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Efficacy of Nets Coated with Different Concentrations of Alpha-Cypermethrin against Two Major Pests of Stored Tobacco

Christos G. Athanassiou [1], Maria K. Sakka [1,*], Christos I. Rumbos [1], Stefan Schaffert [2], Thorsten Sterz [2], Constantinos Bozoglou [3], Panos Klitsinaris [3] and James W. Austin [4]

[1] Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou Str., 38446 Volos, Greece
[2] BASF Crop Protection, BASF Plant Science, Speyerer Straße 2, 67117 Limburgerhof, Germany
[3] BASF Hellas, Paradissou 2 and Kifissias, 15125 Athens, Greece
[4] BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709-3528, USA
* Correspondence: msakka@uth.gr; Tel.: +30-2421093195

Abstract: In the present study, we examined the insecticidal effect of Carifend® (BASF AG, Ludwigshafen, Germany; 163.2 mg m^{-2}), an alpha-cypermethrin-coated polyester net, as well as Carifend-like nets containing different rates of alpha-cypermethrin, specifically 10, 30, 80, and 325 mg m^{-2}, against two major stored tobacco insect pests, i.e., *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) and *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae). The nets were glued at the bottom of plastic Petri dishes, and adults of *L. serricorne* and *E. elutella* were exposed to the nets for different exposure times (e.g., 1, 5, 15, 30, 60, 90, and 120 min, as well as 12 and 24 h). After each exposure interval, insect mortality was recorded, whereas after the final evaluation, alive individuals were transferred to untreated dishes, and delayed mortality was recorded after an additional period of 1, 3, 5, and 7 d. Based on our results, efficient control of both insect species was achieved with all nets, even the one containing a lower concentration of alpha-cypermethrin (10 mg m^{-2}). High mortality rates were recorded for all treatments, indicating that even short exposure of adults of both tested species to the alpha-cypermethrin-coated nets tested can lead to mortality. For instance, at 30 mg m^{-2}, mortality of *E. elutella* reached 40% 1 d after exposure for 30 min. Moreover, at the highest concentration (325 mg m^{-2}), mortality reached 70% and 99% 7 d post exposure for 24 h of *L. serricorne* and *E. elutella*, respectively. Based on these data, Carifend®, as well as the rest of the Carifend-like nets tested, can be used as an alternative insecticide method for the control of two major stored tobacco insect species, i.e., *L. serricorne* and *E. elutella*. Insecticide treated nets can be used in areas in which fumigants and contact insecticides are not accessible.

Keywords: coated net; cypermethrin; *Ephestia elutella*; *Lasioderma serricorne*; stored-product insects

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Citation: Athanassiou, C.G.; Sakka, M.K.; Rumbos, C.I.; Schaffert, S.; Sterz, T.; Bozoglou, C.; Klitsinaris, P.; Austin, J.W. Efficacy of Nets Coated with Different Concentrations of Alpha-Cypermethrin against Two Major Pests of Stored Tobacco. *Agronomy* **2023**, *13*, 40. https://doi.org/10.3390/agronomy13010040

Academic Editor: Ivo Toševski

Received: 16 October 2022
Revised: 29 November 2022
Accepted: 16 December 2022
Published: 22 December 2022

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1. Introduction

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), and the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), are polyphagous species, causing serious losses and quantitative degradation of a wide spectrum of commodities [1–3]. Apart from the other commodities that they infest, they are considered to be the most important pests of stored tobacco [1,4,5]. For their control, several measures have been used in different types of facilities and commodities, such as insecticides, cold treatment, control atmospheres, heat treatment, insecticide-treated nets, and pheromones for mating disruption [1,6–16]. For instance, Amoah and Mahrroof [10] tested ozone against all life stages of *L. serricorne* and found that eggs are the most tolerant stage to ozone exposure. Athanassiou et al. [14] tested nitrogen treatment against eggs and larvae of *E. elutella* and found complete control at 38–43 °C after 3 d of exposure at <1% oxygen. Moreover, Athanassiou et al. [15] tested two commercially available pheromone traps for *L. serricorne* and *E. elutella* and found them effective in terms of insect capture. However, the most

popular method is fumigation. Phosphine fumigation can control different life stages of stored product insects and enables a residue-free treatment [1]. Nevertheless, populations of both species, but especially *L. serricorne*, have been found to be resistant to many of the currently most-used active ingredients for their control, such as phosphine [16–19]. For instance, Saglam et al. [16] noted that specific populations of *L. serricorne* were highly resistant to the fumigant phosphine, at exposures that are usually recommended for the control of this species in stored tobacco.

Recent studies have shown that an alternative way to apply contact insecticides, i.e., in impregnated or coated nets, can provide a viable control method in stored product protection. For instance, Ranabhat et al. [20] reported that exposure to controlled release materials with deltamethrin significantly reduced the movement of the lesser grain borer, *Rhyzopetha dominica* (Fabricius) (Coleoptera: Bostrihidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). In the case of stored tobacco, Carifend®, a net that is coated with the pyrethroid alpha-cypermethrin, has been successfully evaluated against both *L. serricorne* and *E. elutella*, in extensive laboratory, semi-field, and field tests [20,21]. The principle of using Carifend® is simple: the net is placed in such a way as to cover stored tobacco that has been previously fumigated. Athanassiou et al. [20] found that the application of Carifend® in a commercial storage facility was effective against both species even four months after its initial application.

The evaluation of insecticide-impregnated or -coated nets meets with a specific challenge: the estimation of the right amount of insecticide that should be used in order to obtain the desired efficacy level. Hence, higher concentrations of a given active ingredient may provide a rapid knockdown, which is not always desirable, as it can lead to the termination of the contact of the insect body with the toxic agent, which may cause increased recovery [22,23]. At the same time, high doses of certain insecticides may have a repulsive action that can force insects to seek alternative infestation pathways [24]. On the other hand, low insecticide doses may not be effective, and they can also cause sub-lethal effects and increased survival [25–27]. Thus, these nets should contain a "threshold" concentration of insecticide that will provide sufficient control without the shortcomings indicated above. In this context, the aim of the current study was to evaluate nets with different concentrations of alpha-cypermethrin than Carifend®, the commercial coated net that is registered for the protection of stored tobacco, in order to indicate immediate and delayed mortality patterns of *L. serricorne* and *E. elutella*. In this way, we study whether the standard Carifend® net can be effective at concentrations of alpha-cypermethrin that are different than those of the label dose.

2. Materials and Methods

2.1. Insects

Rearing of *L. serricorne* and *E. elutella* was carried out at the laboratory of Entomology and Agricultural Zoology, Department of Agriculture Crop Production and Rural Environment, University of Thessaly, at 26 °C and 55% relative humidity (r.h.) and continuous darkness. The rearing medium for both species was wholemeal wheat flour with 5% brewer's yeast. For both species, adults (<1 month old for *L. serricorne* and <1 week for *E. elutella*) were used in the bioassays.

2.2. Dish Preparation

Laboratory-made polyester net samples containing alpha-cypermethrin at different concentrations (10, 30, 80, 163.2, and 325 mg m^{-2} alpha-cypermethrin, respectively) were adjusted at the bottom of plastic petri dishes (1.5 cm high and 9 cm in diameter), with the internal layers covered with fluon to avoid escapes. Moreover, plastic dishes containing an untreated net served as controls. There were 3 dishes for each exposure and treatment combination, while the entire experiment was repeated 3 times (3 subreplicates × 3 replicates = 9 dishes for each treatment). All tests were carried out in incubator chambers set at

continuous darkness, 25 °C, and 75% relative humidity, using saturated salt solutions of sodium chloride.

