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ASSESSMENT OF POTENTIAL LURES FOR FRUIT FLIES (DIPTERA: TEPHRITIDAE) OF ECONOMIC IMPORTANCE IN URUGUAY

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ABSTRACT

Within Uruguay's fruit plantations, both *Anastrepha fraterculus* Wiedemann and *Ceratitis capitata* Wiedemann are reported as species of economic importance. These species are polyphagous and cause economic problems in fruit orchards. Currently, different control methods and techniques are combined, but in some cases, they are insufficient to prevent the damage caused by these species. We evaluated the attractiveness of different baits for fruit flies, as well as their selectivity toward non-target arthropods, in two peach and mandarin farms in southern Uruguay. Four treatments with three replicates were evaluated: diammonium phosphate (DAP), torula yeast (PBX®), torula yeast 'type B' and Cera Trap®/Plustrap®. Captured arthropods were separated into three groups: tephritids, beneficial and other. Tephritids were sexed, dissecting females to determine the presence of developed ovaries. All treatments were effective in capturing young females of *C. capitata,* while captures of *A. fraterculus* were very low. Captures of beneficial arthropods were relatively low, with DAP capturing the highest number. Lastly, torula yeast 'type B' was the one that captured the greatest number of non-target insects.

Keywords: *Ceratitis capitata, Anastrepha fraterculus*, DAP, traps.

RESUMEN

Evaluación de potenciales atrayentes para moscas de la fruta (Diptera: Tephritidae) de importancia económica en Uruguay. En las plantaciones frutícolas de Uruguay, tanto *Anastrepha fraterculus* Wiedemann como *Ceratitis capitata* Wiedemann se reportan como especies de importancia económica. Estas especies son polífagas y generan problemas económicos en predios frutales. Actualmente, se combinan diferentes métodos y técnicas de control que, en algunos casos, son insuficientes para prevenir el daño ocasionado por estas especies. En este estudio, evaluamos la atracción de diferentes cebos para moscas de la fruta y su selectividad hacia artrópodos no objetivo en dos predios de durazno y mandarina en el sur de Uruguay. Se evaluaron cuatro tratamientos con tres réplicas: fosfato diamónico (DAP), levadura torula (PBX®), levadura torula 'type B' y Cera Trap®/Plustrap®. Los artrópodos capturados se separaron en tres grupos: tefrítidos, benéficos y otros. Los tefrítidos fueron sexados, disecando a las hembras para determinar la presencia de ovarios desarrollados. Todos los tratamientos fueron efectivos en la captura de hembras jóvenes de *C. capitata*, mientras que las capturas de *A. fraterculus* fueron escasas. Las capturas de artrópodos benéficos fueron bajas, siendo DAP el tratamiento que capturó el mayor número. Finalmente, la levadura torula 'type B' fue la que capturó la mayor cantidad de insectos no objetivo.

Palabras clave: *Ceratitis capitata, Anastrepha fraterculus*, DAP, trampas.

INTRODUCTION

Fruit flies are dipterans from the Tephritidae family, whose larvae develop and feed inside fruits (Echeverri & Yepes, 2019; Scatoni, Calvo, Delgado, Duarte & Zefferino, 2019). These insects are considered significant pests on both global and regional scale due to the substantial economic losses they cause (Allwood, Leblanc, Tora Vueti & Bull, 2001; Buenahora & Otero, 2012; Scatoni et al., 2019). In Uruguay, two economically important species have been recorded: *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) (Malavasi, Rohwer & Campbell, 2019; Calvo, Duarte, Delgado, Mello Garcia & Scatoni, 2024). *Ceratitis capitata*, the Mediterranean fruit fly, with an afrotropical origin, is recognized as the most damaging pests in global fruit production due to the large number and variety of hosts it attacks and its cosmopolitan distribution (Malavasi et al., 2019). *Anastrepha fraterculus*, the South American fruit fly, is native to the Neotropical region and forms a complex of polyphagous species with at least eight described morphotypes (Hernández-Ortiz & Aluja, 1993; Scatoni et al., 2019).

