrefied in the field, attracting non-target insects and causing, the disintegration of target flies in the liquid [11]. Several food-based synthetic attractants have been developed using ammonia and its derivatives. This may reduce the number of non-target insects captured [16]. Ineffective and non-selective attractants provide an underestimation of tephritid populations and render the mass trapping system inviable.

Due to the information gap on the impact of non-target insects, in the present study we evaluate the capture of non-tephritids by McPhail traps baited with different lures used for monitoring or mass trapping systems against fruit flies in citrus orchard.

2. MATERIALS AND METHODS

The test was conducted in an eight year old orange, cv. Hamlin (*Citrus sinensis*) grafted onto ‘Swingle’ citrumelo; the site (22º23'51.29"S; 46º59'31.45"O; 634 meters) is situated in the municipality of Mogi Mirim, state of São Paulo, Brazil, a citrus-growing region. The regional climate is characterised as Cwa (humid subtropical) according to the Köppen classification system.

Plants were spaced 6.50 by 3.00 m and were approximately 3.00 m high. The total area of the orchard was 5.85 ha. The trees were managed using broad spectrum synthetic pesticides all year round: cypermethrin, dimethoate and chlorpyriphos (biweekly application), and abamectin and sulphur (monthly application). During the study, the trees were in fructification. The orchard was bordered by acid Tahiti lime (*C. latifolia*) trees and corn crop (Fig. 1).

Plastic yellow McPhail traps (BioControle Ltda.) were positioned within foliage of the canopy, at about 1.60 to 1.80 m high in the northwest quadrant from 02 March to 27 April 2017.

The following fruit fly lures were tested: 1) the liquid protein Cera trap® (Bioiberica Company); 2) Cera trap® + Acetone (LABSYNTH Ltda.,

Fig. 1. Map of study site of orange grove in Mogi Mirim, SP, Brazil

99.5%); 3) Cera trap® + Ammonium phosphate dibasic (APD; LABSYNTH Ltda., 98.0%); 4) Cera trap® + Brazilian orange oilphase essence (BOE; Citrosuco S.A.); 5) Cera trap® + Trimetylamine (TMA; Sigma - Aldrich, 98.0%); 6) TMA; and 7) APD + BOE. Acetone, APD, BOE and TMA were disposed into open 2mL Eppendorf tubes filled with cotton rolls (0.05g) and taped onto the underside of the top of the McPhail traps. We pipetted 1.5 mL of acetone, APD, BOE and TMA diluted in water at 99.5%, 66.0% (w/v), 5.00% (v/v) and 3.33% (w/v) into each tube. The synthetic lures were replaced weekly, and Cera trap® was kept for eight weeks. During the collection we replaced a volume of 350 mL of Cera trap® in each trap if necessary. The initial pH of Ceratrap was 6.87.

McPhail traps baited with APD, BOE and TMA contained 350 mL of water plus detergent solution. The liquid was the only retention method used for capturing inside the traps.

The contents of each trap were sieved weekly; the insects were transferred into 500-mL glass container and immediately taken to the laboratory in Campinas (SP), where they were counted and classified by Order.

We used five replications for each treatment, and each plot contained 35 orange trees (five rows of seven trees). The McPhail trap was installed in the centre of the plot (Fig. 1). The positions of the traps were randomized and rotated weekly.

Data on the captured insects were transformed by SQRT (x + 0.5) and analysed by F and ANOVA, with mean separation obtained using Tukey test ($P < 0.05$) [17]. We compared effects of attractants and insects using 2-way ANOVA.

3. RESULTS

During the study period, 8,296 non-tepritid insects were captured in McPhail traps baited with different compounds; these are related to the Orders *Diptera*, Hymenoptera, Hemiptera, Lepidoptera, Neuroptera and Coleoptera. A significant number of *Diptera* specimens were captured in the study (Table 1) using all attractants. A very low population of *Tephritidae* was captured during the study. The majority of *Diptera* belonged to the families Drosophilidae, Muscidae and Calliphoridae. The numbers of Hymenoptera, Hemiptera, Lepidoptera, Neuroptera and Coleoptera insects were statistically similar for each attractant (Table 1). Considering all traps, 98.12%, 0.66%, 0.16%, 0.90%, 0.06% and 0.10% of the total corresponded to *Diptera*, Hymenoptera, Hemiptera, Lepidoptera, Neuroptera and Coleoptera, respectively.