2.3. Bioassays

Twenty adult beetles or moths of mixed sex were placed in each dish, with separate dishes for each species. Insects were exposed to the treated and untreated nets for 1, 5, 15, 30, 60, and 120 min and for 12 and 24 h. Mortality and knockdown effect were measured after the termination of each exposure. After these intervals, all alive individuals were removed and placed in untreated plastic dishes with 0.5 g of wheat flour as a food source, and delayed mortality was recorded after 1, 3, 5, and 7 d.

2.4. Statistical Analysis

For each species and treatment (0, 10, 30, 80, 163.5, and 325 mg m^{-2} of alpha-cypermethrin), mortality and knockdown data were submitted to one-way ANOVA to determine differences among exposure intervals, and means were separated by the Tukey–Kramer (HSD) test at the 5% level to compare mortality and knockdown counts from the different treatments. As the same dishes were examined for delayed mortality after 1, 3, 5, and 7 d, the mortality and knockdown counts of both species were analyzed using a repeated-measure ANOVA with exposure as the repeated measure variable and treatment and exposure interval as the main effects. SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA) was used for all analyses.

3. Results

3.1. Lasioderma serricorne

Generally, in most of the cases, the exposure interval did not significantly affect mortality levels, as in all cases low mortality levels (<1%) were recorded immediately after exposure (Table 1). In contrast, higher knockdown levels were noted for longer exposure intervals and for nets with higher alpha-cypermethrin concentration (Tables 1 and 2). At the highest doses (80, 163.5, and 325 mg m^{-2}), delayed mortality levels were higher than 70% 7 d after exposure for 24 h (Table 2). There was a general trend for high knockdown percentages at the first day of evaluation, which were gradually transformed to mortality. However, especially at the low dose rates, a high proportion of the knocked-down individuals managed to overcome the effect of the exposure to the treated nets when they were transferred to the untreated net (Table 2).

3.2. Ephestia elutella

As above, right after the exposure, mortality was low, whereas high knockdown percentages were observed even after the shorter periods of exposure (Table 1). As in the case of *L. serricorne*, the high knockdown percentages of *E. elutella* adults directly after exposure were gradually transformed to mortality after their removal from the treated net (Table 3). In this context, three days after exposure, in the vast majority of the cases, there were significant differences between the different exposure intervals in the different types of nets (Table 3). For instance, mortality 3 d after exposure for different intervals to the net with 325 mg m^{-2} ranged between 24% and 87%, whereas 24 h after exposure to different nets (0–325 mg m^{-2}) varied between 36% and 96% with significant differences noted among treatments (Table 3).

Table 1. Mean mortality and knock-down effect (% ± SE) of *Lasioderma serricorne* and *Ephestia elutella* adults exposed for 1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h to nets with different concentrations of alpha-cypermethrin (0, 10, 30, 80, 163.5, and 325 mg m^{-2}), evaluated immediately.

Exposure/Treatment	*Lasioderma serricorne*		*Ephestia elutella*	
	Mortality (%)	**Knocked-Down (%)**	**Mortality (%)**	**Knocked-Down (%)**
0 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0
5 min	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0
15 min	0.0 ± 0.0	0.0 ± 0.0bB	0.0 ± 0.0b	0.0 ± 0.0
30 min	0.0 ± 0.0	0.6 ± 0.6abB	0.0 ± 0.0b	0.0 ± 0.0C
60 min	0.0 ± 0.0	0.0 ± 0.0bC	0.0 ± 0.0b	0.0 ± 0.0B
90 min	0.0 ± 0.0	0.6 ± 0.6abC	0.0 ± 0.0b	0.0 ± 0.0C
120 min	0.0 ± 0.0	0.0 ± 0.0bC	0.0 ± 0.0b	0.0 ± 0.0C
240 min	0.0 ± 0.0	1.1 ± 0.7abC	0.0 ± 0.0b	0.0 ± 0.0D
12 h	0.0 ± 0.0	3.3 ± 1.2aC	0.0 ± 0.0bB	0.0 ± 0.0C
24 h	0.0 ± 0.0	2.8 ± 1.5abC	3.9 ± 2.2a	0.0 ± 0.0A
F	-	3.2	3.2	-
P	-	0.002	0.002	-
10 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c
5 min	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c
15 min	0.0 ± 0.0	0.0 ± 0.0bB	0.0 ± 0.0b	0.0 ± 0.0c
30 min	0.0 ± 0.0	7.2 ± 4.9bB	0.0 ± 0.0b	1.7 ± 0.8cC
60 min	0.0 ± 0.0	21.7 ± 9.3bC	0.0 ± 0.0b	28.9 ± 9.3 bcB
90 min	0.0 ± 0.0	56.1 ± 10.8B	0.0 ± 0.0b	43.9 ± 6.9 abB
120 min	0.0 ± 0.0	65.0 ± 6.9B	0.0 ± 0.0b	53.3 ± 10.9 abB
240 min	0.0 ± 0.0	67.2 ± 5.5B	0.0 ± 0.0b	40.0 ± 7.0abC
12 h	0.0 ± 0.0	71.7 ± 4.4B	3.9 ± 1.6aA	32.2 ± 6.4bB
24 h	0.0 ± 0.0	80.6 ± 3.7B	3.9 ± 1.8a	63.9 ± 8.8aA
F	-	34.3	4.5	14.2
P	-	<0.001	<0.001	<0.001
30 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0c
5 min	0.0 ± 0.0	0.6 ± 0.6c	0.0 ± 0.0b	0.0 ± 0.0c
15 min	0.0 ± 0.0	10.0 ± 5.3cB	0.0 ± 0.0b	0.0 ± 0.0c
30 min	0.0 ± 0.0	67.2 ± 9.8abA	0.0 ± 0.0b	40.0 ± 10.4 bB
60 min	0.0 ± 0.0	66.1 ± 8.5bB	0.0 ± 0.0b	89.4 ± 3.0aA
90 min	0.0 ± 0.0	76.7 ± 7.7abAB	0.0 ± 0.0b	93.3 ± 2.2aA
120 min	0.0 ± 0.0	77.8 ± 7.6abAB	0.0 ± 0.0b	92.8 ± 2.4aA
240 min	0.0 ± 0.0	86.7 ± 3.4abA	0.0 ± 0.0b	73.9 ± 5.1aB
12 h	0.0 ± 0.0	88.9 ± 3.4abA	1.7 ± 0.8bAB	78.3 ± 6.0aA
24 h	0.0 ± 0.0	93.3 ± 2.6aA	9.4 ± 3.2a	86.1 ± 4.9aA
F	-	41.7	8.2	79.2
P	-	<0.001	<0.001	<0.001
80 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0c
5 min	0.0 ± 0.0	1.7 ± 1.7b	0.0 ± 0.0b	0.0 ± 0.0c
15 min	0.0 ± 0.0	18.3 ± 6.6bA	0.0 ± 0.0b	33.3 ± 14.8b
30 min	0.0 ± 0.0	87.2 ± 4.0A	0.0 ± 0.0b	80.0 ± 4.1aA
60 min	0.0 ± 0.0	85.6 ± 5.3AB	0.0 ± 0.0b	84.4 ± 8.8aA
90 min	0.0 ± 0.0	87.2 ± 4.5A	0.0 ± 0.0b	75.6 ± 7.6aA
120 min	0.0 ± 0.0	89.4 ± 3.7A	0.0 ± 0.0b	88.9 ± 6.5aA

