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Identification of Drosophilidae and Ephydridae species damaging pepper cultivated under greenhouse in Algeria

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Abstract

The Diptera fauna colonizing cultivated pepper in Algerian greenhouse was identified and damages were studied. The results let to the identification, of three Diptera species: *Scaptomyza pallida*, *Scaptomyza unipunctum* (Drosophilidae) and *Scatella tenuicosta* (Ephydridae). insects monitoring in Debidibi and El Ogla stations revealed early infestation in March caused by *S. pallida* and *Spodoptera exigua*. Dynamic population studies of *S. pallida* over three generations showed that the duration of the larval stage and pupation is temperature-dependent, with the larval stage decreasing by 2.3 days with a 3.1°C temperature increase. Examination of adult lifetime revealed a significant difference between male and female lifetimes in the first generation (5.87 days for males and 7.26 days for females). The male-female ratio was lowest in the second generation (0.44) compared to the first (0.85) and third generations (0.83). The examination of damaged pepper fruits over three years demonstrated that *S. pallida* and *Spodoptera exigua* were responsible for significant damage, with an average infestation rate of 26.17% and 26.15% respectively at El Ogla stations. Their association caused 25.26% infestation at Debidibi station and 21.06% at El Ogla station. However, the association of *S. pallida* with *Ceratitis capitata* didn't exceed 10% at either station.

These findings are crucial for implementing integrated control strategies against insects from Drosophilidae, Ephydridae, Noctuidae and Tephritidae family in greenhouses.

Keywords: lifetime; *Scaptomyza unipunctum*; *Scatella tenuicosta*; *Scaptomyza pallida;* association*;* Damages

1. Introduction

Diptera, commonly known as true flies or two-winged flies, is one of the most species-rich orders, accounting for 10-15% of all known animal species. This order is subdivided into two main suborders: Nematocera and Brachycera, encompassing a total of 150 families [1]. In the Mediterranean region six families of Ephydroidea are identified: Ephydridae (200 species), Drosophilidae (45 species), Campichoetidae (5 species), Camillidae (4 species), Diastatidae (3 species) and Curtonotidae (2 species) [2].

Ephydridae, commonly referred to as “shore” or “brine flies”, comprise approximately 2000 species organized into 5 subfamilies and 19 tribes. Most of these species grow in aquatic or semi-aquatic environments, feeding on algae, bacteria, yeasts, decomposing animal matter and faeces; or exhibiting phytophagous behaviors such as leaf or stem mining ([3], [4], [5]).

Beyond their impressive morphological diversity, Drosophilidae species occupy a wide array of ecological niches. While, many cosmopolitan *Drosophila* species, commonly known as vinegar flies, are not considered pests [6], others exhibit varied behaviors such as leaf mining or saprophagy, feeding on microorganisms like yeasts found in fermented fruits and decomposing foliage. Some genera even include entomophagous species ([2], [7]).

Some species within the Ephydridae and Drosophilidae families are recognized as pest in specific crops, such *Hydrellia griseola* [8], *Hydrellia Robineau-Desvoidy* [9], *Zaprionus indianus* [10], *Drosophila suzukii* [11].

The drosophilid fauna of North Africa remains relatively understudied, although recent investigations in Tunisia highlights the occurrence of *Zaprionus indianus* and *Z. tuberculatus* on *Carissa macrocarpa* (Apocynaceae) fruits [12]. Additionally, the invasive pest, Spotted Wing Drosophila *Drosophila suzukii* has been reported in Morocco and Algeria over the past two years ([13], [14]).

This paper is a contribution to study damages caused by Ephydridae and Drosophilidae species attacking pepper in greenhouse cultures in Algeria.

2. Material and methods

2.1. Survey locations

Our surveys were conducted on pepper crops in greenhouses situated in two stations within the Souf region: El Ogla (33.2526667 N, 6.9444611 E) and Debidibi (33.1878889N, 6.8742777E). These locations fall under the Saharan bioclimatic stage, characterized by mild winters and sandy soils.

2.2. Morphological Identification of Ephydridae and Drosophilidae specimens

The specimens collected from infested peppers fruits were identified based on various morphological characteristics including body color, body length, wings, male’s tarsal comb, and the structure of the ovipositor. This identification process involved dissecting and observing the specimens under a microscope at French Agency for Food, Environmental, and Occupational Health & Safety, France) (ANSES, France).

