



## Article

# Occurrence and Behavior Analysis of *Duponchelia fovealis* on Strawberry Plants: Insights for Integrated Pest Management

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**Abstract:** The European pepper moth, *Duponchelia fovealis* (Lepidoptera: Crambidae), is a key pest to strawberries in America and Europe. Understanding its behavior in the field can support integrated management strategies. In this work, field surveys were conducted to confirm the presence of this pest in commercial areas within the State of Paraná (Brazil) and to determine on which plant organ it prevailed. Semi-field experiments evaluated oviposition preference as a function of strawberry cultivar. Based on pest behavior, insecticide distribution was assessed following conventional field applications. Our results determined that *D. fovealis* spread within a 400 km radius from the site in which it was first recorded in Paraná, and the infested area reached 68.2% by the end of the survey. This species concentrated on basal leaves and crowns, where more than 90% of the larvae were collected. Moreover, the number of eggs per plant was significantly higher in the ‘Albion’ cultivar. The sprayed insecticide remained in the upper and middle thirds of the strawberry plant canopy, not reaching the organs where *D. fovealis* larvae were mainly detected. This study provides useful information on the cryptic habit of this pest that may help in designing efficient monitoring and control strategies.

**Keywords:** European pepper moth; oviposition preference; cryptic habit; canopy spraying limitations; pest spatial distribution; integrated pest management; *Fragaria × ananassa*



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## 1. Introduction

Strawberry (*Fragaria × ananassa*, (Weston) Duchesne) is commercially grown in more than 70 countries worldwide, with the USA, Mexico, Turkey, and Spain being the top producers [1]. Brazil is the largest producer in South America and the seventh in the world, with almost 219,000 tons per year [2]. Nevertheless, most growers are small farmers with acreages of between 0.5 and 1 ha [3]. The strawberry production in Brazil is concentrated in three states: Minas Gerais, Paraná, and Rio Grande do Sul. The main strawberry cultivars grown in Brazil include ‘Albion’, ‘Aromas’, and ‘San Andreas’ [4], which are day-neutral varieties that produce fruit for a longer time than short-day cultivars, leading to higher economic revenues.

The incidence of mites and insect pests causes relevant yield losses in Brazilian strawberry plantations. Among them, the European Pepper Moth, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), is a polyphagous pest native to Southern Europe and the Eastern Mediterranean, but nowadays it is widespread in several continents [5–12]. This species feeds on a wide variety of vegetables, fruits, ornamentals, and uncultivated plants of over 143 species [13]. Among the most damaged agricultural crops are peppers, squash, tomatoes, corn, cucumbers, and strawberries [5]. In Brazil, where it was first observed in 2010 in São José dos Pinhais (Paraná), *D. fovealis* has become an important pest of strawberries [7].

The *D. fovealis* larvae can feed on flowers, fruits, leaves and the crowns of strawberry plants [6,7,14], compromising bud break and sap flow, thus leading to vigor loss, leaf senescence and, in some extreme cases, plant death [15]. In addition, when larvae perforate the fruit surface, they create potential pathways for fungal infections, and the entry of other pathogens [6,11]. The economic relevance of the damages caused by this pest and the lack of studies under field conditions to develop specific control protocols pose scientific and technical challenges [13]. Most research on *D. fovealis* is limited to laboratory assays and there is currently no solid information on the effective management practices for *D. fovealis* infestations [13]. Field studies on this pest are challenging due to its cryptic habit and the small size of the larvae. In this context, understanding the behavior of *D. fovealis* in strawberry fields is essential to protect this crop against the attacks of this pest, in view of the significant economic losses it causes.

In general, strawberries are grown as an annual plant; however, the main practice among growers is to maintain the crop for two years, while it is still productive. Conversely, older plants can favor more generations of insect pests [7]. Since *D. fovealis* has a cryptic habit, keeping strawberry plants for two years favors the development of *D. fovealis* colonies. The tolerance of several strawberry cultivars to *D. fovealis* attacks was assessed under laboratory conditions [16], which reported that ‘Albion’, ‘Aromas’ and ‘Portola’ cultivars had some sort of antibiosis behavior. In contrast, under field conditions, there is no study about the preference of this moth for specific strawberry cultivars or for a given plant organ. In this sense, evidence was reported about Lepidoptera larvae feeding differently on specific plant organs [15]. Therefore, field surveys focusing on pest behavior could provide useful information for the development of appropriate monitoring strategies and optimizing control practices [17]. Moreover, in Brazil, there are no chemical pesticides available to control this pest specifically [16], and general pesticides are currently used. Even though biocontrol strategies against *D. fovealis* have been successfully tested [18–21]. For instance, entomopathogenic fungi have caused high mortality rates in first-instar larvae of *D. fovealis* when applied at concentrations around  $10^6$  conidia mL<sup>-1</sup> but required higher concentrations to affect pupae [21]. When combined with predators, increased the efficiency of entomopathogenic fungi to control *D. fovealis* larvae [20]. These studies were carried out in the laboratory and have not been upscaled to field conditions due to the technical challenges mentioned previously. Consequently, strawberry growers still rely on pesticides (either chemical or biological) that are specific to other pests and may not be effective due to the particular life habits of this insect [13].

In this context, to improve our understanding about the behavior of *D. fovealis* and direct the development of adequate strategies for its control, the aims of this study were to (i) determine the prevalence and distribution of *D. fovealis* in strawberry commercial fields in the Paraná State (Southeast Brazil), (ii) assess the spatial distribution of *D. fovealis* larvae in the different aboveground organs of strawberry plants, (iii) determine the preference of this pest to oviposit on a given strawberry cultivar, and (iv) evaluate the efficiency of pesticide spraying against this pest in one-year-old strawberry plants.

## 2. Materials and Methods

### 2.1. Occurrence of *Duponchelia Fovealis* in Commercial Strawberry Fields in Paraná, Brazil

The occurrence of *D. fovealis* was assessed through visual sampling [22] in commercial strawberry fields both organic and conventional, located in the State of Paraná, one of the main producing regions of Brazil, over the periods of May–June (autumn/winter), August–September (winter/spring), November–December (spring/summer) of 2013 and May–June (autumn/winter) of 2014.