Table 1. *Cont.*

Exposure/Treatment	*Lasioderma serricorne*		*Ephestia elutella*	
	Mortality (%)	**Knocked-Down (%)**	**Mortality (%)**	**Knocked-Down (%)**
240 min	0.0 ± 0.0	84.4 ± 5.9A	0.0 ± 0.0b	98.3 ± 1.7aA
12 h	0.0 ± 0.0	89.4 ± 3.6A	0.0 ± 0.0bB	94.4 ± 3.4aA
24 h	0.0 ± 0.0	93.3 ± 2.0A	16.1 ± 5.6a	82.8 ± 5.4aA
F	-	90.0	8.3	31.6
P	-	<0.001	<0.001	<0.001
163.5 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0d	0.0 ± 0.0b	0.0 ± 0.0c
5 min	0.0 ± 0.0	5.6 ± 3.8d	0.0 ± 0.0b	0.0 ± 0.0c
15 min	0.0 ± 0.0	62.2 ± 8.0cA	0.0 ± 0.0b	30.0 ± 15.0bc
30 min	0.0 ± 0.0	76.7 ± 5.5bcA	0.0 ± 0.0b	57.8 ± 11.8abAB
60 min	0.0 ± 0.0	80.6 ± 6.7abcAB	0.0 ± 0.0b	66.1 ± 9.3abA
90 min	0.0 ± 0.0	86.1 ± 2.7abA	0.0 ± 0.0b	77.2 ± 8.6aA
120 min	0.0 ± 0.0	82.8 ±3.4abAB	0.0 ± 0.0b	73.3 ± 6.2aAB
240 min	0.0 ± 0.0	96.7 ± 2.2abA	0.0 ± 0.0b	78.3 ± 4.2aB
12 h	0.6 ± 0.6	98.3 ± 0.8A	0.0 ± 0.0bB	88.9 ± 4.0aA
24 h	0.0 ± 0.0	96.1 ± 2.7A	17.2 ± 17.3a	82.8 ± 5.8aA
F	1.0	71.2	8.9	17.8
P	0.447	<0.001	<0.001	<0.001
325 mg m^{-2}				
1 min	0.0 ± 0.0	0.0 ± 0.0c	0.0 ± 0.0b	0.0 ± 0.0c
5 min	0.0 ± 0.0	12.2 ± 6.1c	0.0 ± 0.0b	0.0 ± 0.0c
15 min	0.0 ± 0.0	47.2 ± 11.7bA	0.0 ± 0.0b	0.0 ± 0.0c
30 min	0.0 ± 0.0	77.2 ± 42.2	0.0 ± 0.0b	26.1 ± 9.7bBC
60 min	0.0 ± 0.0	93.9 ± 3.2A	0.0 ± 0.0b	69.4 ± 5.7aA
90 min	0.0 ± 0.0	82.2 ± 3.9A	0.0 ± 0.0b	75.5 ± 4.7aA
120 min	0.0 ± 0.0	92.8 ± 2.2	0.0 ± 0.0b	69.4 ± 3.6aAB
240 min	0.0 ± 0.0	95.0 ± 2.6A	0.0 ± 0.0b	78.3 ± 6.3aB
12 h	0.0 ± 0.0	99.4 ± 0.5A	0.6 ± 0.6bB	88.9 ± 3.6aA
24 h	0.6 ± 0.6	97.8 ± 1.2A	12.8 ± 5.1a	86.1 ± 4.9aA
F	1.0	56.4	6.1	60.6
P	0.447	<0.001	<0.001	<0.001

For each treatment (0, 10, 30, 80, 163.5, and 325mg m^{-2}), within each column, means followed by the same lowercase letter do not differ significantly (in all cases, df = 9, 89; Tukey HSD test at $P = 0.05$). Where no letters exist, no significant differences were noted. Within each column, for each exposure interval (1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h), means followed by the same uppercase letter do not differ significantly (in all cases, df = 5, 53; Tukey HSD test at $P = 0.05$).

Table 2. Mean mortality and knock-down effect (% ± SE) of *Lasioderma serricorne* adults exposed for 1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h to nets with different concentrations of alpha-cypermethrin (0, 10, 30, 80, 163.5, and 325mg m^{-2}) evaluated 1, 3, 5, and 7 d after exposure (delayed effect).

Exposure/ Treatment	Delayed Effect							
	Day 1		Day 3		Day 5		Day 7	
	Mortality (%)	**Knocked-Down (%)**	**Mortality (%)**	**Knocked-Down (%)**	**Mortality (%)**	**Knocked-Down (%)**	**Mortality (%)**	**Knocked-Down (%)**
0 mg m^{-2}								
1 min	0.0 ± 0.0	0.0 ± 0.0 C	0.0 ± 0.0	2.8 ± 1.2C	2.8 ± 1.5	4.4 ± 1.5B	7.8 ± 3.8	2.8 ± 1.5B
5 min	1.1 ± 0.7	5.0 ± 2.9 B	5.6 ± 2.1	9.4 ± 4.2B	12.8 ± 3.9	8.3 ± 2.6	16.1 ± 3.4	11.7 ± 3.1
15 min	0.0 ± 0.0	5.0 ± 1.4 B	7.2 ± 2.5	8.9 ± 2.6B	16.7 ± 4.6	7.8 ± 2.1C	16.7 ± 4.4	6.7 ± 2.4
30 min	0.6 ± 0.6	1.1 ± 1.1 C	5.0 ± 2.2	4.4 ± 1.8C	11.7 ± 2.4AB	5.0 ± 2.2C	13.9 ± 4.0CD	4.4 ± 2.1C
60 min	0.6 ± 0.6	1.7 ± 0.8 B	3.9 ± 1.4	4.4 ± 1.8B	10.6 ± 2.8	11.1 ± 2.9BC	17.8 ± 4.7	10.6 ± 2.7AB
90 min	0.0 ± 0.0	2.8 ± 1.2 C	2.8 ± 1.2	2.8 ± 1.7C	8.3 ± 0.8B	6.1 ± 2.3B	16.7 ± 2.4B	7.8 ± 2.2B

