Mortality of Fruit Flies (Diptera: Tephritidae) Exposed to Spinetoram Toxic Bait in the Laboratory

ABSTRACT

Fruit flies (Diptera: Tephritidae) cause significant losses during the production and marketing of horticultural products. Brazilian growers usually adopt full-coverage insecticide spraying to control fruit flies, but toxic bait is a more strategic technique. We tested toxic baits in the laboratory, using commercial hydrolysed corn protein (10% v/v) plus 90 g, 120 g, 150 g and 180 g dilutions of spinetoram 250 WG (commercial product/1,000 litres of water). All toxic baits were compared with an untreated control (only protein) for the adults of females and males of *Anastrepha obliqua* (Macquart) and *Ceratitis capitata* (Wiedemann) up to 30 hours of exposure. Dry food for adults was included in all dilutions (5% w/v). In addition, we tested the residual effect of toxic baits applied to the leaves of mandarin seedlings. We used the same treatments of the earlier bioassay without dry food, collecting treated leaves and exposing them to *C. capitata* (medfly) females for 24 hours in the laboratory. Leaves were collected 1, 3, 7, 15 and 30 days after application. Overall, medfly adults were more susceptible to spinetoram baits than *A. obliqua*. All toxic baits resulted in 100% *C. capitata* mortality 24 hours after initial exposure, and the toxic bait at 150 g/1,000 L of water resulted in the maximum mortality (96%) in *A. obliqua*. Except for 90 g of spinetoram bait at 30 days after application, all spinetoram bait concentrations resulted in significantly more dead *C. capitata* females than the control over all tested periods in the residual bioassay. At 30 days after application, spinetoram baits at 120 g, 150 g and 180 g resulted in 85%, 87% and 86% mortality in *C. capitata*, respectively. Spinetoram toxic baits have proven promising for long-term fruit fly management.

Keywords: *Insecta; Tephritoidea; insecticide; spinosyn; adulticide; residual effect*

1. INTRODUCTION

Fruit flies (Diptera: Tephritidae) are insects that cause significant losses in the global fruit production industry and their commercial hosts are subjected to quarantine restrictions during regional and international trade [1,2].

Ceratitis capitata (Wiedemann), known as the Mediterranean fruit fly (medfly), is an introduced species, with 115 host plant species recorded in Brazil [3]. The West Indian fruit fly, *Anastrepha obliqua* Macquart occurs in Mexico, Central and South America and several Caribbean islands [4], with 70 host plants reported in Brazil [5].

Insecticides delivered with bait stations and cover sprays have typically been used to control fruit flies on all continents [6,7]. Ground insecticide bait spray and toxic bait stations are similarly effective for reducing fruit fly populations [8]. Toxic bait is a lure-and-kill method that acts through ingestion and contact. Compared with cover spray, the use of toxic baits reduced the insecticide contact with beneficial insects [9] and the toxicant volume needed to treat an area.

Despite the serious financial losses caused by tephritid species, relatively little research into the effects of new insecticides has been published regarding this group of pests in Brazil (personal information). The lack of interest in alternative insecticides was due to the intensive use of organophosphates, especially malathion, in the past couple of decades [10, [11,12,13]. Furthermore, field resistance of fruit flies to organophosphates and pyrethroids were reported by many authors [14, 15, 16, 17,18].

The management of fruit flies in orchards or area-wide systems calls for eco-friendly strategies, such as toxic bait using insecticides with low environmental impacts. Spinosyns are a family of broad-spectrum insecticides, including spinosad and spinetoram, isolated from the actinomycete soil bacterium *Saccharopolyspora spinosa* [19]. Spinosad is the first active ingredient in the Naturalyte product line for insect control [20], including fruit flies [6, 21, 22]. The semi-synthetic insecticide spinetoram is a broad-spectrum pesticide that is more active and longer-lasting than spinosyn A, which is the main active component of spinosad [23, 24]. Spinetoram is a mixture of two synthetically modified spinosyns that overexcites insect´ nervous system by modifying the function of GABA receptors. This active ingredient presents low risks to the environment and humans [25] and acts both through ingestion and contact [26]. *Ceratitis capitata* adults collected in different regions and from different fruit crops in Brazil were highly susceptible to the ingestion of spinetoram in the laboratory [27].