In light of the damage and the increasing restrictions imposed by export destinations, early detection, pest control and the development of new managment alternatives are necessary (Arroyo et al., 2013; Delgado, Calvo, Duarte, Borges & Scatoni, 2022; Delgado, Duarte, Yakimik & Calvo, 2024). Currently, various methods and control techniques are combined to manage this species, including physical, biological, ethological (mass trapping), cultural, chemical, and autocidal approaches (Delgado et al., 2022; Duarte, Calvo, Delgado, Garcia & Scatoni, 2021; Sabater-Muñóz et al., 2012). In Uruguay, pest management is primarily based on chemical control and mass trapping, often implemented in combination due to the high pest populations observed (Buenahora, 2015; Delgado et al., 2022, 2024).

Mass trapping uses a high density of traps with species-specific attractants to capture the largest possible number of adult flies (Buenahora, 2015). An efficient attractant must meet certain criteria: primarily capturing immature females to prevent oviposition damage (Vilajeliu, Batellori & Escudero, 2007), being selective towards non-target arthropods (Porcel, Campos, Ruano, Sanllorente & Caballero, 2009; Son, Suh & Choi, 2019), and having a cost-effective application for use in fruit fly monitoring and mass trapping programs (Kouloussis et al., 2022). On the other hand, in Uruguay, the attractants available on the market for mass trapping were designed for *C. capitata*, resulting in lower development of lures for *A. fraterculus*. In the search for attractants that meet the aforementioned criteria, the efficacy of two compounds, torula yeast 'type B' and diammonium phosphate (DAP), was evaluated. Torula yeast 'type B' has been used in tephritid diets and could serve as a new attractant for monitoring populations, while DAP is an inexpensive phosphorus fertilizer that releases ammonia when dissolved, which has shown certain attractiveness to fruit flies (Sadraoui-Ajmi et al., 2022). The volatile compounds derived from ammonia are perceived by insects as an indicator of a food source (Lasa & Williams, 2021). Regionally, there have been limited studies on its efficacy, but its use is beginning to be recommended for informal fruit fly control (Triadani & Buxmann, 2019).

MATERIALS AND METHODS

The study was conducted from January to March of 2023, on two fruit farms in the southern part of Uruguay (Canelones), both with a history of high fruit fly infestation. The orchards selected were: peach trees 'Moscato Delicia' and 'Moscato Tardío' (34°34'26.72"S, 56°18'28.03"W), and mandarin trees 'Afourer' (34°37'15.90"S, 56°21'21.40"W). During the study, ripe fruit, which is attractive to tephritids, was observed on the selected orchards. While late peach varieties are harvested in February or March, the 'Afourer' variety is harvested in June or July (Instituto Nacional de Investigación Agropecuaria, 2019). Nonetheless, the orchard with 'Afourer' mandarin trees was used due to the presence of early-ripening fruit, as it was a year of uneven ripening and high captures were recorded in traps installed by the fruit fly surveillance network of the Ministry of Livestock, Agriculture, and Fisheries (Uruguay). This information is relevant considering that, according to the label recommendations, lures are installed in orchards 45 days before harvest.

Four treatments were evaluated with three repetitions on each site: DAP (ISUSA, Uruguay), torula yeast/borax ('PBX®', SUSBIN, Argentina), torula yeast 'type B' (Lallemand, Argentina) and CeraTrap® (Bioiberica S.A., Spain)/PlusTrap® (SUSBIN, Argentina). PBX® and CeraTrap®/PlusTrap® were used as controls, as both treatments have been extensively studied and are commercially available in Uruguay. CeraTrap® was used from week 16/01 - 22/01 until week 13/02 - 19/02 in the peach orchard (5 weeks) and from week 23/01 - 29/01 to week 20/02 - 26/02 in the mandarin orchard (5 weeks), when it was replaced by PlusTrap®. This change was due to the lack of CeraTrap® at the time of replenishing.

The attractant solutions of DAP, PBX®, and torula yeast 'type B' were evaluated in McPhail traps. DAP, following the regional recommendation (Instituto Nacional de Tecnología Agropecuaria, s.f.), was prepared 24 hours prior to the trial installation by mixing 12.5 grams in 300 mL of water. For PBX® and 'type B' yeast, four pellets and 12 g respectively (equivalent to the weight of four torula pellets) were dissolved in 300 mL of water, following the manufacturer's recommendations. CeraTrap® and PlusTrap® are commercial products ready for use, consisting of the trap and the attractant liquid, that last 45 days (Garrido & Simón, 2019). All traps were placed in the tree canopies, 1.5 meters above the ground, and were evenly distributed (FAO/International Atomic Energy Agency, 2018), separated by at least 15 meters from each other. Also, traps were inspected and rotated every other week, and re-baited every week, or after 45 days in the case of the treatment CeraTrap®/PlusTrap®, over a period of 10 weeks.