2.3. *Scaptomyza pallida* rearing

Scaptomyza pallida adults were reared on healthy peppers fruits wihin three glass cages (75 cm x 35 cm x 40 cm), each covered with plastic film featuring mesh openings for ventilation. Plastic containers (17 cm x17 cm x1 cm), filled with 1 cm of sand, were placed in each cage to provide support for pupation. These cages were placed in greenhouse at the El Ogla station in Algeria. Temperature readings were recorded three times daily (morning, midday and sunset) to calculate the average daily temperature. Daily monitoring was conducted to observe and record the duration of eggs incubation, larval instars, pupal development, and the emergence of adult flies. Additionally, observations were made regarding the male and female lifetime and male-to-female ratios were calculated for three generations using the following formula:

Sex ratio = (number of *S. pallida* males/ number of *S. pallida* female)

2.4. Damage evaluation

The study was performed for three years (2019, 2020 and 2021) in two greenhouses cultivated with pepper *Capsicum annum* (Linnaeus 1753) var. Beskri. Each greenhouse was divided into nine rows, each one including 70 plants. From each greenhouse, ten plants were randomly selected from three rows, and five fruits were randomly chosen from each plant to estimate the percentage of attacked fruits. Eight samplings were performed weekly from the El Ogla station from February to the end of May of each year, and the same number of samples were collected weekly from Debidibi station of the same period for each year.

All fruits were examined using a stereomicroscope, and the percentage of infested fruits was calculated for each station and each year. Infested fruits were thoroughly examined to identify the insect species present. The relative abundance (RA) was calculated using this formula

RA = (the amount of species/the total amount of Drosophilidae) x 100.

2.5. Statistical analysis

Male and female lifetime and sex-ratio of *S. pallida* were compared using analysis of variance (ANOVA) followed by a Duncan test for multiple-comparison when significant differences were observed at $p<0.05$.

3. Results

3.1. Insect identification

Fifty Ephydridae and Drosophilidae specimens were morphologically examined. resulting in the first identification in Algeria of one *Scaptomyza* species and one *Scatella* species. Among the specimens, forty-five specimens (97.8% of Drosophilidae species) were identified as *Scaptomyza pallida* (Zetterstedt, 1847) (Diptera, Drosophilidae) (Fig. 1), one adult specimen (2.17%) as *Scaptomyza unipunctum* (Zetterstedt 1847) (Diptera, Drosophilidae) (Fig. 2), and four as *Scatella tenuicosta* (Collin, 1930) (Diptera, Ephydridae) (Fig. 3).

Fig. 1. Scaptomyza pallida

Fig. 2. Scaptomyza unipunctum

Fig. 3. Scatella tenuicosta

3.2. Life cycle of *S. pallida*

3.2.1. Generation evolution

The life cycle of *S. pallida* was observed over three generations under ambient laboratory conditions. The mean temperature varied slightly, ranging from 28.65°C to 31.24°C, and a similar pattern was noted for humidity, which varied between 72.99% and 76.04% (Table 1). These variations in temperature and humidity did not significantly affect the duration of *S. pallida* generations. Only a slight difference of 1.2 days was observed between the first and third generations. The second generation completed its life cycle 2 days faster than the first and 0.8 days faster than the third, despite a gradual increase in mean temperature from 28.65°C to 31.24°C. This variation in generation duration can be attributed to a decrease in humidity, which reached 73% during the second generation (Table 1).

Table 1. Life Cycle Duration of S. pallida for Each Generation

Stage	1st generation	2nd generation	3rd generation
Duration	15,9	13,9	14,7
Mean T°C	28,6575	29,55	31,2425
Mean H%	74,6525	72,9925	76,045

The duration of each developmental stage for each generation, influenced by temperature and humidity, is detailed in Figure 4. The incubation period for eggs remained consistent across all three generations, lasting 1 day. However, the duration of the larval stages showed a slight decrease from generation to generation: 4.1 days for the first generation, 3.9 days for the second, and 3.5 days for the third. This decrease can be attributed to a temperature increase of 3.1°C during the larval stage. A similar trend was observed for the pupal stage, where a decrease in pupation duration was recorded across the three generations. Regarding adult lifetime, there was no significant difference between the first and third generations, with lifetime of 7.1 and 7.2 days, respectively. However, adults of the second generation had a shorter lifetime of 5.9 days compared to those of the first and third generations (Fig. 4).