The commercial product Success® 0,02CB (formulated spinosad toxic bait) is registered in Brazil for use on ten fruit crops against four fruit fly species: *A. obliqua, Anastrepha fraterculus* (Wiedemann), *Bactrocera carambolae* Drew & Hancock and *C. capitata* [28]. It can be successfully applied, but the high cost discourage application, but fruit growers report that the high cost discourages application. In the laboratory, the mortality resulting from spinetoram toxic bait in *C. capitata* and *A. fraterculus* adults was evaluated over different exposure times. We intend to provide useful information regarding the toxicity of spinetoram bait to two fruit fly species as an alternative to organophosphates and the specific spinosad formulation used in IPM programs for fruit flies.

2. MATERIAL AND METHODS

Insect colonies. Ceratitis capitata and *A. obliqua* adults were obtained from colonies established in 1993 at the Instituto Biológico in Campinas, State of São Paulo, Brazil. The media and other rearing methodology details were described previously [1, 29]. The colonies of both fly species were maintained at 25 °C± 2 °C, 70% ± 10% relative humidity, with a regime of 14:10 light to dark hours.

Chemicals. Spinetoram (Delegate® 250WG – Corteva Agriscience do Brasil Ltda.) was diluted in water at 90.0 g, 120.0 g, 150.0 g and 180.0 g of commercial product per 1,000 L of water. Biofruit (Biocontrole Métodos de Controle de Pragas Ltda., Brazil) was the commercial protein (a condensed fermented corn extract) used as a food attractant when preparing the toxic baits (treatments) and was diluted at 10% (v/v) for each spinetoram treatment. The untreated control consisted only 10% (v/v) Biofruit in water. The pH of solutions was determined with an Alphalab pH meter model PA 200.

Bioassay 1. Five females and five males of *C. capitata* (3-day-old) and *A. obliqua* (5-day-old) were separately exposed to toxic baits in containers in the laboratory. The containers were plastic boxes of 1,000 cc. Five adults of each species (per replication) were captured from rearing jail and placed in a flatbottomed test tube (25 OD × 85 mm) that was capped with cotton. The adults were immediately released into the container, which was covered with voile and anchored with elastic.

To increase of the attractiveness of the toxic baits we added dry food (5% w/v) [1] to the solutions. The control plots consisted only Biofruit 10% (v/v) plus dry food 5% (w/v). About 3.0 mL of bait was dispersed through cotton in 2.7 cm plastic dishes placed in the centre of container floor.

The cumulative mortalities were evaluated at 15, 30, 45, 60, 90, 120, 150, 180, 240 and, 360 minutes and, 24 and 30 hours after initial exposure. Irreversible knockdown followed by the death of the adults was the criterion to determine mortality [11]. During the experiment the room conditions were 22.5 C – 26.0ºC and 42% – 77% RH.

Bioassay 2. Residual contact was used for the bioassay to determine the persistence of toxic baits. We tested the usual toxic bait (food attractant plus insecticide) and the untreated control (only food attractant). The solutions (spinetoram and control) were applied in the laboratory to the abaxial and adaxial leaf surfaces of 80 cm tall mandarin cv. Rio (*Citrus deliciosa* Tenore) seedlings, grafted on citrandarin ‘Riverside’. The solutions were applied with a manual compression sprayer (1.5 L – Rio Chen´s Import & Export

Ltda.). The seedlings (3 per treatment) were sprayed to dripping. They were kept outdoors in a covered location, protected from direct rain and sunlight, in temperatures between 20.3 ºC and 28.3 ºC and RH between 38% and 83%.