There is a significant difference in the quantity of *Diptera* captured by lure type (Table 1). McPhail traps baited with Cera Trap®, Cera Trap® + acetone and Cera Trap® + TMA captured similar *Diptera* specimens. Among all attractants, TMA was the most selective within this Order. BOE +

Cera Trap® decreased the capture of non-target *Diptera* to 57.3%. In addition, APD dispensed onto the exposed side of McPhail traps baited with Cera Trap® reduced the non-target *Diptera* to 25.9%, while APD + BOE captured only 25.4% compared to Cera Trap® alone. No differences were detected amongst treatments for the remaining insect Orders.

The population of *Diptera* gradually increased from 9 March 2017 until 23 March 2017, when the McPhail traps baited with Cera Trap® reached their peak. The remaining Orders displayed diferent peaks of capture (Fig. 2). TMA and APD + BOE were more selective and pratically only capture *Diptera* (Fig. 3).

4. DISCUSSION

Based upon my laboratory data, the tested attractants and their combinations were effective to capture fruit flies. But in the field, Cera Trap® captured a high number of non-target flies on McPhail traps, supporting the hypothesis of the present study. Cera Trap® is derived from the enzymatic hydrolysis of animal proteins; it is used in many countries for monitoring or supressing fruit flies in tropical and subtropical crops. Although in many cases, this product has outperformed hydrolysed protein from plant sources [18], the attraction of non-target insects is a limitation for both methods.

Whereas these baits are useful for survey, numerous non-target insects are captured and

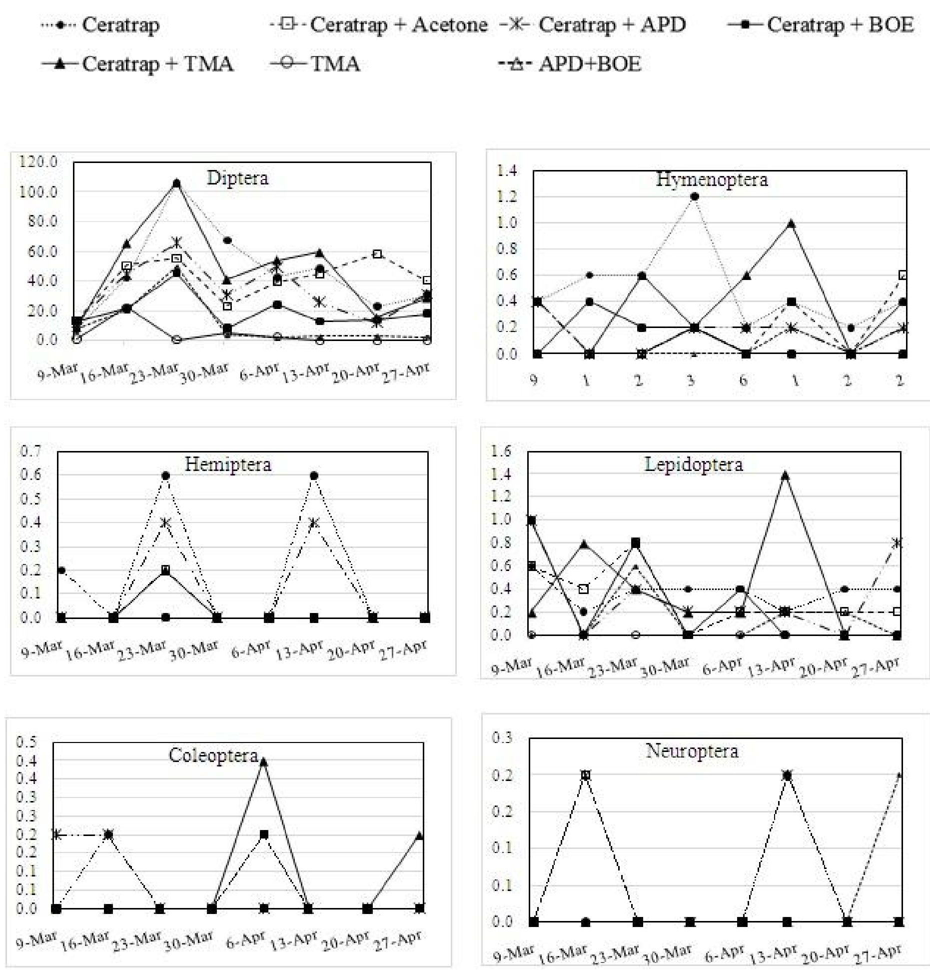

Fig. 2. Seasonal abundance of insects captured by diferent attractants in McPhail traps installed in citrus orchard. Mogi Mirim, SP, Brazil. Ammonium phosphate dibasic (APD), Brazilian orange oilphase essence (BOE), trimethylamine hydrochloride (TMA)