The captured specimens were collected, placed in labeled plastic tubes with 70% alcohol, and transported to the laboratory of the Entomology Laboratory at the

Faculty of Agronomy. The collected specimens were taxonomically identified to species level for Tephritidae using the key by Norrbom et al. (2012), while other arthropods were classified to order and/or family level, following the key by Bentancourt, Scatoni and Morelli (2009). Tephritids were sexed and counted, and females were dissected to check the presence of developed ovaries, an indicator of sexual maturity (Delgado et al., 2022). Selectivity of the attractants was assessed by the number of tephritids and non-target arthropods (beneficials and others) captured.

Statistical analyses were performed using the accumulated capture data. The accumulated means of fruit flies (total captures, and captures of mature and immature females), as well as captures of non-target arthropods between treatments, were analyzed using a Generalized Linear Mixed Model (GLMM), adjusted to a Quasi-Poisson distribution, followed by comparison using the DGC test ($p = 0.05$) (Di Rienzo et al., 2010). The captures of mature and immature females, and the captures of non-target arthropods within each treatment, were analyzed using a Chi-Square test ($p = 0.05$).

RESULTS AND DISCUSSION

Over the 10-week evaluation period a total of 3001 tephritids were recorded, with significantly more females (n = 1907) than males (n = 1094) captured in both trials (GLMM, $p \leq 0.05$). All evaluated attractants captured more females than males, which is desirable in an attractant for mass trapping. This result aligns with findings from previous studies (Hafsi, Harbi, Rahmouni & Chermiti, 2015; Cotoc-Roldán, Vela-Luch, Estrada-Marroquín & Hernández-Pérez, 2021; Ghanim, El-Sharkawy & El-Baradey, 2021; Delgado et al., 2022).

Captures of *C. capitata* accounted for 99% of the total, while *A. fraterculus* represented only 1% (n = 27) (Table 1). The low capture rate of *A. fraterculus* prevented an assessment of the effectiveness of the evaluated attractants for this species. Such low representation could be due to either a low population density during the study (fruit infestation was not evaluated) or possible inefficiencies in the attractants used. Delgado et al. (2022) reported similar results in *A. fraterculus* captures. However, fruit infestation was observed during harvest, suggesting the presence of this species in the area, even if its populations were not fully detected through trapping methods.

For *C. capitata*, no significant differences were observed in accumulated captures between treatments (GLMM, $p \geq 0.05$) (Fig. 1), which may suggest that DAP and torula yeast 'type B' are as effective as the commercial attractants used. Delgado et al. (2020), Buenahora and Otero (2013), and Shelly and Kurashima (2016) observed significant differences in female captures between torula yeast, CeraTrap®, and PlusTrap®, with CeraTrap® being the most effective attractant. These differences with our results may be due to variations in experimental design, specifically regarding trap spatial distribution and density.

Regarding sexual maturity, 71% of captured *C. capitata* females exhibited developed ovaries, while for *A. fraterculus*, of the 12 females captured, eight were mature. In both farms, the treatments that captured the most mature females were DAP and PBX® (χ^2, $p \leq 0.05$). Mangan and Thomas (2014) and Shelly, Kurashima, Nishimoto and Andress (2017) also found that PBX® has a higher capture rate for mature females compared to other attractants. Roh, Kendra and Cha (2021) suggests that mature females prefer the scent of torula yeast due to their increased protein needs for producing the critical egg load required for oviposition. All evaluated treatments were able to capture sexually immature females, although no significant differences were observed compared to captures of mature females (GLMM, $p \geq 0.05$). The search for new attractants is focused on compounds that capture the highest number of females, particularly immature ones, to prevent oviposition punctures in fruits (Vilajeliu et al., 2007).

In terms of total arthropod captures (including tephritids), torula yeast 'type B' and PBX® attracted the highest number of individuals, with most captures belonging to the category of other arthropods (Fig. 2). Selectivity is an essential trait for an attractant, especially in mass trapping due to the high number of traps deployed in crops. Among the attractants evaluated, DAP captured a higher number of beneficial arthropods (GLMM, $p \leq 0.05$) (Table 2). The high capture rate may be attributed to the use of McPhail traps, which have a larger opening (approximately a diameter of 8 cm) than those typically used in mass trapping (1 cm diameter holes). Braham (2013) showed that DAP captured a lower number of beneficial insects (syrphids and lacewings) when using traps with smaller openings than the McPhail trap.