The fields were selected, considering municipalities with areas devoted to strawberry crops of at least 5 ha. In total, 25 municipalities were surveyed (Appendix A). On average, four farms per municipality were surveyed and the surveyed fields were up to 0.5 ha in surface. The surveys were carried out by trained technicians and researchers. The assessments were conducted between 14:00 and 18:00 local time due to the availability of personnel. In each area, 10 equidistant plants (30 m between them) were randomly selected and the presence of *D. fovealis* larvae was recorded separately in six different strata of the plant (flowers, fruits, crown, apical, middle, and basal leaves). The sampling pattern was in zigzag. Insects were collected, identified and sent to the laboratory for species confirmation. The percentage of surface infested was calculated as the number of fields in which *D. fovealis* larvae were observed divided by the total number of fields surveyed.

### 2.2. Vertical Distribution of *Duponchelia fovealis* Larvae in Strawberry Plants

In August 2010, 2012, 2013, and 2014, a survey was conducted in a 2 ha commercial strawberry field (cv. 'Albion') located in São José dos Pinhais, Paraná, Brazil (25°37'5.32" S, 49°4'46" W). Strawberry plants were grown under high tunnels, with four rows per tunnel and the soil was covered with a black mulching (Figure 1). Crop management was organic, and the field was drip irrigated. Due to plant health issues, strawberry plantations rotated from year to year within the area of the farm, leaving bare some zones.



**Figure 1.** Commercial strawberry field (cv. 'Albion') in São José dos Pinhais (Paraná, Brazil) surveyed for the assessment of the vertical distribution of *Duponchelia fovealis* (Lepidoptera: Crambidae) larvae: external view of the protection tunnel (a); strawberry plants grown under high tunnels (b); trained staff conducting the survey (c); and larvae present in the plant crown (d).

The assessment of the spatial distribution of *D. fovealis* larvae on strawberry plants was conducted following a methodology adapted from Fitzgerald et al. [22]. A survey was carried out on 15 plots (33.6 m × 1.2 m) within the farm, consisting of four plant rows each. The plots were randomly distributed on the farm. The measurements were performed in the two inner rows, while the external rows acted as borders. Strawberry plants were 10 to 12 months old, being renewed all year. Plant spacings were 0.3 × 0.3 m (50,000 plants ha<sup>-1</sup>). In each plot, 10 randomly selected plants were surveyed (*n* = 150). Plants were divided in six strata: flowers, fruits, crowns, apical, middle and basal leaves), and the position of *D. fovealis* larvae was recorded.

### 2.3. Free-Choice Oviposition Preference Bioassay

To assess *D. fovealis* preference to oviposit on a given strawberry cultivar, the methodology proposed by Robledo et al. [23] was followed. The experiment was conducted in March and April 2024 with strawberry plants grown under organic management in a vegetation house at the Universidade Federal do Paraná (Curitiba, Paraná, Brazil, 25°44′69.9″ S, 49°23′32.3″ W, 945 m elevation).

For this experiment, adult individuals of *D. fovealis* were obtained from a laboratory colony established from insects locally collected in strawberry fields and reared under controlled conditions (25 ± 2 °C, 70 ± 10% relative humidity, and 14 L:10D photoperiod) and fed on an artificial diet [24]. This colony was established in 2012, and it has been kept under these conditions ever since. However, new individuals collected in strawberry fields are periodically introduced (every four months). Strawberry plants of four neutral-day cultivars were used in this bioassay: ‘Albion’, ‘Aromas’, ‘Monterrey’, and ‘San Andreas’ (Bioagro Agropecuaria Ltd.a, Araucaria, PR, Brazil). The plants were grown in pots containing a mixture of soil, sand, and Plantmax<sup>®</sup> (Den Ouden GrowSolutions B.V., Schijndel, The Netherlands) substrate, and were drip irrigated.

Recently emerged adult individuals of *D. fovealis* from the reared population were isolated in couples (one male and one female) in plastic cages (300 mL) with a hole in its upper part (4 cm in diameter) sealed with voile fabric for allowing gas exchange. These insects were fed on a solution containing honey, beer, and water (1:1:1). After 5–6 days, females were taken from the cages and released into the vegetation house where the experiment was carried out.

In the vegetation house, the experimental design was completely randomized, with 10 repetitions per treatment (strawberry cultivar). Each repetition consisted of a PVC-structured cage (1 × 1 × 1 m) covered with voile fabric. In each cage, four pots with a strawberry plant (8 months of age) of each cultivar were equidistantly distributed. In each pot, the exposed surface of the substrate was covered so only the stem and leaves of the plant surfaced (Figure 2). Five previously mated females of *D. fovealis* were released in each cage. After 48 h, females were removed and strawberry leaves were cut from the base of the stem, tagged and stored in a plastic bag along with voile fabric from the cage. With the aid of a Stemi 508 stereomicroscope (×40 magnification, Carl Zeiss, Oberkochen, Germany), the number of eggs in the leaves and voile fabric from each pot was counted and recorded.



**Figure 2.** Experimental setup for the bioassay conducted in a vegetation house in Curitiba (Paraná, Brazil) for testing the oviposition preference of *Duponchelia fovealis* (Lepidoptera: Crambidae) to different strawberry cultivars: (a) pots in the vegetation house; (b) pot protected with voile fabric; (c) experimental unit with four strawberry cultivars; and (d) PVC cage (1 × 1 × 1 m) covered with voile.

#### 2.4. Evaluation of Pesticide Droplet Deposition Distribution in Strawberry Plants

An experiment was conducted in November and December 2022 to assess the vertical distribution of pesticide droplets sprayed on a strawberry crop (cultivar ‘San Andreas’) located in Colombo (Paraná, Brazil, 25°17′24.9″ S, 49°09′45.8″ W, 1027 m elevation). Strawberry plants were grown on the soil in a greenhouse under organic management.