We collected mature leaves at 1, 3, 7, 15, 22 and 30 days after the application of toxic bait. One leaf and its petiole was inserted perpendicularly into the cotton in 2.7 cm plastic dishes placed in the containers. The average foliar area (each side) of 10 randomized leaves was 16.5 cm^2.

We tested ten 3-day-old *C. capitata* females per container (one replication). The insect exposure to the treatments was the same as described for the previous assay. Mortality was evaluated 24 hours after exposure.

Statistical analysis. Ten replicates were used for each treatment. A two-way ANOVA (Tukey´s test, $P < 0.05$) was performed with Sisvar, version 5.6 [30].

3. RESULTS AND DISCUSSION

Bioassay 1. Only *C. capitata* exposed to spinetoram 150 g and 180 g/1000 litres of water differed from the control at 240 minutes of exposure. Medfly adults were more susceptible to spinetoram baits than *A. obliqua* (Table 1, Fig. 1) at 360 minutes. All doses of spinetoram baits resulted in 100% mortality of *C. capitata* 24 hours after initial exposure, and the toxic bait at 150 g/1,000 L of water resulted in the maximum mortality in *A. obliqua* (96%). *Ceratitis capitata* exhibited greater mortality than *A. obliqua* at 24 hours of exposure to spinetoram bait at 90 g/1,000 L of water. The pH of toxic bait solutions ranged from 4.01 to 4.17 (Figure 2).

The antennae and the maxillary palps, covered by numerous sensilla, are the primary olfactory organs in fruit flies. The multiporous sensilla trichodea are responsible for detecting volatile plant compounds and pheromones [31]. The toxic baits were likely more attractive to medflies or slightly repellent to *A. obliqua* during periods shorter than 360 minutes (Figure 3), and volatile compounds may have reduced their attractiveness. Spinetoram baits at 150 g and 180 g/1,000 L of water resulted in the highest mortality due to the combined effects of toxicity and attractiveness. Except for *A. obliqua* exposed to spinetoram at 90 g/1,000 L of water, the number of females and males affected in each fly species differed from the control plots 24 hours after initial exposure (Table 2), and no susceptibility differences between the sexes of fruit fly species were detected.

However, the dose of 150.0 g of spinetoram/1,000 L of water tended to kill faster than the other tested concentrations (Figure 3), probably due to an appropriate

combination of pesticide and food to attract and kill flies. Age-related patterns of feeding vary among tephritid species [32], but both sexes need sugar and protein to sexually mature [33]. Biofruit, manufactured specifically for use as toxic bait, is a vegetal protein-based syrup. Spinetoram bait also results in 100% mortality of *Rhagoletis indifferens* Curran (Tephritidae) 12 days after exposure in the laboratory [34].

Food-based attractants incorporating an insecticide are an important component of area-wide control programmes for *C. capitata* [35]. Although toxic baits are primarily ingested through the proboscis, the legs and other body parts also contact the toxic bait, and consequently the insecticide.

The concentration of food significantly affected the food handling and foraging time [36]. Before lowering the proboscis to begin feeding on toxic bait, the olfactory sensilla probably stimulate foraging or not. This behaviour influences the dose-response of toxic baits and consequently the curves of mortality (Figure 3).

Bioassay 2. The pH of toxic bait solutions ranged from 4.28 to 4.32 (Figure 2). These values varied compared with the previous bioassay because the dry food for adults was omitted from the toxic baits used in Bioassay 2. Consequently, when we added the dry food to the solutions, the pH values were slightly higher than the corresponding pure spinetoram solution.

All spinetoram bait concentrations resulted in significantly more dead *C. capitata* females than the control for all tested periods, except 90 g spinetoram bait at 30 days after application (Table 3). For up to 22 days after application, we recorded mortalities from spinetoram baits between 77% and 97%, 24 hours after exposure. At 30 days after application, spinetoram baits at 120 g, 150 g and 180 g resulted in 85%, 87% and 86% mortalities of *C. capitata*, respectively. The survivorship of *C. capitata* females exposed to 90 g spinetoram bait was similar to the control (Table 3).