As mentioned above, torula yeast 'type B' treatment showed significant attractiveness to other arthropods (GLMM, $p \leq 0.05$). Torula yeast-based attractants are known for capturing many non-target arthropods (Thomas, 2003; Delgado et al., 2022). Uchida et al. (2006) observed that the attraction of torula yeast is due not only to the attractant itself but also to the decomposition of insects accumulating in the traps. Additionally, in this study, the lack of borax stabilization led to rapid decomposition, producing an unpleasant odor that attracted various Diptera taxa, such as Calliphoridae, Muscidae, and Sarcophagidae, the most frequently captured families (Table 2).

CONCLUSION

DAP emerges as a viable novel option for mass trapping of fruit flies due to its low cost and ease of use, which may make it an efficient attractant based on the

Table 1. Accumulated captures of *A. fraterculus* by the different treatments.

Treatment	Accumulated captures
PBX®	13
Torula yeast 'type B'	4
DAP	9
CeraTrap®/PlusTrap®	1

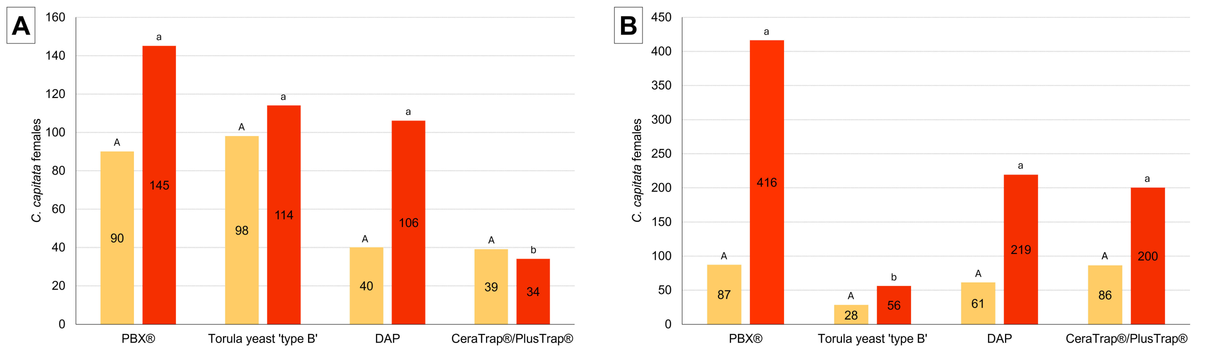

Fig. 1. Accumulated captures of immature (yellow) and mature (red) females of *Ceratitis capitata*. Uppercase letters indicate significant differences between treatments in the captures of immature females, while lowercase letters indicate differences in the captures of mature females (MLGM, $p \leq 0.05$). A. 'Moscato Delicia' and 'Moscato tardío' peach orchard; B. 'Afourer' mandarin orchard.

Table 2. Orders or families of arthropods captured by different treatments during the study period.

Order/Family	PBX®	Torula yeast 'type B'	DAP	CeraTrap®/PlusTrap®	TOTAL
Diptera					
Agromyzidae	24	41	4	32	101
Anthomyiidae	0	137	4	7	148
Calliphoridae	6	1375	6	1	1388
Cecidomyiidae	10	2	113	6	131
Ceratopogonidae	0	0	0	1	1
Chloropidae	999	52	70	49	1170
Culicidae	1	5	2	2	10
Drosophilidae	12	37	6	13	68
Lonchaeidae	334	41	57	8	440
Muscidae/Fanniidae	18	6821	9	13	6861
Mycetophilidae	0	1	6	0	7
Otitidae	24	301	9	25	359
Phoridae	111	104	136	28	379
Psychodidae	0	103	4	1	108
Sarcophagidae	1236	4700	62	96	6094
Scatopsidae	0	2	0	0	2
Sciaridae	3	4	5	1	13
Stratiomyidae	0	0	1	0	1
Syrphidae	0	0	7	1	8
Tachinidae	8	1	28	2	39

Table 2. Cont.