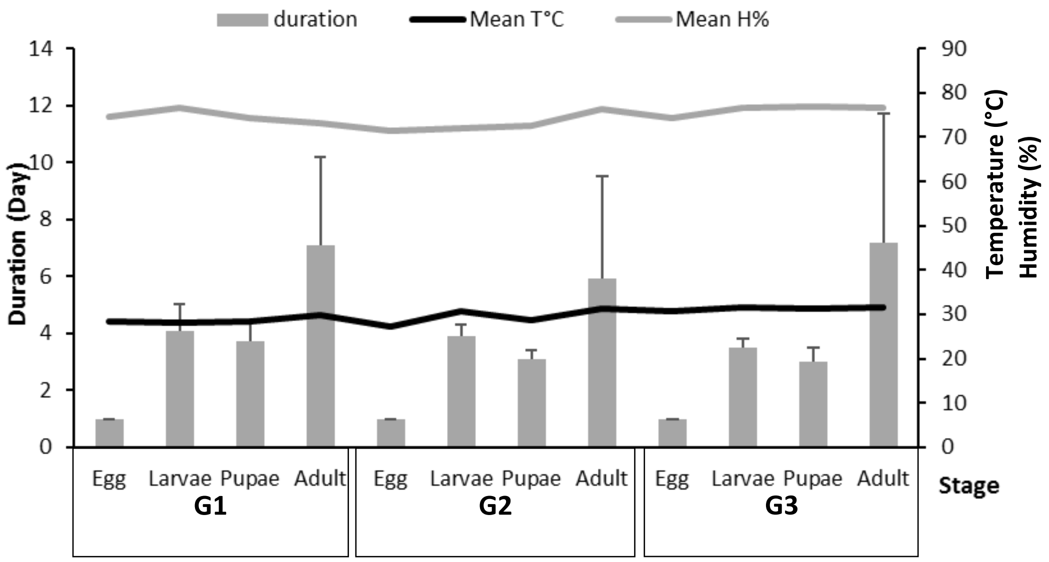


Fig. 4. Duration of S. pallida life cycle throughout three generations in natural conditions

3.2.2. Adult lifetime

The lifetime of male and female *S. pallida* adults was tracked across three generations. The results indicate, on average, males have a shorter lifetime than the females (6.11 and 7.18 days respectively). When comparing the male lifetime with each generation, no significant difference was observed ($p<0.05$). The same observation held true for female lifetime across the generations. However, a highly significant difference ($p<0.05$) was noted between male and female lifetime in the first generation, with males living an average of 5.87 days and females 7.26 days. In contrast, there were no significant differences in male and female lifetime in the second and third generations (Fig. 5).

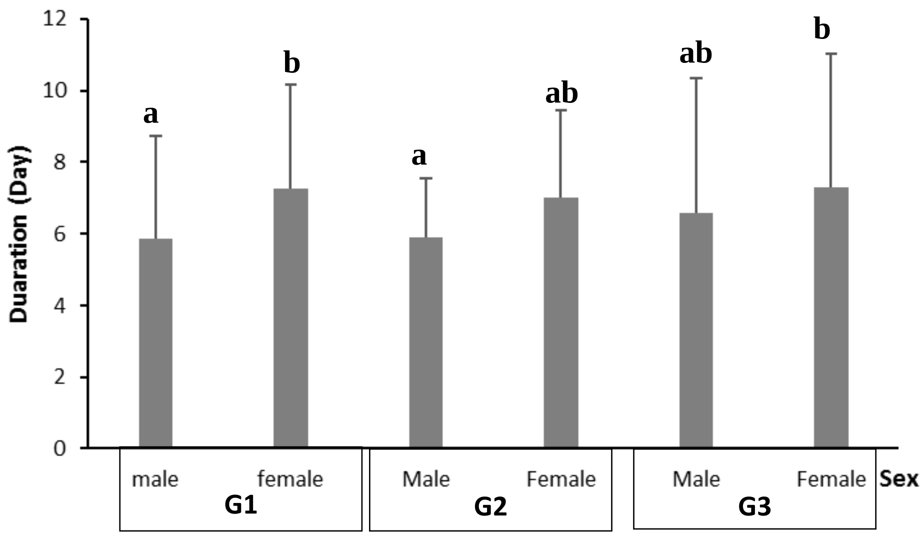


Fig. 5. Male and female lifetime for three generation

3.2.3. Sex ratio

The calculation of male-ration showed that the first and third generation tends respectively 0.85 and 0.83. However, in the second generation the male-ratio decreased to 0.44 where the number of *S. pallida* female was higher than the male (Table 2).