The trial followed the method proposed by Román et al. [25]. The experiment was designed in random blocks, with ten replicates. Experimental plots consisting of three rows of strawberry plants, 7 m long each. Droplet deposition was assessed using 3.8 × 2.6 cm pieces of hydrosensitive paper (WSpaper<sup>®</sup>, Zanagro Agrocomercial Ltd.a., Maringá, PR, Brazil). These papers were attached to polystyrene pieces with the same dimensions and fixed to a wood stick (20 cm height × 0.4 cm width) at heights corresponding to the lower, middle, and upper thirds of the strawberry plant canopy. The wood sticks were positioned in the central row of each repetition. Polystyrene blocks with hydrosensitive paper were alternated in each third of the plant canopy, avoiding superposition of strawberry leaves. Pesticide was applied with a 20 L knapsack sprayer (PEM P20, Kawashima<sup>®</sup>, Changhua Hsien, Taiwan) at a 2.9 L min<sup>-1</sup> flow rate and a maximum pressure of 5 bar, thus simulating the spraying conditions commonly used by farmers in the region. The product used was the botanical insecticide/acaricide Matrine<sup>®</sup> (Dinagro Ltd.a., Ribeirão Preto, SP, Brazil) based on *Sophora flavescens* Aiton at a dose of 3 mL L<sup>-1</sup>. The objective was to simulate the same drop density as a conventional application, but not to evaluate pest mortality.

After spraying, wood sticks were tagged and taken to the laboratory. Each paper was tagged, photographed, and analyzed using the GOTA5 software [26] to obtain the number of droplets, density (droplets cm<sup>-2</sup>) and coverage (%) at the different heights within the plant canopy.

#### 2.5. Statistical Analysis

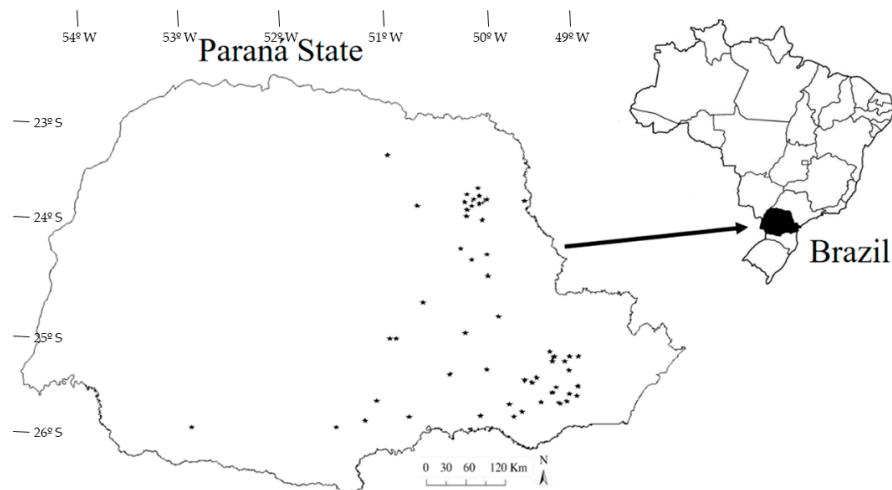
The commercial strawberry fields in which the presence of *D. fovealis* was detected, were georeferenced and plotted on a map of the Paraná State using the Diva-GIS software version 7.1 (<https://diva-gis.org/>, accessed on 20 February 2024). The data obtained from the survey and the experiments were checked for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests, respectively. Since data did not meet these assumptions, non-parametric tests were used for analyzing them, so no data transformation was required. The presence of *D. fovealis* larvae in different plant organs, the effects of cultivar on the number of eggs laid by *D. fovealis* and the differences in vertical distribution of the sprayed pesticide over the strawberry canopy were assessed using the Kruskal–Wallis test, and multiple comparisons were performed with the Dunn test using library

FSA, adjustment method of Bonferroni. Statistical analyses were conducted in the R environment version 4.3.2 [27].

### 3. Results

#### 3.1. Survey on the Presence of *Duponchelia fovealis* in Strawberry Fields of the State of Paraná

A total of 104 strawberry fields were monitored to detect the presence of *D. fovealis* larvae in commercial areas (Figure 3). These fields were located in the East and Northeast of the state of Paraná, the main two areas of strawberry crop production. The presence of *D. fovealis* was confirmed in 93% of the fields surveyed. Appendix A presents the geographical coordinates of the surveyed fields.



**Figure 3.** Commercial strawberry areas (represented as stars in the map) surveyed in 2010, 2012, 2013 and 2014 for monitoring the European pepper moth, *Duponchelia fovealis* (Lepidoptera: Crambidae), in the State of Paraná, Brazil.

Over the course of a year (from May 2013 to June 2014), the percentage of infested fields with *D. fovealis* increased from 15.4% to 68.2%, and the mean infestation in these fields reached 52.7% on average in the last period of measurements (Table 1). In fact, 22% of the surveyed fields had 100% infestation by May–June 2014. The average number of larvae per plant increased from 0.9 to 5.3 over the survey period (Table 1).

**Table 1.** Infested fields and average number of *Duponchelia fovealis* (Lepidoptera: Crambidae) larvae in commercial strawberry fields in the State of Paraná (Brazil) from May 2013 to June 2014.

Period	Infested Fields (%)	Average Infestation (%)	Average Number of Larvae Per Plant ( $\pm$ Standard Error)
May–June 2013	15.4	0.7 (0.5–1.0)	0.9 $\pm$ 1.1
August–September 2013	20.5	22.0 (1.0–50.0)	1.3 $\pm$ 2.0
November–December 2013	33.3	20.3 (1.0–73.0)	1.2 $\pm$ 1.0
May–June 2014	68.2	52.7 (10.0–100.0)	5.3 $\pm$ 0.9

The presence of *D. fovealis* larvae was more common in fields with short-day cultivars than in those with neutral-day cultivars (Table 2). However, the highest number of larvae per plant was observed in May–June 2014 in two fields with ‘San Andreas’, a neutral-day cultivar. The larvae were collected mainly in the crowns and basal leaves of the strawberry plants, but in some cases, they were also found in other organs (Table 2).