Table 2. *Cont.*

Exposure/ Treatment	Delayed Effect							
	Day 1		Day 3		Day 5		Day 7	
	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)
120 min	2.2 ± 1.7	5.6 ± 3.9 C	2.8 ± 1.7	7.8 ± 3.6B	10.6 ± 4.6A	6.7 ± 2.8B	14.4 ± 6.6AB	10.0 ± 4.4B
240 min	0.6 ± 0.6B	5.6 ± 2.8 C	3.3 ± 1.7A	8.3 ± 6.1B	11.7 ± 3.3BC	9.4 ± 4.3B	18.3 ± 4.9BC	12.8 ± 4.1
12 h	0.6 ± 0.6	2.2 ± 1.2 C	2.8 ± 1.5	6.1 ± 3.5C	12.8 ± 5.6C	7.8 ± 3.1B	17.8 ± 7.8B	10.0 ± 3.8
24 h	1.1 ± 0.7	1.7 ± 1.2 C	4.4 ± 1.8B	4.4 ± 1.9C	6.7 ± 2.8C	6.1 ± 2.2	11.7 ± 6.1B	5.0 ± 2.2
F	0.9	1.1	1.2	0.6	1.2	0.6	0.4	1.2
P	0.528	0.400	0.262	0.773	0.331	0.816	0.918	0.275
10mg m^{-2}								
1 min	0.6 ± 0.6	3.3 ± 1.9 cC	3.9 ± 1.8	10.0 ± 3.3C	10.0 ± 3.3	14.4 ± 5.4AB	21.7 ± 6.4	7.8 ± 3.5AB
5 min	2.8 ± 1.9	5.6 ± 4.0 cB	11.0 ± 1.1	9.4 ± 2.8B	5.6 ± 2.3	15.6 ± 3.8	17.2 ± 4.5	11.7 ± 4.9
15 min	0.0 ± 0.0	11.1 ± 5.0 cB	5.0 ± 1.7	8.9 ± 3.0B	6.7 ± 1.4	13.9 ± 5.8BC	13.9 ± 2.3	13.9 ± 4.9
30 min	0.0 ± 0.0	11.1 ± 5.3 cC	4.4 ± 2.1	5.6 ± 3.4C	4.4 ± 1.8B	3.9 ± 2.3C	8.3 ± 2.8D	5.6 ± 2.7BC
60 min	1.1 ± 0.7	5.6 ± 1.8 cB	3.9 ± 1.1	6.7 ± 1.9B	9.4 ± 2.7	3.9 ± 1.8C	13.9 ± 4.1	3.3 ± 1.7B
90 min	0.0 ± 0.0	11.7 ± 4.4cC	5.6 ± 2.1	15.6 ± 6.4C	12.8 ± 2.4AB	9.4 ± 3.4B	18.9 ± 2.5B	11.1 ± 3.0B
120 min	0.6 ± 0.6	23.3 ± 5.8bcBC	1.1 ± 1.1	7.2 ± 2.5B	6.7 ± 2.8	8.3 ± 3.3B	13.3 ± 5.2B	8.9 ± 2.9B
240 min	0.6 ± 0.6B	16.1 ± 3.1cC	3.9 ± 1.7A	8.9 ± 4.4B	8.9 ± 4.6C	13.9 ± 5.0B	12.8 ± 5.6C	8.9 ± 3.1
12 h	0.6 ± 0.6	37.8 ± 6.7bB	3.9 ± 2.3	12.8 ± 5.5C	6.1 ± 3.2C	18.9 ± 10.3AB	11.1 ± 4.8B	13.3 ± 6.1
24 h	0.6 ± 0.6	59. 4 ± 6.0aB	3.9 ± 2.0B	16.7 ± 7.3BC	10.6 ± 5.1C	13.9 ± 6.4	12.2 ± 5.3B	9.4 ± 4.5
F	1.2	14.3	0.7	0.7	0.7	0.9	0.7	0.7
P	0.311	<0.001	0.711	0.694	0.716	0.531	0.671	0.701
30 mg m^{-2}								
1 min	0.0 ± 0.0	4.4 ± 2.3dC	2.2 ± 1.2	6.7 ± 2.6C	7.8 ± 2.5	10.0±4.9bAB	16.1±4.5	10.0 ± 4.0AB
5 min	1.1 ± 0.7	5.0 ± 1.9dB	3.3 ± 1.7	7.8 ± 2.4B	8.3 ± 2.2	15.0 ±4.6ab	15.6±2.7	7.2 ± 2.8
15 min	0.6 ± 0.6	31.1± 9.9bcdB	1.7 ± 0.8	10.6 ± 2.8B	7.2 ± 2.2	16.1 ± 5.4abABC	10.6±2.7	16.7 ± 5.9
30 min	0.0 ± 0.0	45.6 ± 12.4bcB	12.2 ± 4.8	26.7±10.3BC	17.8 ± 6.8AB	10.0 ± 4.7bC	22.2 ± 7.0BCD	11.1 ± 3.1BC
60 min	0.6 ± 0.6	24.4 ± 10.9cdB	10.0 ± 4.5	3.3 ± 1.9B	13.3 ± 6.0	5.6 ± 2.3bC	21.7 ± 8.3	12.2 ± 3.5AB
90 min	1.7 ± 1.2	40.6 ± 9.6bcdB	12.8± 4.6	24.4 ± 9.9BC	19.4 ± 6.2AB	21.7± 8.9abAB	28.3 ± 8.9AB	16.1 ± 4.0AB
120 min	1.1 ± 0.7	46.1 ± 11.8bcB	15.6 ± 5.4	17.2 ± 6.1B	23.9 ± 5.7	16.1 ± 7.4abAB	38.9 ± 10.2AB	13.9 ± 2.7AB
240 min	0.6 ± 0.6B	58.9 ± 6.9abcB	11.7 ± 5.5A	8.9 ± 2.9B	16.1 ± 6.2ABC	8.3 ± 1.9bB	21.7 ± 7.3ABC	8.9 ± 2.2
12 h	0.0 ± 0.0	66.1± 8.2abAB	9.4 ± 5.4	20.6±5.9BC	16.7 ± 6.7BC	15.6 ± 3.5abAB	20.6 ± 6.2B	12.8 ± 2.4A
24 h	0.0 ± 0.0	91.7 ± 3.2aA	12.2 ± 5.1AB	61.7 ± 8.2bA	30.0 ± 6.6BC	35.6 ±6.9a	32.8±6.3B	22.8 ± 6.7
F	1.0	9.9	1.3	7.7	1.8	2.4	1.5	1.3
P	0.439	<0.001	0.242	<0.001	0.061	0.017	0.151	0.264
80 mg m^{-2}								
1 min	1.1 ± 0.7	30.6 ± 5.4cB	2.8 ± 1.9	21.7 ±4.2bBC	10.0 ± 1.7c	24.4 ± 4.1cAB	18.9 ± 4.1c	13.9 ±3.4AB
5 min	2.2 ± 2.2	61.7 ± 7.1bA	9.4 ± 4.2	35.6 ±6.9abA	20.0 ± 6.4bc	28.9 ± 7.8bc	22.8 ± 7.2c	18.9 ± 5.1
15 min	6.1 ± 3.8	75.0 ± 6.0bA	12.2 ± 5.9	37.8 ±4.3bA	29.4 ± 6.7abc	38.9 ± 8.0abcA	36.7 ± 7.2abc	27.8 ± 6.7