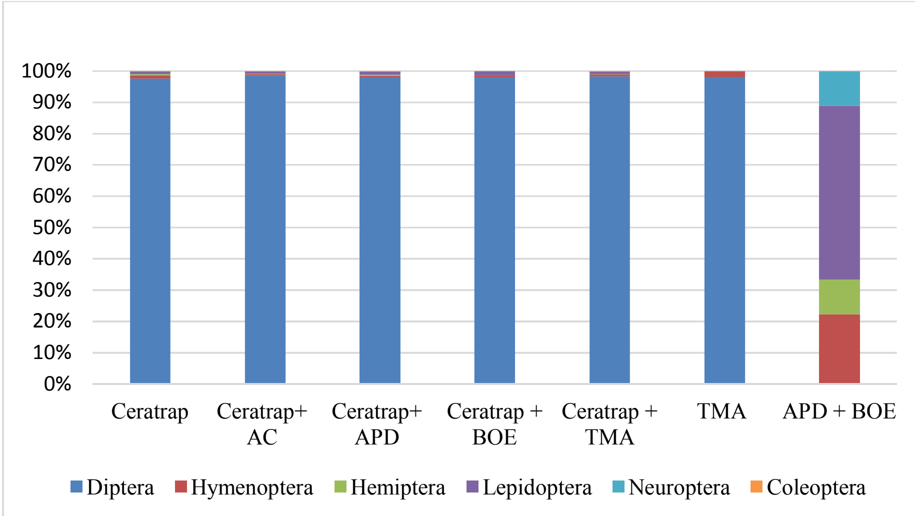

Fig. 3. Comparative diversity of insect Orders among different attractants captured by McPhail traps in citrus orchard. Acetone (AC), Ammonium phosphate dibasic (APD), Brazilian orange oilphase essence (BOE) and trimethylamine hydrochloride (TMA)

have to be sorted [19]. In citrus orchards, hydrolysed corn proteins captured three times as many *Zaprionus indianus* (Gupta) as *C. capitata* and *Anastrepha* specimens combined [12]. Immature phases inside fruits on the ground are likely not affected during the pesticide application, in contrast to arthropod species that live exclusively in the canopy.

A chemically complex odour, such as that emitted by fermented food baits, exhibit volatile cues that are attractive to numerous insect species, as opposed to a simple chemical blend optimised for a single species [19]. *Muscina stabulans* (Fallen) and *Fannia canicularis* (L.) (Muscidae) are attracted to etanol and acetic acid from sucrose baits [20,21]. Ammonia attract *Diptera*, including members of the family *Tephritidae* [22] and it is considered the main volatile from protein lures. Probably, the capture of insects of different Orders causes changes in the composition of the volatiles from liquid protein sources over time. In the present study, adding acetone, APD, BOE or TMA to McPhail luring with Cera Trap® we detected changes of *Diptera* abundance among attractants (Table 1).

Cera Trap® is a liquid bait consiting of enzymatic hydrolised proteins that release mostly amines and organic acids [18]. The high capture rate of non-*Tephritidae* by Cera Trap® may be due to the mutualistic or symbiotic relationships between microbes growing in fermented liquid and flies, according to the hypothesis suggested to responses of adults of Muscidae to sugar - rich sources [21].

In field cages, 5% to 28% of *Anastrepha ludens* (Loew) females were observed escaping from the MultiLure traps after feeding on the CeraTrap® bait [23]. When the liquid attractant surface is partially filled for non-target insects inside the trap, is likely that a physical barrier makes it difficult to capture the tephritids. In addition, the probability of the fruit flies dipping into liquid decreases greatly, evading capture. In many cases, insects captured by McPhail traps installed in tropical and subtropical areas should be collected twice a week.

We suggesting improve the efficacy and selectivity of lures based on protein sources, in order to avoid capture non-target arthropods and consequently chemical and biological deterioration of the attractant. For this, it is necessary to add other synthetic components to the protein attractive and to provide them in a stable and long-lasting formulation.