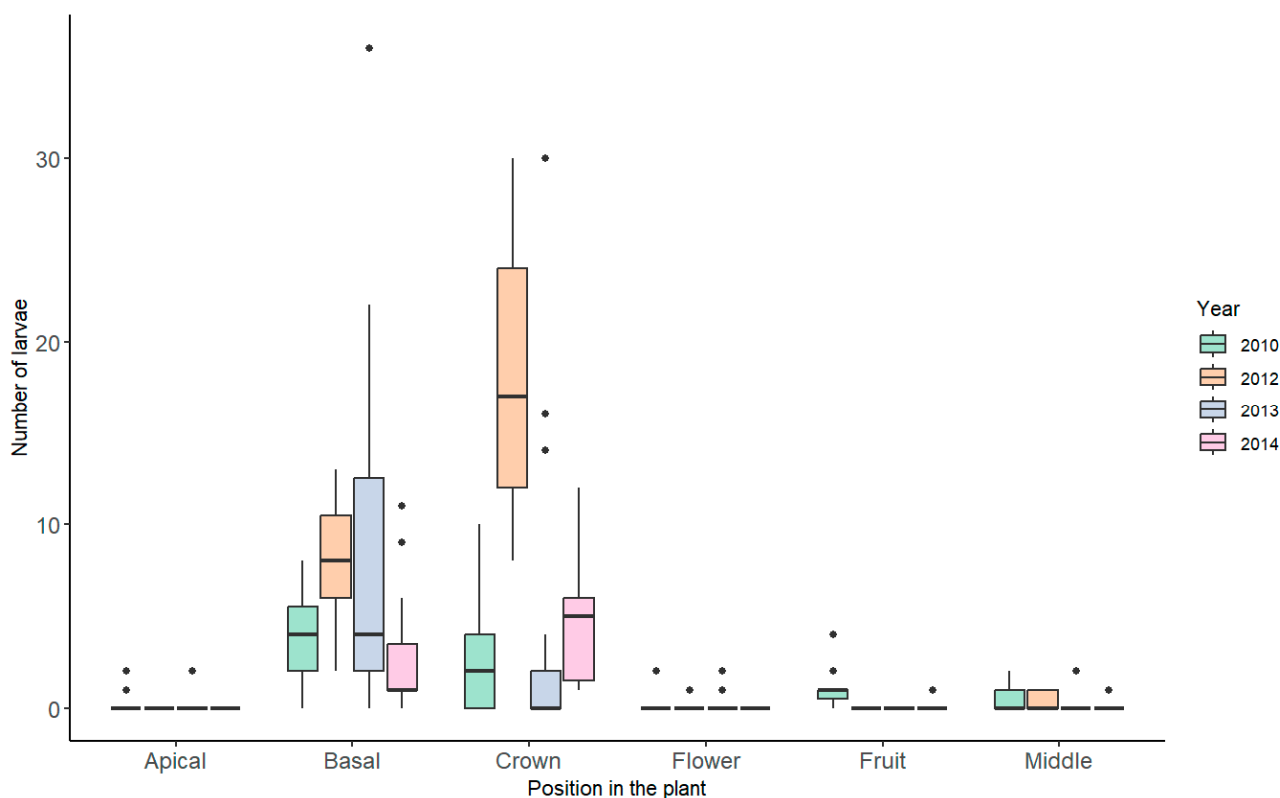
**Table 2.** Average number ( $\pm$ standard error) of *Duponchelia fovealis* (Lepidoptera: Crambidae) larvae in strawberry plants of neutral and short-day cultivars grown in commercial fields in the state of Paraná (Brazil) over the period from May 2013 to June 2014.

	Strawberry Cultivar	Average Number of <i>Duponchelia fovealis</i> Larvae						Location Within the Plant			
		May–June 2013	n <sup>2</sup>	August–September 2013	n	November–December 2013	n	May–June 2014	n		
Photoperiod	Neutral-day	Albion (27) <sup>1</sup>	-	-	1.6 $\pm$ 1.1	2	1.6 $\pm$ 0.6	3	1.4 $\pm$ 0.7	1	Flower, fruit, crown, basal leaves
		Aromas (3)	-	-	-	-	-	-	-	-	
		Monterey (3)	-	-	-	-	-	-	-	-	
		Portola (6)	-	-	-	-	0.1 $\pm$ 0.0	1	-	-	Basal leaves
		San Andreas (17)	0.8 $\pm$ 0.3	2	-	-	0.1 $\pm$ 0.0	1	9.6 $\pm$ 1.2	2	Crown, basal leaves
	Short-day	Camarosa (18)	-	-	3.3 $\pm$ 1.6	3	3.4 $\pm$ 2.7	2	3.4 $\pm$ 0.4	5	Crown, apical, middle, and basal leaves
		Camino Real (22)	-	-	3.0 $\pm$ 0.0	1	1.2 $\pm$ 1.0	2	6.7 $\pm$ 1.0	7	Crown, apical, middle, and basal leaves
		Cristal (1)	-	-	-	-	0.6 $\pm$ 0.3	3	-	-	Crown, basal leaves
		Dover (1)	-	-	1.5 $\pm$ 0.0	1	-	-	-	-	Middle and basal leaves
		Festival (2)	-	-	1.5 $\pm$ 0.5	2	0.1 $\pm$ 0.0	1	-	-	Crown, apical, and basal leaves
	Oso Grande (2)	-	-	-	-	2.0 $\pm$ 0.0	1	-	-	Basal leaves	
	Saborosa (2)	0.5 $\pm$ 0.7	1	2.3 $\pm$ 1.6	2	1.0 $\pm$ 0.0	1	-	-	Fruit, crown, basal leaves	

<sup>1</sup> Number of surveyed fields between parentheses. <sup>2</sup> Number of surveyed fields with presence of the insect.

### 3.2. Spatial Distribution of *Duponchelia fovealis* Larvae in Strawberry Plants

The larvae of *D. fovealis* concentrated significantly on the basal leaves and crowns of strawberry plants (Figure 4). The observations on the occurrence of larvae were consistent over the years; however, significant differences were detected between the survey in 2012 and that in 2013 ( $p$ -value = 0.037). In 2013, there were fewer larvae counted in the crown of the strawberry plants when compared to those observed in 2012 (Figure 4).