30 min	1.1 ± 0.7	86.1 ± 4.5A	14.4 ± 3.7	60.0 ± 6.9A	24.4 ± 3.6bcAB	56.1 ± 6.7aA	38.9 ± 5.8abcABC	33.3 ± 5.1A
60 min	2.8 ± 1.9	84.4 ± 4.9A	21.1 ± 9.9	52.8 ± 8.9abA	29.4 ± 22.2abc	43.9 ± 10.4abcA	34.4 ± 11.1bc	33.3 ± 8.2A
90 min	3.9 ± 1.8	83.9 ± 3.7A	22.2 ± 10.4	57.8 ± 10.0AB	41.7 ± 9.8abcAB	47.2 ± 9.2abA	50.6 ± 7.7abcA	34.4 ± 7.0A
120 min	1.7 ± 1.2	90.6 ± 2.3 A	25.0 ± 8.3	58.9 ± 9.1A	40.6 ± 9.7abc	48.9 ±10.2abA	52.8 ± 9.9abcA	23.9 ±7.2AB
240 min	0.0 ± 0.0B	84.4 ± 5.4 A	16.1 ± 5.8A	65.6 ± 6.4A	30.0± 10.0abcABC	53.9 ± 8.8aA	34.4 ± 9.5bcABC	31.1 ± 4.7
12 h	3.3 ± 1.9	87.8 ± 1.9 A	23.3 ± 9.2	58.3 ± 7.8AB	49.4 ± 9.4 abAB	33.9 ± 6.6abcAB	62.2 ± 6.4abA	21.1 ± 4.8
24 h	2.2 ± 1.5	90.6 ± 2.6 A	34.4 ± 5.9AB	50.6 ± 5.7abAB	63.3 ± 7.1aA	27.8 ± 6.0bc	72.2 ± 6.2aA	17.8 ± 4.8
F	0.9	16.0	1.6	3.6	3.6	2.0	4.7	1.6
P	0.561	<0.001	0.119	0.001	0.001	0.052	<0.001	0.125
163.5 mg m^{-2}								
1 min	0.0 ± 0.0	71.7 ± 7.3A	22.2 ± 10.8	32.2 ± 8.6AB	31.1 ± 13.0	20.0 ± 6.1AB	36.7 ± 12.4	22.8 ± 4.9A
5 min	5.6 ± 2.9	65.0 ± 4.2A	22.2 ± 10.6	35.6 ± 6.7A	31.7 ± 11.4	23.3 ± 6.7	36.1 ± 12.4	17.8 ± 4.6
15 min	7.2 ± 5.0	70.6 ± 5.5A	12.2 ± 7.0	51.7 ± 7.2A	26.1 ± 11.0	34.4 ± 6.6AB	36.1 ± 11.4	25.6 ± 5.7
30 min	8.3 ± 4.8	78.3 ± 4.5A	24.4 ± 8.6	62.2 ± 7.6A	40.6 ± 11.6A	38.9 ± 8.3AB	52.8 ± 9.2A	18.3 ± 5.1ABC
60 min	11.7 ± 5.1	74.4 ± 3.7A	26.7 ± 11.8	53.9 ± 9.5A	39.4 ± 12.1	36.7 ± 8.3AB	46.1 ± 11.9	32.8 ± 7.4A
90 min	10.6 ± 5.4	76.7 ± 7.1A	27.2±13.0	63.3 ± 12.8A	40.6 ± 12.1AB	47.2 ± 10.5A	56.1 ± 10.0A	29.4 ± 6.9AB
120 min	11.7 ± 4.7	76.7 ± 6.3A	26.1 ± 9.8	59.4 ± 10.4A	30.0 ± 11.2	48.9 ± 10.8A	42.8 ± 11.0AB	35.6 ± 8.1A
240 min	11.1 ± 4.5A	86.1 ± 4.3A	30.6 ± 9.8A	61.7 ± 10.8A	46.1 ± 13.0AB	49.4 ± 12.9A	56.7 ± 13.2A	30.6 ± 10.9
12 h	20.0 ± 10.1	76.1 ± 9.5A	41.7 ± 14.6	56.1 ± 14.1AB	66.7 ± 9.0A	26.1 ± 7.8AB	82.8 ± 5.8A	12.8 ± 4.1
24 h	18.9 ± 10.5	80.6 ± 10.4AB	33.3 ± 16.6AB	63.3 ± 11.1A	60.0 ± 11.7AB	35.0 ± 11.5	77.2 ± 8.7A	17.2 ± 8.9
F	0.9	0.7	0.5	1.2	1.3	1.3	2.4	1.2
P	0.484	0.659	0.872	0.279	0.258	0.248	0.018	0.300
325 mg m^{-2}								
1 min	0.0 ± 0.0	75.0 ± 4.6A	14.4 ± 7.9	47.2 ± 7.2A	23.9 ± 9.6	27.2 ± 5.8A	31.1 ± 9.6	18.9 ± 4.9AB
5 min	4.4 ± 2.9	57.8 ± 8.2A	17.8 ± 7.7	38.9 ± 3.9A	30.6 ± 10.5	23.3 ± 3.8	42.8 ± 10.1	17.2 ± 4.6
15 min	6.1 ± 4.5	62.2 ± 7.6A	16.1 ± 7.8	45.0 ± 6.9A	28.9 ± 10.9	18.9 ± 5.0ABC	34.4 ± 10.3	16.7 ±4.0
30 min	11.7 ± 6.1	75.0 ± 9.6AB	22.8 ± 10.7	54.4 ± 10.8AB	33.3 ± 13.0AB	23.3 ± 6.9BC	45.0 ± 11.1AB	25.6 ± 8.1AB
60 min	12.8 ± 6.9	79.4 ± 6.4A	25.6 ± 12.0	63.9 ± 10.8A	38.3 ± 14.2	42.2 ± 11A	45.0 ± 13.4	33.9 ± 9.4A
90 min	8.9 ± 4.5	73.9 ± 5.7A	23.9 ± 9.3	61.7 ± 8.4AB	46.1 ± 10.4A	34.4 ± 7.9AB	57.2 ± 8.1A	24.4 ± 7.6AB
120 min	11.7 ± 5.4	82.2 ± 5.7A	22.8 ± 9.8	66.7 ± 9.7A	38.3 ± 12.2	46.7 ± 11.6A	46.7 ± 11.2AB	32.2 ± 7.8AB
240 min	6.7 ± 3.7AB	86.1 ± 3.0A	28.3 ± 10.4	61.7 ± 8.7A	48.3 ± 10.7A	31.1 ± 6.9AB	55.0 ± 10.0AB	26.7 ± 5.8
12 h	25.0 ± 12.7	71.1 ± 11.9A	31.7 ± 15.2	60.0 ± 13.7A	48.3 ± 12.9AB	40.6 ± 10.5A	58.9 ± 11.3A	27.2 ±7.1
24 h	15.6 ± 7.4	82.2 ± 7.1AB	42.2 ± 12.0A	52.2 ± 11.4A	58.9 ± 10.1AB	31.7 ± 9.4	70.6 ± 8.3A	20.6 ± 7.9
F	1.2	1.4	0.6	0.9	0.9	1.2	1.3	0.7
P	0.305	0.174	0.790	0.508	0.550	0.303	0.251	0.666