The spinetoram baits exhibited considerable residual effects against fruit flies. The combined effects of contact and ingestion likely increased insecticide performance of toxic baits against tephritids. Fruit flies intensively probed the surface of the substrate with their proboscises [37], especially *C. capitata* adults*.* The feeding of *C. capitata* on toxic baits over the 24-hour laboratory exposure likely also increased the absorption of insecticidal residues when flies walked on the leaf surface.

The long-lasting residual effect of spinetoram bait reveals the persistence of this insecticide on the mandarin nursery trees were protected from exposition of direct sunlight and rain. Additionally, the spinetoram toxic baits tested showed an appropriate chemical combination between the insecticide and the food attractant. The hydrolysed corn used as a protein attractant for fruit flies [38] exhibited significantly reduced attractiveness after 10

days of exposure, as proven in tests with McPhail traps [39]. The behaviour of females sponging the leaf surface was consistent over all evaluation periods.

Adult fruit flies obtain nutrients by foraging for plant exudates, honeydew and bird droppings scattered on leaves and fruits, which provide the with carbohydrates and nitrogen [40]. In an orchard we must attract resident or immigrant fruit fly adults as quickly as possible during the pre-ovipositional period. Consequently, potent toxic baits are essential to avoid fruit losses and successive fruit fly generations in local or regional production areas.

The few cases of fruit fly resistance to spinosad reported in the literature, were attributed to topical applications and, probably due to reduced penetration of the cuticle (Vontas et al., 2011). The concomitant ingestion of spinosyns by flies during foraging activities probably reflects the improved the mortality resulting from spinetoram toxic baits.

4. CONCLUSION

The ingestion and contact with spinetoram results in adequate mortality 24 hours after initial exposure to the toxic baits. We detected different curves of mortality between fruit fly species, and spinetoram baits at 360 minutes result in a significant increase in *C. capitata* mortality. The residual effects of spinetoram toxic baits (120 g, 150 g and 180 g/1,000 L of water) against *C. capitata* were consistent for up to 30 days after application. Further studies should be conducted to more accurately assess the mortality of fruit fly adults exposed to spinetoram toxic baits in different field conditions to determine the most efficacious for the purpose to suggest the best efficacy and economic dosage of spinetoram and, the minimum protection period of the orchard.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1. The number of dead adults (*n* = 10) of *Anastrepha obliqua* (AO) and *Ceratitis capitata* (CC) at three times after initial exposure to spinetoram toxic baits (g of commercial product/1,000 liters of water) in the laboratory.

Treatment	Time of exposure					
	240 min		360 min		24h	
	AO	CC	AO	CC	AO	CC
Spinetoram 90 g	0.1 aA	3.3 bcA	0.3 aB	6.1 aA	2.4 bcB	10.0 aA
Spinetoram 120 g	1.7 aA	2.8 bcA	1.9 aB	7.8 aA	9.0 aA	10.0 aA
Spinetoram 150 g	2.4 aB	8.3 aA	3.3 aB	9.2 aA	9.6 aA	10.0 aA
Spinetoram 180 g	0.2 aB	5.0 abA	0.4 aB	8.4 aA	6.8 abA	10.0 aA
Control	0.0 aA	0.0 cA	0.0 aA	0.0 bA	0.0 cA	1.2 bA

Original means within columns followed by the same lower case letter are not significantly different (ANOVA – Tukey´s test, *P<0.05*). Means within rows followed by the same upper case letter in each time of exposure are not significantly different (ANOVA – Tukey´s test, *P<0.05*).

Table 2. The number of dead females and males (*n* = 5) of *Anastrepha obliqua* (AO) and *Ceratitis capitata* (CC), 24 hours after initial exposure to spinetoram toxic baits (g of commercial product/1,000 liters of water) in the laboratory.