Order/Family	PBX®	Torula yeast 'type B'	DAP	CeraTrap®/PlusTrap®	TOTAL
Other Tephritidae	0	0	1	0	1
Hymenoptera					
Apidae	1	0	0	3	4
Bethylidae	6	2	1	13	22
Braconidae	1	0	2	0	3
Chalcididae	0	3	0	1	4
Encyrtidae	0	2	0	0	2
Figitidae	0	9	1	0	10
Formicidae	162	82	26	170	440
Ichneumonidae	2	0	0	0	2
Pompilidae	1	0	5	0	6
Proctotrupidae	1	0	0	0	1
Pteromalidae	5	17	5	51	78
Vespidae	41	20	138	2	201
Coleoptera					
Anthribidae	1	0	0	0	1
Carabidae	1	0	0	0	1
Cerambycidae	0	0	0	1	1
Chrysomelidae	2	1	0	1	4
Coccinellidae	2	1	1	0	4
Curculionidae	0	0	0	6	6
Laemophloeidae	0	0	0	1	1
Nitidulidae	0	2	0	7	9
Staphylinidae	10	2	3	0	15
Araneae					
Cheiracanthiidae	0	1	0	1	2
Dictynidae	0	1	0	0	1
Lycosidae	1	0	0	0	1
Oxyopidae	0	1	0	0	1
Salticidae	7	6	4	1	18
Sparassidae	1	0	1	0	2
Thomisidae	0	1	0	1	2
Hemiptera					
Delphacidae	0	0	0	1	1
Derbidae	0	0	4	0	4
Dictyopharidae	3	0	0	0	3
Neuroptera					
Chrysopidae	5	2	22	0	29
Lepidoptera	31	103	126	13	273
Mantodea	0	1	0	0	1
Blattodea	0	3	0	7	10
Orthoptera	0	2	0	0	2

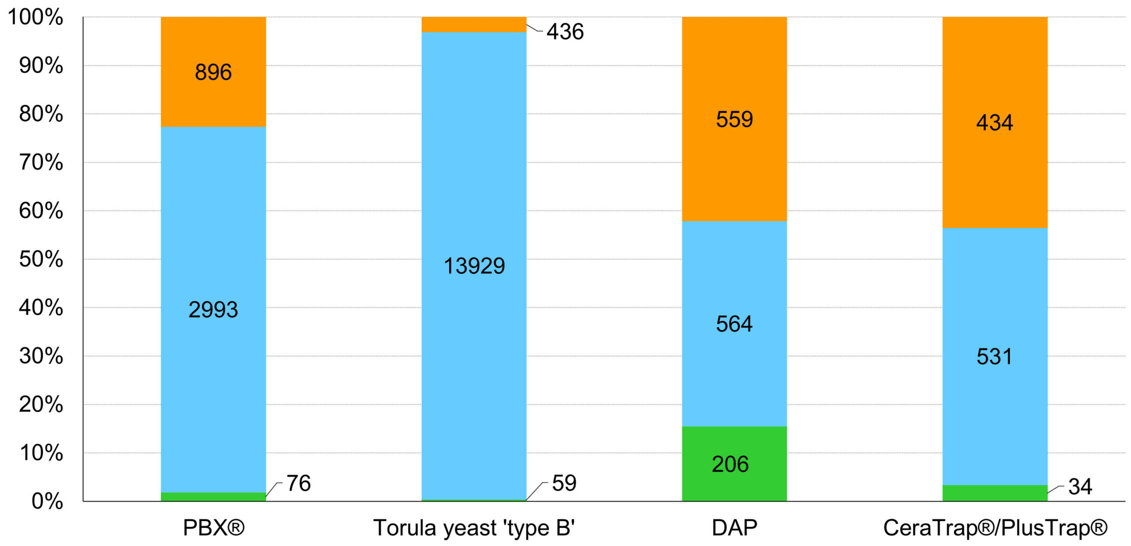

Fig. 2. Percentage of Tephritidae (orange), other arthropods (blue), and beneficial arthropods (green) captured by the different treatments.

required dosages. However, further research is needed to evaluate its durability in the field, its attractiveness to *A. fraterculus*, and assess its selectivity concerning beneficial arthropods. For torula yeast 'type B', future studies should focus on testing a stabilized form of the attractant, as this could improve its efficacy in capturing fruit flies, although it is necessary to evaluate its cost-effectiveness in mass trapping. Additionally, it is essential to advance the research on effective attractants for controlling *A. fraterculus*.

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