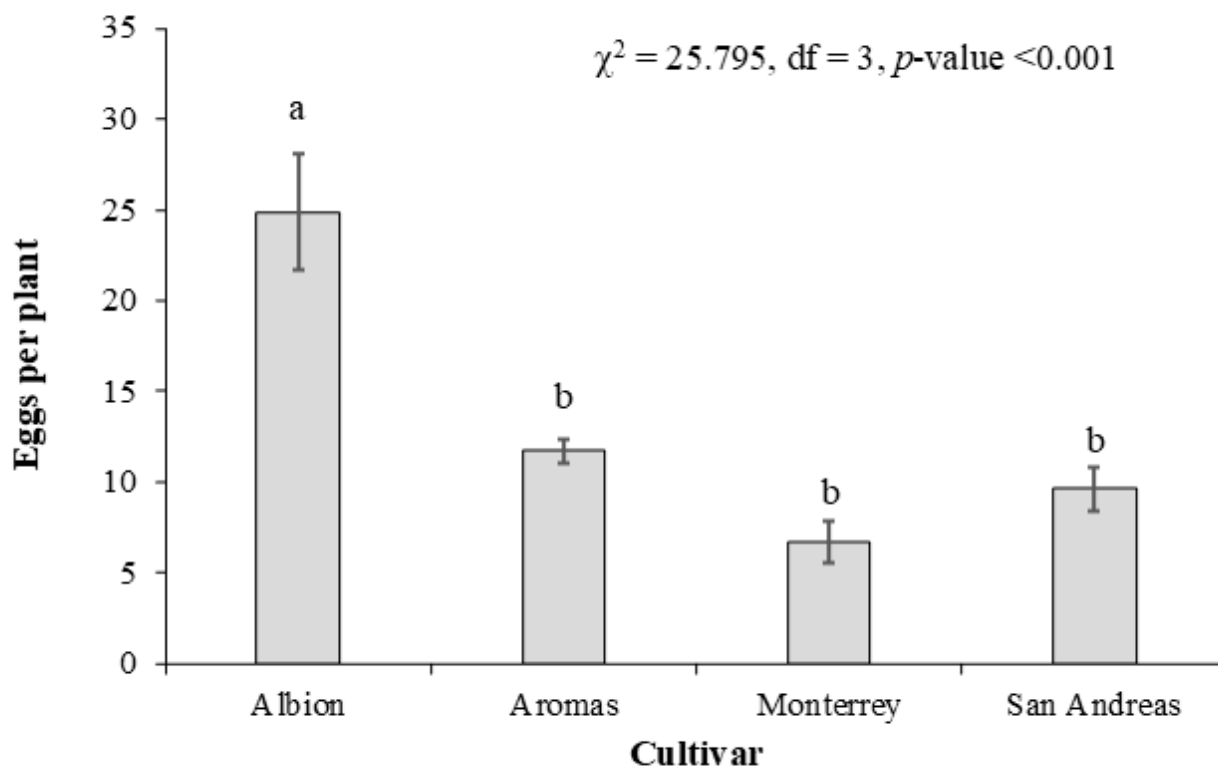
**Figure 4.** Boxplot of the number of *Duponchelia fovealis* (Lepidoptera: Crambidae) larvae in different organs of strawberry plants (cv. 'Albion') grown in the field under conventional management during the period from 2010 to 2014.

Basal leaves and crowns had significantly higher numbers of *D. fovealis* larvae than the rest of the plant organs (Figure 4). In fact, 94.2% of the larvae were recorded on these two organs, with 41.4% in basal leaves and 52.8% in crowns, while the remaining 5.8% was observed in fruits (2.0%), middle leaves (1.9%), flowers (1.2%), and apical leaves (0.8%). The average number of larvae varied from year to year:  $0.142 \pm 0.024$  in 2010,  $0.450 \pm 0.081$  in 2012,  $0.229 \pm 0.068$  in 2013, and  $0.128 \pm 0.029$  in 2014, being significantly higher in 2012 ( $p$ -value = 0.04). Considering the plant density in this orchard (50,000 plants ha<sup>-1</sup>), the number of larvae per hectare would have been 7100, 22,500, 11,450, and 6400, respectively, in 2010, 2012, 2013, and 2014.

### 3.3. Preference of *Duponchelia fovealis* for Laying Eggs on a Given Strawberry Cultivar

In the conducted bioassay, the 'Albion' cultivar had the highest number of *D. fovealis* eggs per plant, when compared to the rest of the strawberry cultivars considered ( $p$ -value < 0.001; Figure 5). In contrast, 'Monterrey' had the lowest number of eggs, although not significantly different from those in 'Aromas' and 'San Andreas' ( $p$ -value = 0.24; Figure 5).





**Figure 5.** Average number of eggs of *Duponchelia fovealis* (Lepidoptera: Crambidae) in four strawberry cultivars (‘Albion’, ‘Aromas’, ‘Monterrey’ and ‘San Andreas’) grown in pots under organic management. Error bars represent standard errors. Different letters on the bars indicate significant differences according to the Dunn test. The Kruskal–Wallis  $\chi^2$  value, degrees of freedom (df) and  $p$ -value are also shown.

### 3.4. Pesticide Spraying Distribution on the Strawberry Plant Canopy

The number of pesticide droplets and their coverage were significantly greater in the upper and middle sections than in the lower third of the strawberry plant canopy (Table 3). The density of these droplets had a great variability, and this caused the upper and the lower sections of the canopy to differ significantly (Table 3).

**Table 3.** Number, density, and coverage of droplets (mean  $\pm$  standard error) of sprayed pesticide on different sections of the canopy of strawberry plants (cv. ‘San Andreas’) grown organically under protection tunnels in Colombo (Paraná, Brazil).