For each treatment (0, 10, 30, 80, 163.5, and 325mg m^{-2}), within each column, means followed by the same lowercase letter do not differ significantly (in all cases, df = 9, 89; Tukey HSD test at $P = 0.05$). Where no letters exist, no significant differences were noted. Within each column, for each exposure interval (1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h), means followed by the same uppercase letter do not differ significantly (in all cases, df = 5, 53; Tukey HSD test at $P = 0.05$).

Table 3. Mean mortality and knock-down effect ± SE of *Ephestia elutella* adults exposed for 1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h to nets with different concentrations of alpha-cypermethrin (0, 10, 30, 80, 163.5, and 325mg m^{-2}) after 1, 3, 5, and 7 d of exposure (delayed effect).

Exposure/ Treatment	Delayed Effect							
	Day 1		Day 3		Day 5		Day 7	
	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)	Mortality (%)	Knocked-Down (%)
0 mg m^{-2}								
1 min	0.0 ± 0.0bB	0.0 ± 0.0bB	12.2 ± 3.6	5.6 ± 2.1CD	36.1 ± 8.1B	16.1 ± 4.5AB	68.3 ± 6.8	7.8 ± 1.9
5 min	0.0 ± 0.0b	0.0 ± 0.0bC	16.1 ± 4.0B	13.9 ± 3.8	41.7 ± 7.6B	27.8 ± 6.2A	82.8 ± 3.7	3.9 ± 1.8
15 min	0.0 ± 0.0bB	0.0 ± 0.0bC	17.8 ± 5.0B	6.7 ± 2.3	37.8 ± 6.3B	17.2 ± 2.1	74.4 ± 7.3	4.4 ± 2.1
30 min	0.0 ± 0.0b	2.2 ± 1.2abB	17.8 ± 3.1C	7.8 ± 2.6	45.6 ± 8.9D	18.3 ± 5.6	77.2 ± 4.9C	5.0 ± 2.8
60 min	0.6 ± 0.6ab	2.8 ± 1.5abD	23.3 ± 4.7B	15.0 ± 5.1	57.8 ± 6.6C	14.4 ± 3.5	86.7 ± 2.2BCD	4.4 ± 1.0
90 min	2.2 ± 1.2abB	0.0 ± 0.0bD	25.0 ± 7.0C	20.5 ± 4.7	51.7 ± 9.5B	10.6 ± 2.7	75.6 ± 5.5B	2.8 ± 1.2
120 min	2.8 ± 1.7abB	1.1 ± 1.1abC	29.4 ± 6.1B	15.0 ± 2.2	63.3 ± 4.4B	11.7 ± 2.9AB	86.7 ± 2.5B	6.7 ± 1.9AB
240 min	2.2 ± 1.2abA	0.6 ± 0.6bC	23.3 ± 6.1C	6.7 ± 1.7	53.9 ± 6.0C	10.0 ± 3.1	72.2 ± 2.9B	5.6 ± 1.3AB
12 h	6.1 ± 2.8abB	1.1 ± 0.7abB	25.6 ± 3.3C	7.8 ± 3.2B	51.1 ± 3.1C	20.0 ± 4.5	80.5 ± 2.8C	3.9 ± 1.8AB
24 h	6.7 ± 2.6aD	6.1 ± 2.7abC	35.6 ± 7.4C	15.0 ± 4.5	64.4 ± 7.2C	10.0 ± 3.9	88.3 ± 3.6	4.4 ± 2.3
F	3.1	2.8	1.7	2.2	2.0	1.9	2.2	0.6
P	0.003	0.006	0.097	0.030	0.048	0.063	0.029	0.792
10 mg m^{-2}								
1 min	1.7 ± 1.2cAB	1.1 ± 0.7bB	23.3 ± 5.2bc	17.2 ± 4.4ABC	53.3 ± 7.0AB	15.0 ± 4.4AB	84.4 ± 3.2	9.4 ± 2.7
5 min	5.0 ± 2.2bc	1.7 ± 1.2bC	20.6 ± 4.5cAB	16.7 ± 5.3	52.2 ± 7.4AB	13.9 ± 6.0AB	81.7 ± 4.8	4.4 ± 1.7
15 min	3.9 ± 2.3bcAB	3.9 ± 1.8bC	30.0 ± 8.0abcAB	9.4 ± 2.4	60.0 ± 8.0AB	12.8 ± 4.6	84.4 ± 3.8	8.3 ± 3.8
30 min	7.8 ± 3.5bc	13.9 ± 5.8abB	29.4 ± 7.7abcC	20.6 ± 5.2	58.9 ± 8.2CD	15.0 ± 6.2	77.2 ± 4.2C	11.7 ± 5.1
60 min	7.8 ± 2.1bc	24.4 ± 9.7abCD	35.6 ± 9.0abcB	24.4 ± 5.9	65.5 ± 7.2BC	8.9 ± 2.8	81.7 ± 5.4D	8.9 ± 3.8
90 min	7.8 ± 1.5bcAB	12.8 ± 4.6abCD	27.2 ± 5.8bcBC	20.5 ± 3.6	47.8 ± 5.7B	23.9 ± 7.8	77.8 ± 3.1B	7.2 ± 1.9
120 min	8.3 ± 4.2bcB	12.8 ± 4.4abC	29.4 ± 6.2abcB	22.2 ± 5.6	63.3 ± 5.0B	20.6 ± 5.6A	85.5 ± 3.2B	7.8 ± 2.2A
240 min	13.3 ± 2.6bcAB	23.3 ± 8.2abBC	52.8 ± 7.8abBC	12.2 ± 2.8	76.1 ± 6.2ABC	6.7 ± 4.0	86.7 ± 4.9AB	5.0 ± 1.7AB
12 h	17.8 ± 6.2 abAB	16.1 ± 4.5abB	32.8 ± 7.0 abcBC	22.2 ± 7.7AB	63.9 ± 5.1BC	18.9 ± 4.5	84.4 ± 3.5BC	10.0 ± 3.6A
24 h	30.6 ± 4.4aCD	34.4 ± 4.3aAB	60.6 ± 5.8aBC	13.3 ± 2.9	76.7 ± 5.3BC	3.9 ± 1.8	86.7 ± 4.9	3.9 ± 2.2
F	6.3	4.1	3.4	1.0	2.1	1.5	0.7	0.7
P	<0.001	<0.001	0.001	0.415	0.040	0.155	0.745	0.720
30 mg m^{-2}								
1 min	7.2 ± 2.6bcA	3.9 ± 2.2f	28.9 ± 9.3abc	7.2 ± 2.9BCD	44.4 ± 10.3bcAB	20.0 ± 5.9AB	77.8 ± 5.3	10.0 ± 4.4
5 min	4.4 ± 2.3bc	8.3 ± 3.5efBC	19.4 ± 4.0cAB	16.1 ± 5.8	46.1 ± 4.8bcAB	12.8 ± 4.0AB	73.9 ± 5.0	10.5 ± 3.5
15 min	3.9 ± 1.4cAB	13.3 ± 4.1defBC	24.4 ± 6.5bcAB	7.2 ± 3.9	40.0 ± 8.7cB	18.9 ± 5.1	69.4 ± 8.1	14.4 ± 6.3
30 min	19.4 ± 8.9abc	19.4 ± 5.2cdefB	40.6 ± 11.6abcBC	17.8 ± 6.2	63.3 ± 10.4abcBCD	21.1 ± 7.9	83.3 ± 5.6BC	13.3 ± 5.1
60 min	14.4 ± 6.0abc	29.4 ± 4.6bcdeCD	38.3 ± 10.1abcB	24.4 ± 10.7	63.9 ± 7.1abcBC	17.8 ± 6.8	85.6 ± 1.9CD	2.8 ± 1.2
90 min	27.8 ± 7.4abA	33.3 ± 5.8abcdBC	58.3 ± 12.2abcAB	18.3 ± 3.6	73.9 ± 10.9abcAB	12.2 ± 5.5	85.6 ± 5.6AB	10.0 ± 4.3
120 min	33.3 ± 7.9abA	37.8 ± 4.8abcB	65.6 ± 12.4abA	17.8 ± 7.1	88.3 ± 5.4aA	6.7 ± 3.6AB	93.9 ± 3.2AB	5.0 ± 2.9AB