Treatment	AO		CC	
	Female	Male	Female	Male
Spinetoram 90 g	0.7 bc	1.7 bc	5.0 a	5.0 a
Spinetoram 120 g	4.4 a	4.6 a	5.0 a	5.0 a
Spinetoram 150 g	4.9 a	4.7 a	5.0 a	5.0 a
Spinetoram 180 g	3.3 ab	3.5 ab	5.0 a	5.0 a
Control	0.0 c	0.0 c	1.0 b	0.2 b

Original means within columns followed by the same lower case letter are not significantly different (ANOVA – Tukey´s test, *P<0.05*).

Table 3. The number of dead females (n = 10) of *Ceratitis capitata* 24 hours after exposure to collected mandarin leaves with spinetoram toxic baits (g of commercial product/1,000 liters of water) under laboratory conditions (residual effects).

Treatment	Days after application					
	1	3	7	15	22	30
Spinetoram 90 g	8.7 aA	9.8 aA	8.8 aA	9.6 aA	9.1 aA	0.2 bB
Spinetoram 120 g	9.0 aA	9.7 aA	9.1 aA	9.7 aA	7.7 aA	8.5 aA
Spinetoram 150 g	8.7 aB	9.6 aA	9.5 aA	9.1 aA	9.3 aA	8.7 aA
Spinetoram 180 g	8.4 aB	9.8 aA	8.8 aB	9.9 aA	8.6 aA	8.6 aA
Control	0.1 bA	0.1 bA	0.0 bA	0.0 bA	0.2 bA	0.0 bA

Original means within columns followed by the same lower case letter are not significantly different (ANOVA – Tukey´s test, $P<0.05$). Means within rows followed by the same upper case letter in each time of exposure are not significantly different (ANOVA – Tukey´s test, $P<0.05$).

Fig. 1. Females of *Anastrepha obliqua* (A) and *Ceratitis capitata* (B); feeding of *C. capitata* (C); Dead adults inside the container (D).

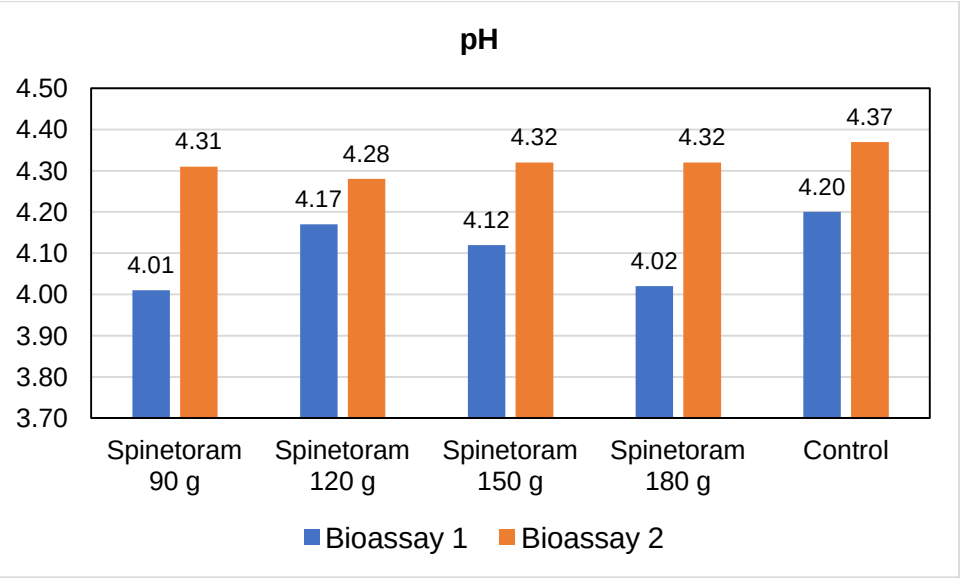

Fig. 2. Initial pH values of spinetoram toxic bait solutions (g of commercial product/1,000 liters of water) presented to fruit flies in the laboratory. Bioassay 1 included dry food.