Vertical Distribution of Strawberry Canopy	Droplet <sup>1</sup>	Density	Coverage
	(n)	(droplet cm <sup>-2</sup> )	(%)
Upper third	284.6 $\pm$ 20.9 a	28.8 $\pm$ 2.5 a	40.7 $\pm$ 4.3 a
Middle third	339.1 $\pm$ 31.2 a	26.7 $\pm$ 6.2 ab	32.6 $\pm$ 6.7 a
Lower third	140.2 $\pm$ 20.3 b	15.8 $\pm$ 2.3 b	2.7 $\pm$ 0.8 b

<sup>1</sup> Means followed by the same letter in the columns do not differ significantly between strawberry canopy portions according to the Dunn’s test at the 5% level of significance.

## 4. Discussion

The current study comprehensively described the occurrence of *D. fovealis* in strawberry fields located in one of the main producing states of Brazil. Moreover, it yielded relevant information regarding the preference of this insect for laying eggs on a given strawberry cultivar, as well as pointed out operational gaps in pesticide spraying. Therefore, it

provides useful information for understanding the behavior of this moth and designing efficient management practices for controlling this pest.

#### 4.1. Incidence and Dispersion of *Duponchelia fovealis* in the State of Paraná, Brazil

The results from this study proved the spread of *D. fovealis* infestations, since this pest was detected up to 400 km away from the site in which it was first recorded in the State of Paraná, the municipality of São José dos Pinhais [7]. There are no other surveys of this type reported for additional states in Brazil, and the records and damages caused by this pest are described for specific strawberry fields [14].

The presence of *D. fovealis* larvae was more common in fields with short-day strawberry cultivars, than in those growing neutral-day cultivars, likely because short-day cultivars are planted about four months earlier (in February compared to June) and this could lead to more favorable conditions for the development of this insect, such as a higher availability of food resources. Under high temperatures, as those when short-day strawberry cultivars are planted, vegetative growth is favored against reproductive growth [28], developing more leaf area close to the mulch. In this scenario, in case the farmer did not prune for removing senescent plant material, *D. fovealis* larvae would encounter a favorable environment for its development [29].

From the beginning of this survey, there was an increase in the population of *D. fovealis* within the strawberry fields. In the first assessment, conducted in May–June 2013, the presence of larvae was confirmed in 15.4% of the fields, while in the last assessment (May–June 2014), 68.2% of the fields had larvae. Moreover, mean infestation also increased over time (from 0.7% to 52.7%). This increase in population poses a high risk to agricultural production, aggravated by the widespread distribution of this insect over the state of Paraná, which can be extended to the rest of the country as reported for other exotic species [30]. For instance, *D. fovealis* presence and spread over Costa Rica has recently been reported [10], leading to a great concern due to the lack of organic and chemical pesticides specifically aiming at this pest. Therefore, the rapid expansion and populational increase of *D. fovealis* verified in the current study represents a considerable risk not only for strawberry crops, but also to other host plants because of the polyphagous character of this moth [13]. Due to the proximity of strawberry fields to other areas with different crops, mainly vegetables and ornamentals, it is possible that this pest colonizes other hosts. In addition, due to the lack of knowledge on the biological potential of this insect under the Brazilian conditions, levels of control, and monitoring technologies, *D. fovealis* can become a key pest for Brazilian horticulture, as occurred with other exotic lepidoptera [31].

In the current study, the highest incidence of *D. fovealis* larvae was observed on basal leaves and crowns of strawberry plants. This can be explained by the preference of the insect to lay its eggs in these organs [6]. Moreover, larvae were also observed in the soil surface layers and mulching located close to the plant crowns, which make control practices based on pesticides difficult [5,13]. Despite the low incidence of *D. fovealis* larvae in strawberry fruits observed in the current study, this pest can cause important damages, compromising postharvest management and limiting marketability of the produce due to the dispersal of the insect inside the fruits [10]. The knowledge on the spatial distribution of a given pest within the plant canopy is essential for planning control strategies [32]. This distribution depends on several factors, including host quality for oviposition [33], which conversely affects offspring survival in the plant [34].

Nevertheless, the survey presented in this study was carried out few years after the first detection of *D. fovealis* in Brazil (between 2010 and 2014) and this species is now a key pest of strawberries in Brazil, being widely spread in the country, as it has been detected in other Brazilian states [14]. Since cultivation practices did not change much

during the time span between this survey and nowadays and considering that no specific chemical pesticide is currently available for this pest, we can speculate that the percentage of infested fields reported in this study could have increased in the last 10 years. Therefore, additional monitoring is required to follow the pest status and determine efficient strategies for its control.

#### 4.2. Preference of *Duponchelia fovealis* for a Given Strawberry Cultivar

In addition, the results from this study suggest that *D. fovealis* have a certain preference to lay their eggs on 'Albion' when compared to other three strawberry cultivars. Previous research under laboratory conditions reported no differences in the number of eggs on three out of the four strawberry cultivars used in the current study [16]. Moreover, *D. fovealis* adults consumed more leaf surface from the 'Albion' cultivar than from the other three cultivars, which suggests a certain preference for this specific cultivar; however, the lowest average number of eggs laid was recorded in 'Albion' [16], which can be due to anatomical characteristics of the leaves in this cultivar, which had a higher number of glandular trichomes. However, there were slight differences in the density of glandular trichomes between strawberry cultivars [16], and these may form barriers that can affect the oviposition capacity of *D. fovealis* by hampering the access to the leaf or interfering with egg fixation [35,36]. These partially contrasting results between the report by Bischoff et al. [16] and the current study can be explained by the different conditions in which they were performed (laboratory using detached leaves vs. semi-field using whole plants).

In general, the behavioral events leading to oviposition by a gravid moth follow a sequence of searching, orientation, encounter, landing, surface evaluation, and acceptance, and they depend on a wide variety of sensory cues [37]. Among the mechanisms behind oviposition preference in Lepidopterans, chemicals have received particular attention, as the number of compounds identified as oviposition stimulants or deterrents has increased in the last decades. However, no specific study involving *D. fovealis* and strawberry has been conducted on this topic yet, thus, further research is needed to elucidate the mechanisms behind *D. fovealis* preference for oviposition on a given strawberry cultivar.