240 min	18.3 ± 7.2abcAB	52.8 ± 5.6aAB	54.4 ± 10.6abcBC	15.0 ± 5.7	64.4 ± 9.9abcA	15.6 ± 5.8	80.0 ± 7.6B	13.3 ± 7.0A
12 h	21.1 ± 5.1abcAB	49.4 ± 5.9abA	48.3 ± 8.2abcABC	25.0 ± 9.5AB	73.9 ± 5.8abcAB	15.6 ± 6.3	90.0 ± 2.6ABC	5.0 ± 2.0AB
24 h	37.2 ± 8.7aBC	43.9 ± 5.4abA	71.1 ± 7.7aAB	10.5 ± 3.8	81.1 ± 5.6abABC	8.9 ± 3.2	90.0 ± 4.6	5.0 ± 2.2
F	3.4	12.8	3.3	0.9	3.8	0.7	2.1	1.0
P	0.001	<0.001	0.002	0.497	<0.001	0.686	0.040	0.480
80 mg m^{-2}								
1 min	3.3 ± 1.7cAB	8.9 ± 5.0dB	25.6 ± 5.8d	20.6 ± 4.2abAB	46.7 ± 7.5cAB	31.7 ± 5.1aA	78.3 ± 6.0b	8.3 ± 3.5
5 min	15.5 ± 7.3bc	38.3 ± 4.3bcdA	48.3 ± 9.6cdA	23.9 ± 5.6ab	72.8 ± 7.6bA	13.3 ± 4.7abcAB	87.8 ± 4.1ab	7.8 ± 3.6
15 min	11.1 ± 3.2cAB	48.3 ± 8.2abcA	56.7 ± 9.3bcdA	16.1 ± 5.5b	77.2 ± 9.1abA	19.4 ± 8.9ab	92.2 ± 4.9ab	4.4 ± 3.3
30 min	17.8 ± 5.3bc	70.0 ± 5.5aA	78.3 ± 6.0abcA	14.4 ± 4.7b	91.7 ± 2.2abAB	4.4 ± 1.9bc	97.2 ± 0.9aAB	2.2 ± 0.9
60 min	11.1 ± 3.2c	73.9 ± 7.4aA	75.0 ± 6.6abcA	20.0 ± 5.7ab	93.3 ± 2.2abA	4.4 ± 1.5bc	97.8 ± 1.7aAB	1.7 ± 1.7
90 min	13.9 ± 3.5bcAB	52.2 ± 5.6abcAB	61.1 ± 6.8bcA	23.3 ± 4.3ab	85.0 ± 2.6abA	7.8 ± 2.5bc	93.9 ± 2.2aA	4.4 ± 2.3
120 min	22.2 ± 6.9bcAB	56.7 ± 9.6abAB	73.3 ± 9.5abcA	7.2 ± 4.8b	86.7 ± 5.3abA	6.7 ± 3.7abAB	98.9 ± 1.1aA	1.1 ± 1.1AB
240 min	41.1 ± 8.9bA	54.4 ± 8.0abcAB	87.8 ± 4.6abA	10.6 ± 4.7b	94.4 ± 1.9abA	4.4 ± 1.9bc	98.9 ± 1.1aA	0.0 ± 0.0B
12 h	27.8 ± 10.1bcAB	65.0 ± 8.1abA	51.1 ± 9.6cdABC	43.3 ± 9.5aA	84.4 ± 4.2abA	12.2 ± 3.9bc	96.1 ± 1.8aA	2.2 ± 1.7AB
24 h	74.4 ± 4.2aA	23.3 ± 4.1cdBC	96.1 ± 1.6aA	3.3 ± 1.4b	100.0 ± 0.0aA	0.0 ± 0.0c	100.0 ± 0.0a	0.0 ± 0.0
F	11.6	9.0	8.0	4.3	3.3	1.1	4.9	1.8
P	<0.001	<0.001	<0.001	0.001	0.002	0.375	<0.001	0.083
163.5 mg m^{-2}								
1 min	3.9 ± 1.4cAB	25.6 ± 0.0bA	39.4 ± 8.8b	22.8 ± 3.3A	68.3 ± 8.2bA	8.3 ± 1.9abB	83.9 ± 4.3ab	6.7 ± 2.9
5 min	12.2 ± 4.9bc	26.1 ± 9.4bAB	40.0 ± 11.7bAB	19.4 ± 4.4	67.8 ± 9.4bAB	5.6 ± 1.3bB	77.2 ± 9.6b	3.9 ± 2.0
15 min	13.9 ± 5.4bcAB	32.8 ± 9.9abAB	53.9 ± 11.5abAB	18.3 ± 4.5	70.0 ± 8.8abAB	21.7 ± 6.1a	87.2 ± 4.8ab	6.7 ± 2.9
30 min	8.3 ± 3.1bc	57.2 ± 10.1abA	72.8 ± 6.3abA	20.6 ± 4.0	95.5 ± 1.9aA	3.3 ± 1.9b	99.4 ± 0.6aA	0.6 ± 0.6
60 min	10.0 ± 4.4bc	65.0 ± 7.7abAB	68.9 ± 4.5abA	18.9 ± 5.6	83.9 ± 3.5abAB	13.3 ± 3.7b	98.3 ± 0.8A	1.1 ± 0.7
90 min	16.7 ± 7.6bcAB	68.3 ± 106aA	72.8 ± 6.2abA	11.7 ± 4.4	83.9 ± 6.3abA	7.8 ± 3.1ab	95.6 ± 1.5a	3.3 ± 1.4
120 min	16.1 ± 4.0bcAB	73.3 ± 6.4aA	78.9 ± 8.4abA	11.7 ± 4.1	95.6 ± 2.4aA	3.3 ± 1.7bB	95.8 ± 2.2aA	0.0 ± 0.0B
240 min	30.6 ± 10.6abcAB	63.3 ± 11.4abA	81.1 ± 4.2aAB	16.1 ± 3.2	93.9 ± 2.3abA	3.9 ± 1.6b	98.9 ± 1.1aA	0.0 ± 0.0B
12 h	40.6 ± 13.3abA	55.6 ± 12.1abA	66.1 ± 12.4abAB	31.7 ± 12.0AB	86.1 ± 4.5abA	7.2 ± 2.8b	94.4 ± 2.3abAB	2.8 ± 1.2AB
24 h	58.3 ± 9.0aAB	33.3 ± 6.1abAB	81.7 ± 8.3aAB	13.9 ± 5.8	91.7 ± 5.1abAB	3.9 ± 2.2b	97.2 ± 1.7a	2.2 ± 1.2
F	5.5	4.2	3.3	3.6	3.6	3.8	3.8	2.3
P	<0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.023
325 mg m^{-2}								
1 min	1.1 ± 0.7bAB	2.2 ± 1.2dB	24.4 ± 6.9e	2.8 ± 1.2bD	62.8 ± 2.6dAB	3.9 ± 1.1bB	82.2 ± 3.0b	4.4 ± 2.6ab
5 min	13.3 ± 4.8b	6.1 ± 2.5dC	39.4 ± 5.5deAB	18.9 ± 6.2ab	67.2 ± 6.8cdAB	17.8 ± 4.9aAB	87.2 ± 3.2ab	9.4 ± 3.3a
15 min	17.2 ± 6.8bA	23.9 ± 4.9cdABC	46.7 ± 10.3cdeAB	16.1 ± 2.7ab	72.2 ± 8.2bcdAB	6.7 ± 2.2ab	83.9 ± 5.2b	2.2 ± 1.2ab
30 min	3.3 ± 1.4b	62.8 ± 10.5abA	64.4 ± 7.4abcdAB	23.3 ± 5.3a	86.7 ± 4.3abcdABC	7.2 ± 2.4ab	93.9 ± 1.8abAB	3.9 ± 1.4ab
60 min	13.9 ± 5.4b	38.3 ± 7.4bcBC	52.2 ± 5.8bcdeAB	22.8 ± 4.7a	81.1 ± 5.4abcdABC	10.5 ± 3.3ab	94.4 ± 1.9abABC	3.9 ± 1.6ab
90 min	24.4 ± 7.7bAB	62.2 ± 5.9abA	77.2 ± 6.8abcA	13.9 ± 4.8ab	92.2 ± 3.0abA	5.0 ± 2.8ab	98.9 ± 0.7aA	0.5 ± 0.5b
120 min	20.0 ± 5.6bAB	65.6 ± 4.7abA	79.4 ± 7.2abcA	12.8 ± 4.0ab	95.6 ± 2.4abA	2.8 ± 1.5bB	98.8 ± 0.7aA	0.5 ± 0.5bAB
240 min	26.1 ± 11.1bAB	48.9 ± 8.4abcAB	74.4 ± 9.5abcAB	7.8 ± 3.2ab	82.2 ± 8.4abcdAB	6.1 ± 3.3ab	90.5 ± 4.7abAB	1.1 ± 1.1bAB
12 h	5.0 ± 2.3bB	77.2 ± 7.8aA	80.6 ± 6.3abA	7.8 ± 2.4abB	87.8 ± 4.5abcA	9.4 ± 3.4ab	98.9 ± 0.7aA	0.0 ± 0.0bB
24 h	55.0 ± 4.4aABC	39.4 ± 3.9bcAB	87.2 ± 6.3aAB	10.0 ± 5.2ab	96.7 ± 2.2aAB	1.1 ± 0.7b	98.9 ± 0.7a	1.1 ± 0.7b
F	7.1	16.2	8.1	2.5	5.0	2.8	5.4	3.2
P	<0.001	<0.001	<0.001	0.013	<0.001	0.006	<0.001	0.003