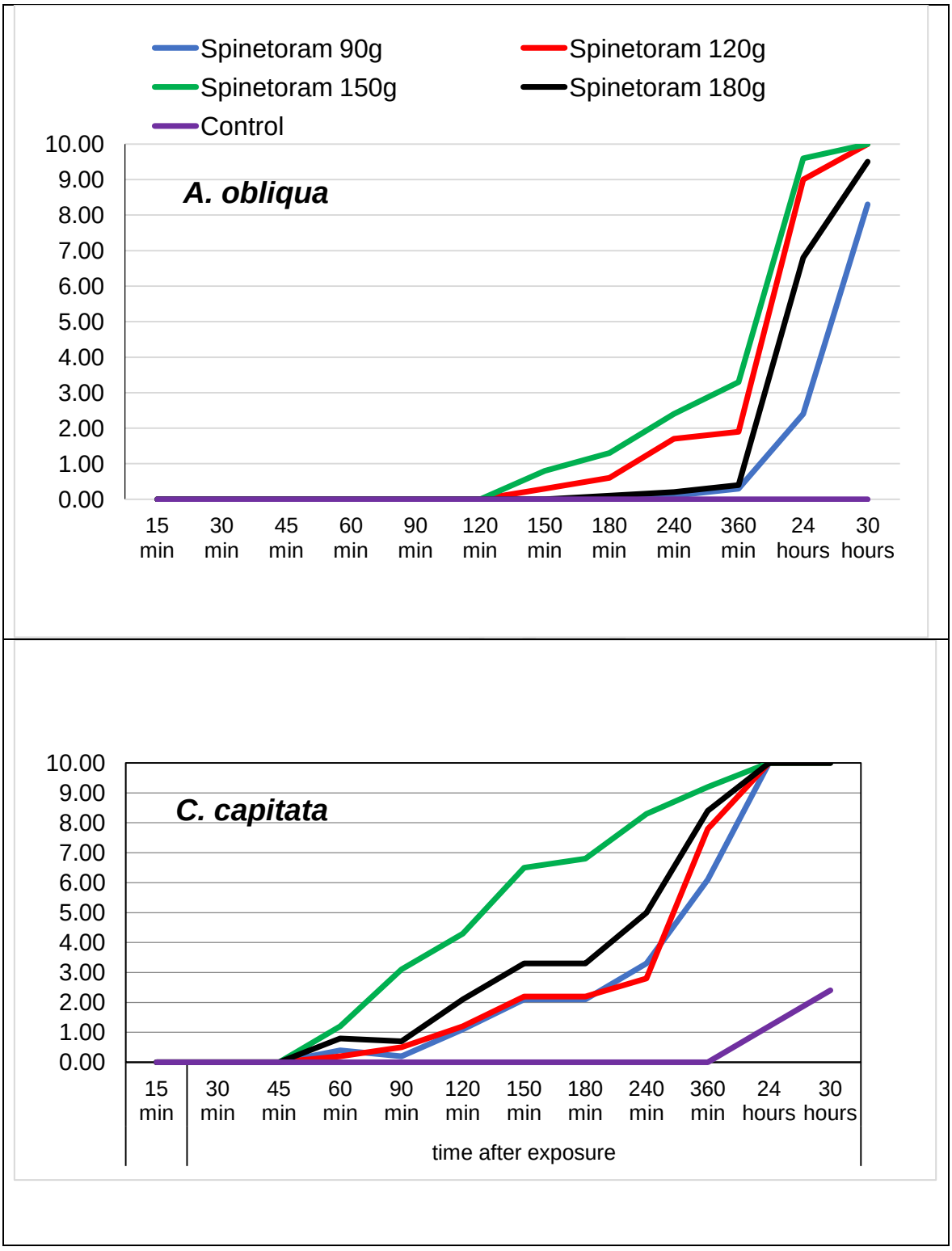

Fig. 3. The cumulative number of dead females and males of *Anastrepha obliqua* and *Ceratitis capitata* at different times after exposure to spinetoram toxic bait in the laboratory