#### 4.3. Chemical Control Limitations

As previously mentioned, *D. fovealis* larvae were mainly detected in the lower section of the strawberry plants (crown and basal leaves), pointing out the difficulty of reaching them with pesticide spraying. Our results indicated that most of the pesticide sprayed remained in the upper section of the plants, while the lower section was practically untouched by the pesticide, similarly to previous reports on other crops [25]. This can be explained by the "umbrella" effect of leaf density in the upper layers of the canopy [38]. Consequently, the vertical distribution of the pesticide was heterogeneous and its efficacy for pest control was compromised. This fact can lead farmers to increase the volume of pesticide sprayed, which would raise production costs and environmental risks [39]. Therefore, improvements should be made in adapting spraying equipment to plant architecture, enhancing the efficiency of pesticide applications [40,41]. Additionally, previous studies indicated that using spinning disc/cage nozzles with air assistance improved leaf coverage through the plant canopy and provided better coverage to the underside leaves [40]. However, in small farms, such as those commonly encountered in Brazil (less than 2 ha), manual hydraulic sprayers are mostly used due to economic constraints, but these have been proven less efficient than air-sleeve sprayers [40]. Therefore, alternative management practices are required to control this pest [42].

#### 4.4. Management Alternatives for Controlling *Duponchelia fovealis* in Strawberry Fields

The current study showed that conventional applications of pesticides, like those performed by farmers in the region, may not be efficient for controlling larvae population dwelling in the crowns of strawberry plants because the sprayed product concentrates mainly on the upper section of the plant canopy. Consequently, alternative strategies should be used for controlling the outbursts of this pest. In this sense, recent studies suggest the use of biological control agents, such as predators [20,43], parasitoids [19,44], and entomopathogenic fungi [18,21]. These agents were proven successful under laboratory conditions, but their efficiency in the field remains to be tested. Laboratory assays confirmed that entomopathogenic fungal strains from the *Beauveria* and *Cordyceps* genus caused high mortality rates in the first instar larvae of *D. fovealis* at a concentration of  $10^6$  conidia  $\text{mL}^{-1}$ , while  $10^9$  conidia  $\text{mL}^{-1}$  was needed for effective action against the rest of the larval instars and pupae [21]. Additionally, it was observed that the predation capacity of *Podisus nigrispinus* and *Harmonia axyridis* was not affected by the residual contact with these entomopathogenic fungal strains, thus combining these agents increased the effectiveness of the biocontrol strategy [20]. In contrast, when applying high concentrations of entomopathogenic fungi, the action of certain egg parasitoids was reduced [19]. Nevertheless, four to eight parasitoids per egg of *D. fovealis* are considered ideal for field release [44]. Despite these promising results under controlled conditions, upscaling these strategies to the field involves technical challenges that need to be solved, such as the fact that this pest is difficult to detect until its population is high, its cryptic behavior and its small size [10,13].

Furthermore, among the control strategies based on microorganisms, the use of the endosymbiont *Wolbachia* has application potential for controlling agricultural pests [45,46]. However, this organism has not been tested yet for controlling *D. fovealis* and further research is needed to unravel its potential as a biocontrol agent against this pest.

Additionally, the use of traps and sex pheromones [6,10] can enhance integrated pest management strategies for controlling *D. fovealis* populations. In this context, the sex pheromone of *D. fovealis* was identified and isolated [47], but it has only been used to detect the presence of this pest in some strawberry fields in Costa Rica [10]. Nevertheless, the use of this pheromone for controlling or reducing *D. fovealis* populations has not been reported yet. Therefore, further research is needed to assess the effectiveness of these management alternatives.

## 5. Conclusions

This study verified and quantified the presence and the extent to which *Duponchelia fovealis* is distributed in the State of Paraná, Brazil. The cryptic habit of this pest was confirmed, as most of the larvae were captured in basal leaves and crowns of strawberry plants, pointing out the difficulty of reaching these zones using conventional pesticide sprayings. In our experiment, most of the products applied did not reach the lower canopy of strawberry plants. Moreover, it was determined that *D. fovealis* preferred the cultivar 'Albion' for laying its eggs when compared to 'Aromas', 'Monterey' and 'San Andreas'. From these results, some practical recommendations can be extracted: (1) monitoring of this pest should be directed to the basal region of strawberry plants, and (2) removing old leaves is essential for improving the efficiency of pesticide sprayings. Further research should be directed to the use of biological control agents for improving management strategies. Finally, the survey presented here was carried out during the few years after the first detection of *D. fovealis* in Brazil, thus additional monitoring is required to follow the status of this pest and determine efficient strategies for its control. Additional studies of the pest's population dynamics under different climatic conditions within the main strawberry

production regions of the country, and field evaluations of alternative control methods constitute research hotspots.

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**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors upon request.

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## Appendix A

Geographical coordinates of commercial strawberry fields for monitoring and recording the European pepper moth *Duponchelia fovealis* (Lepidoptera: Crambidae) in locations from the Paraná State (Brazil) during 2013 and 2014.