For each treatment (0, 10, 30, 80, 163.5, and 325mg m^{-2}), within each column, means followed by the same lowercase letter do not differ significantly (in all cases, df = 9, 89;

Tukey HSD test at $P = 0.05$). Where no letters exist, no significant differences were noted. Within each column, for each exposure interval (1, 5, 15, 30, 60, 90, 120, and 240 min and 12 and 24 h), means followed by the same uppercase letter do not differ significantly (in all cases, df = 5, 53; Tukey HSD test at $P = 0.05$).

4. Discussion

The results of the present study demonstrate that the tested Carifend-like nets can be effective for the control of both *L. serricorne* and *E. elutella*, which stands in accordance with the initial results that have been reported by Rumbos et al. [21] for the exposure of both species to the standard Carifend® formulation. Similarly, in a series of field and semi-field tests, Athanassiou et al. [20] reported that Carifend® can provide long-term protection of tobacco against both *L. serricorne* and *E. elutella*, without a loss in efficacy over time. Moreover, Agrafioti et al. [28] examined different stored product insect species for long storage periods and found that Carifend® is able to control the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *T. castaneum*, and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae). Our results clearly indicate that even nets that contained lower concentrations of alpha-cypermethrin can provide efficient control of these two pest species.

Alpha-cypermethrin has been shown to be a very effective active ingredient for the control of a wide range of stored product insects under different application scenarios, particularly when applied to different types of surfaces [29,30]. For instance, larvae and adults of the khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae), were found to be susceptible to a commercial formulation of this active ingredient applied to surfaces, with larvae being more tolerant than adults [31]. Agrafioti and Athanassiou [32] found that alpha-cypermethrin was more effective against laboratory populations than field populations of two major storage insects. In addition, Agrafioti et al. [23] found that alpha-cypermethrin provided a quick knockdown on the adults of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), and *O. surinamensis*. Given that alpha-cypermethrin has been registered for surface treatment in various parts of the world and has been widely used for this purpose, a net that contains this active ingredient will be ideal in terms of both regulatory aspects and efficacy. Carifend® can thus serve as a viable paradigm towards this direction [22].

We have selected the immediate exposure intervals based on a realistic scenario of short exposures through interception with the net during the flight of either *L. serricorne* or *E. elutella*. As expected, even the longest exposure interval, which was 24 h, did not result in a considerable mortality level. Apparently, as most of the surface of the Carifend® net is placed vertically (as a curtain) to cover the stored tobacco, the contact with the treated substrate is more likely to be minimal, and that is not sufficient to cause high mortality. In previous studies with the exposure of stored product insects to various pyrethroids, it has been reported that after short exposures, despite the initial immobilization, recovery is very likely to occur [33,34]. For instance, Athanassiou et al. [34] tested three pyrethroids (deltamethrin, beta-cyfluthrin, and alpha-cypermethrin) and found satisfactory protection levels for wheat against *T. confusum* for approximately 3.5 months. In addition, Athanassiou et al. [35] noted that exposure of *T. castaneum* and *T. confusum* adults to the pyrethroid beta-cyfuthrin that had been applied on concrete surfaces caused very rapid immobilization of the exposed insects, which was not always related to mortality, in contrast with the pyrrole chlorfenapyr, for which there was no initial immobilization, but high mortality for both species. However, we found that both *L. serricorne* and *E. elutella*, despite their negligible initial mortality, immobilized at a rate that was proportional to the containment of alpha-cypermethrin and the exposure interval. For instance, approximately one-half of the *L. serricorne* adults that had been exposed to 10 mg m^{-2} were recorded as knocked down after 90 min of exposure, while this percentage was exceeded after 15 min of exposure to the 163.5 mg m^{-2} net. Gerken et al. [36] found that short exposure even after 5 min to a long-lasting insecticide net resulted in a reduction in adult progeny production of *T. casta-*

neum and the warehouse beetle, *Trogoderma variabile* (Ballion) (Coleoptera: Dermestidae). Recently, Agrafioti et al. [37] tested four different nets coated with SiO2 nanoparticles and found effective control of the black bean aphid, *Aphis fabae* (Scopoli) (Hemiptera:Aphididae), *S. oryzae*, and *T. confusum*.

However, adults of *E. elutella* required higher exposure intervals to achieve knockdown higher than 50%. For example, at 10 and 163.5 mg m^{-2}, this level was reached only after 120 and 30 min of initial exposure, respectively. Nevertheless, *E. elutella* adults exhibited an increased initial mortality when they were exposed for 24 h, which could be attributed to increased stress of the moths that were forced to move in a confined area [21]. This initial knockdown can be considered an important component of the application of Carifend®, as the nets should be deployed in such a way as to maximize insect exposure. Athanassiou et al. [20] underlined the need to have an extra part of the net that is placed right above the ground in the lower part of the curtain that will provide enough landing space for the knocked down adults. Through this technique, the majority of the exposed individuals will be in continuous contact with the net, which will reduce their chances for recovery.

It is apparent that the bioassay protocol that was used here was designed in such a way to provide continuous exposure of the insects to the nets, while in reality, a percentage of these insects might not be in continuous exposure. However, unsurprisingly, there were noticeable delayed effects of the coated nets on the exposed insects, as has already been proven for the standard Carifend® formulation [21]. Some mortality was also recorded to the dishes that contained the net without the insecticide, which was due to stress. In the nets that contained alpha-cypermethrin, the delayed effects were proportional to the initial exposure, and thus, they were more vigorous in the case of the 24 h initial contact. For *L. serricorne* adults that had been exposed for only 15 min to 30 mg m^{-2}, mortality was >30% at the 1 d post-exposure interval, while for the same immediate and delayed interval, this figure for 325 mg m^{-2} was >60%. In contrast, delayed mortality of adults of *E. elutella* at the 1 d post-exposure interval was much lower than that of the corresponding levels of *L. serricorne*, but it was much increased at longer post-exposure intervals. These findings clearly suggest that even if insects were not dead after the termination of the exposure, delayed mortality is likely to be high, especially when initial exposure exceeds 15 min. For adults of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae), Doganay et al. [28] found that delayed mortality for surfaces that were treated with alpha-cypermethrin was extremely high, and that eventually the majority of the exposed individuals were dead after their removal from the treated surface.

Based on the above, even surfaces that were underdosed could be lethal for the exposed stored product insects. For three species of stored product psocids (Psocoptera: Liposcelididae), mortality was extremely high after exposure to surfaces treated with chlorfenapyr at concentrations that were lower than those of the label dose [38]. The concept of insecticidal underdosing in stored product protection has also been examined with success in the case of grain protectants, including some pyrethroids [39–41]. Our results show that even if the concentration of the alpha-cypermethrin net is lower than that of the label dose, as in the case of Carifend®, slightly longer exposures are likely to lead to a satisfactory level of insect control.

The current series of bioassays show that nets coated with alpha-cypermethrin can be effective against insect species that infest stored tobacco and that the vast majority of the exposed adults of *L. serricorne* or *E. elutella* will die after a certain exposure interval, even if the exposure occurs for a very short period of time, which can be even lower than one hour. The concept of nets that contain insecticides is considered an important technology in stored product protection with various active ingredients [42–44]. At the same time, this technique prevents the insecticide from being in contact with the commodity itself, in contrast with sprayable formulations.

5. Conclusions

The results of our work demonstrate that the nets coated with alpha-cypermethrin can effectively control *L. serricorne* and *E. elutella* and could be further utilized as an alternative method to traditional insecticides (such as repeated fumigations) on tobacco. Our results clearly show that nets can provide protection even after short exposures to alpha-cypermethrin to protect the commodity. Thus, the results are of interest for the protection of the commodity, and additional experimental work is needed to evaluate more aspects of long-term protection of alpha-cypermethrin nets.

Author Contributions: Conceptualization and methodology, C.G.A.; investigation and formal analyses, C.I.R. and M.K.S.; writing original draft, C.G.A., C.I.R. and M.K.S.; writing-review and editing, C.G.A., C.I.R., M.K.S., S.S., T.S., C.B., P.K. and J.W.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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