In Mexican citrus orchards, grape juice captured sevenfold higher Neuroptera (lacewings) than Cera Trap® [24]. Although Cera Trap® captured non-target *Diptera*, very low populations of others Orders were detected in the present study. The product should definitely be chemically enhanced to avoid attract non-*Tephritidae* specimens.

Table 1. Mean number and standard error of the mean (SEM) of nontarget insect Orders captured on McPhail traps baited with different compounds on orange orchard cv. Hamlin. Municipality of Mogi Mirim, São Paulo state, Brazil. March-April 2017

Attractant	*Diptera*	Hymenoptera	Hemiptera	Lepidoptera	Neuroptera	Coleoptera
Ceratrap	367.6 ± 42.8aA	4.0 ± 1.4 aB	1.4 ± 0.9 aB	3.0 ± 0.7 aB	0.2 ± 0.2 aB	0.4 ± 0.2 aB
Ceratrap + Acetone	326.0 ± 73.8abA	1.6 ± 0.5 aB	0.2 ± 0.2 aB	2.6 ± 1.2 aB	0.2 ± 0.2 aB	0.2 ± 0.2 aB
Ceratrap +APD	272.4 ± 42.6 bA	1.2 ± 0.7 aB	0.8 ± 0.5 aB	2.8 ± 1.2 aB	0.4 ± 0.2 aB	0.4 ± 0.2 aB
Ceratrap + BOE	157.0 ± 20.0 cA	0.8 ±0.5 aB	0.0 ± 0.0 aB	2.2 ± 1.3 aB	0.0 ± 0.0 aB	0.0 ± 0.0 aB
Ceratrap + TMA	380.0 ± 50.5 aA	2.4 ± 0.5 aB	0.2 ± 0.2 aB	3.2 ± 0.4aB	0.0 ± 0.0 aB	0.6 ± 0.4 aB
TMA	31.6 ± 11.2eA	0.6 ± 0.2 aB	0.0 ± 0.0 aB	0.0 ± 0.0 aB	0.0 ± 0.0 aB	0.0 ± 0.0 aB
APD + BOE	93.4 ± 38.8 dA	0.4 ± 0.2 aB	0.2 ± 0.2 aB	1.0 ± 0.3 aB	0.2 ± 0.2 aB	0.0 ± 0.0 aB

Means followed by the same lowercase letter in the column and upper case in the line within a same variable do not differ from one another by Tukey ($p<0.05$).Ammonium phosphate dibasic (APD), brazilian orange oilphase essence (BOE), trimethylamine hydrochloride (TMA)

The attraction of *Drosophila suzukii* (Matsumura) to fermented food appears to be due to a relatively small fraction of volatile chemicals emitted by the bait [19]. Our study was conducted during the rainy season, when saprophagous insects attack decaying fruits that were hanging on trees or have fallen to the ground. The *Diptera* peaks coincided with an increase of fallen fruit, when drosophilids are drawn to the orchard for feeding and oviposition.

Among crops, orchards have higher than average uniformity because trees of the same cultivar are clones produced via vegetative propagation, and grafted on to rootstocks that are now commonly produced vegetatively as well [25]. In peach orchards, Chrysopidae, Coccinellidae and Miridae families were more abundants in plots treated with Ceranock® and AAL&K® bait stations against *C. capitata* than in those treated with conventional insecticides [26]. Likewise, as for toxic baits, the mass trapping system should be able to provide minimal impact on arthropod community.

When PET bottles were baited exclusively with hydrolysed protein (without malathion) in Mexican mango orchards, the results were to those in the control condition, possibly due to the escape of the *A. ludens* and *A. obliqua* specimens [27]. Torula yeast also capture significantly more non-target insects than Ammonium acetate [28]. Lures that provide low release rates of ammonia can help to avoid non-target insects as it occurs for western cherry fruit fly, *Rhagoletis indifferens* Curran [22].

5. CONCLUSION AND RECOMMENDATIONS

The findings of the present work showed non-specific insects attraction of Cera trap® in comparison with synthetic lures. The attractants tested separately with Cera trap® in McPhail traps did not provide selectivity to the product. These attractants should be tested mixed with Cera trap® in further studies. In terms of integrated pest management (IPM), broad spectrum attractants should be avoided, in order to properly estimate fruit fly migration or economic threshold based on trapping systems. Mass trapping should obviously reduce or eliminate cover spray insecticides for fruit flies, with the additional advantages of preserving natural enemies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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