## Commercial Strawberry Fields Monitored

Commercial Strawberry Fields Monitored											
Sampling Period											
May–June 2013			August–September 2013			November–December 2013			May–June 2014		
Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)
Almirante Tamandaré	25°18'44.1"	49°19'87.0"	Almirante Tamandaré	25°18.449"	49°19.570'	Almirante Tamandaré	25°18.449'	49°19.570'	Araucária	25°32'18.25"	49°30'35.58"
Almirante Tamandaré	25°19'75.6"	49°20'31.8"	Almirante Tamandaré	25°19.756'	49°20.318'	Almirante Tamandaré	25°19.756'	49°20.352'	Araucária	26°53'15.71"	48°44'0.12"
Antônio Olinto	25°53'34.9"	50°02'67.7"	Antônio Olinto (3)	25°53'34.9"	50°02'67.7"	Antônio Olinto (3)	25°53'34.9"	50°02'67.7"	Araucária	26°54'15.71"	48°43'12"
Araucária	25°33'35.9"	49°33'34.6"	Araucária	25°33'35.9"	49°33'34.6"	Araucária	25°33.591'	49°33.584'	Araucária	26°33'15.7"	48°44'92"
Balsa Nova	25°32'87.4"	49°36'63.8"	Balsa Nova	25°32.874'	49°36.638'	Balsa Nova	25°32.906'	49°36.642'	Pinhalão	23°56'49.3"	50°02'12.5"
Cambé	23°18'19.4"	50°58'58.2"	Cambé	23°18'19.4"	50°58'58.2"	Cambé	23°18'19.4"	50°58'58.2"	Pinhalão	23°56'50.4"	50°0.2'12.7"
Castro	24°54'29.5"	49°53'23.1"	Castro	24°54'29.5"	49°53'23.1"	Castro	24°54'29.5"	49°53'23.1"	Pinhalão	23°50'50.4"	50°12'10"
Colombo	25°18'11.9"	49°04'43.5"	Colombo	25°18'11.9"	49°04'43.5"	Colombo (2)	25°18.203'	49°10.499'	Jaboti	23°44'22.4"	50°05'11"
Cruz Machado	26°00'19.5"	51°29'28.2"	Cruz Machado (3)	26°00'19.5"	51°29'28.2"	Cruz Machado (3)	26°00'19.5"	51°29'28.2"	Jaboti	23°41'56.1"	50°04'46.6"
Fazenda Rio Grande	25°40'20.2"	49°19'54.3"	Cruz Machado (4)	25°55'76.9"	51°11'89.2"	Cruz Machado (4)	25°55'76.9"	51°11'89.2"	Pinhalão	23°47'08"	50°03'15.6"
Fazenda Rio Grande	25°40'12.4"	49°20'42.4"	Fazenda Rio Grande	25°40'20.2"	49°19'54.3"	Fazenda Rio Grande	25°40.324'	49°19.909'	Japira	23°48'48.5"	50°08'40.9"
Guamiranga	25°08'17.4"	50°53'57.6"	Fazenda Rio Grande	25°40'12.4"	49°20'42.4"	Fazenda Rio Grande	25°40.202'	49°20.709'	Jaboti	23°45.39'43.6"	50°13'8.4"
Imbituva	25°09'36.0"	50°35'35.0"	Francisco Beltrão	26°00'30.2"	52°55'20.0"	Francisco Beltrão	26°00'30.2"	52°55'20.0"	Ibaiti	23°54'39"	50°11'97"
Jaboti	24°44'58.4"	51°05'24.7"	Guamiranga	25°08'17.4"	50°53'57.6"	Guamiranga	25°08'17.4"	50°53'57.6"	Ibaiti	23°42'25"	50°4'22"
Japira	23°46'16.1"	50°08'12.3"	Imbituva	25°09'36.0"	50°35'35.0"	Imbituva	25°09'36.0"	50°35'35.0"	Ibaiti	23°45'47"	50°5'25"
Londrina	23°27'32.4"	51°07'53.4"	Jaboti (9)	23°44'58.4"	50°0.5'24.7"	Jaboti (4)	23°44'58.4"	50°0.5'24.7"	Ibaiti	23°42'25"	50°4'22"
Mallet	25°54'06.9"	50°46'05.5"	Japira	23°46'16.1"	50°08'12.3"	Japira (6)	23°46'16.1"	50°08'12.3"	Ibaiti	23°4'25"	50°41'22"
Mandirituba	25°46'09.8"	49°17'34.1"	Londrina	23°27'32.4"	51°07'53.4"	Londrina	23°27'32.4"	51°07'53.4"	Colombo	25°21'19"	49°12'59"
Palmeira	25°26'18.0"	50°00'15.3"	Mallet	25°54'06.9"	50°46'05.5"	Mallet	25°54'06.9"	50°46'05.5"	Colombo	25°20'30"	49°11'22"
Pinhalão	23°47'23.0"	50°04'15.3"	Mandirituba	25°46'09.8"	49°17'34.1"	Mandirituba	25°46'09.8"	49°17'34.1"	São José dos Pinhais	25°40'60"	49°09'51"

## Commercial Strawberry Fields Monitored

Commercial Strawberry Fields Monitored											
Sampling Period											
May–June 2013			August–September 2013			November–December 2013			May–June 2014		
Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)	Location	Latitude (S)	Longitude (E)
Pirai Do Sul	24°29'50.3"	49°59'07.3"	Palmeira	25°22'54.4"	50°00'44.9"	Palmeira	25°22'54.4"	50°00'44.9"	São José dos Pinhais	25°75'42"	49°21'72"
Ponta Grossa	25°04'32.0"	50°12'07.2"	Pinhalão (3)	23°47'23.0"	50°04'15.3"	Pinhalão (2)	23°47'23.0"	50°04'15.3"	São José dos Pinhais	25°45'13"	49°12'43"
Santana do Itararé	24°46'02.2"	50°37'46.8"	Pirai Do Sul	24°29'50.3"	49°59'07.3"	Pirai Do Sul	24°29'50.3"	49°59'07.3"			
São José Dos Pinhais	25°35'44.5"	49°05'04.9"	Ponta Grossa	25°04'32.0"	50°12'07.2"	Ponta Grossa	25°04'32.0"	50°12'07.2"			
São José Dos Pinhais	25°36'50.7"	49°04'43.5"	Santana Do Itararé (4)	23°46'02.2"	49°37'46.8"	Santana do Itararé (3)	23°46'02.2"	49°37'46.8"			
Teixeira Soares	25°29'00.0"	50°20'57.3"	São José Dos Pinhais	25°36'50.7"	49°04'43.5"	São José Dos Pinhais	25°36.839'	49°04.722'			
			São José Dos Pinhais	25°35'44.5"	49°05'04.9"	São José Dos Pinhais	25°35.743'	49°05.074'			
			Teixeira Soares	25°29'00.0"	50°20'57.3"	Teixeira Soares	25°29'00.0"	50°20'57.3"			

The numbers between parentheses indicate the number of times in which the location was visited over a given period.

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