Table 2. Sex ratio for three generations

	1stgeneration	2ndgeneration	3rdgeneration
Male-ratio	0.85	0.44	0.83

3.3. Damages evaluation

Pepper fruits cultivated in the greenhouses at El Ogla and Debidibi are inspected during maturation. The collected fruits are classified as either unaffected or infested. Infested fruits are characterized by rot and softness, and examination revealed the presence of *Scaptomyza* larvae. The larvae of *Scaptomyza* create winding tunnels within the fruit as they feed, causing further internal damage and decay. Additionally, infested fruits may exhibit premature ripening, discoloration, and a general decline in fruit quality. (Fig. 6).

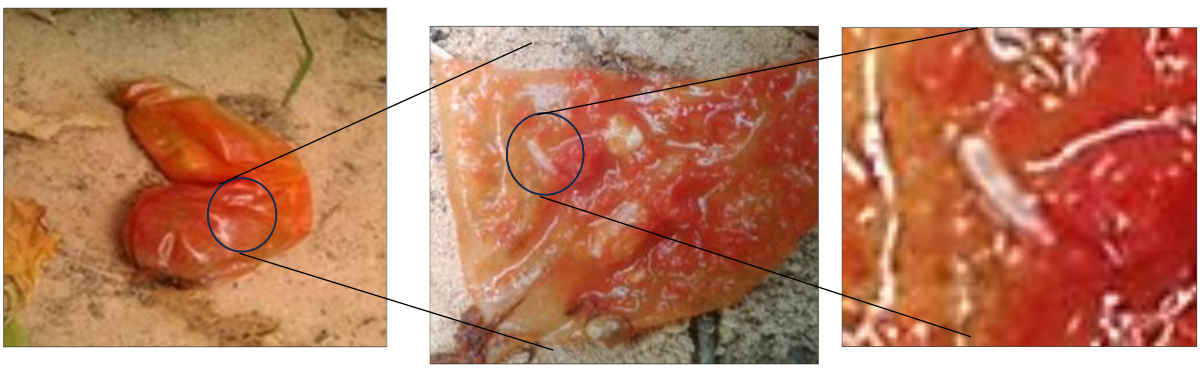


Fig. 6. Symptoms of pepper fruits attacked by the Scaptomyza genus

The percentage of infested fruits is subsequently calculated for both stations. The results indicate that the overall percentage of soft fruits at the two stations does not exceed 6%. In fact, El Ogla station records a 4% infestation rate, while Debidibi station shows a slightly higher rate of 5.23% (Table 3).

Table 3.

Station	Total fruits	Damaged fruits	Damage level (%)
El Ogla	2256	118	4
Debidibi	2012	80	5.23

Damage level on pepper fruits in El Ogla and Dbidibi stations

The examination of damaged pepper fruits collected from El Ogla and Debidibi showed that one fruit can be attacked by one or two insects. The identification of damaging insects demonstrated that pepper fruits are infested by three insects: *S. pallida*, *Ceratitis capitata* (Wiedemann, 1824) (Diptera, Tephritidae) and *Spodoptera exigua* (Hübner, 1808) (Lepidoptera, Noctuidae). The same fruit can be infested by one or two insects. In fact, an association of *S. pallida* and *C. capitata* or *S. pallida and S. exigua* was observed in greenhouses at the two stations. For three years The infestation levels showed that *S. pallida* and *S. exigua* were dominant in El Ogla station with an average of 26.17% and 26.15% respectively. Otherwise, in Debidibi station *S. exigua,* was the most abundant insect pest (40.37% in 2019) responsible of damage on pepper greenhouse. Moreover, the high percentage of pest association in the two stations was noted for *S. exigua* and *S. pallida*, it was 25.26% in Debidibi station and 21.06% in El Ogla station (Fig. 7).

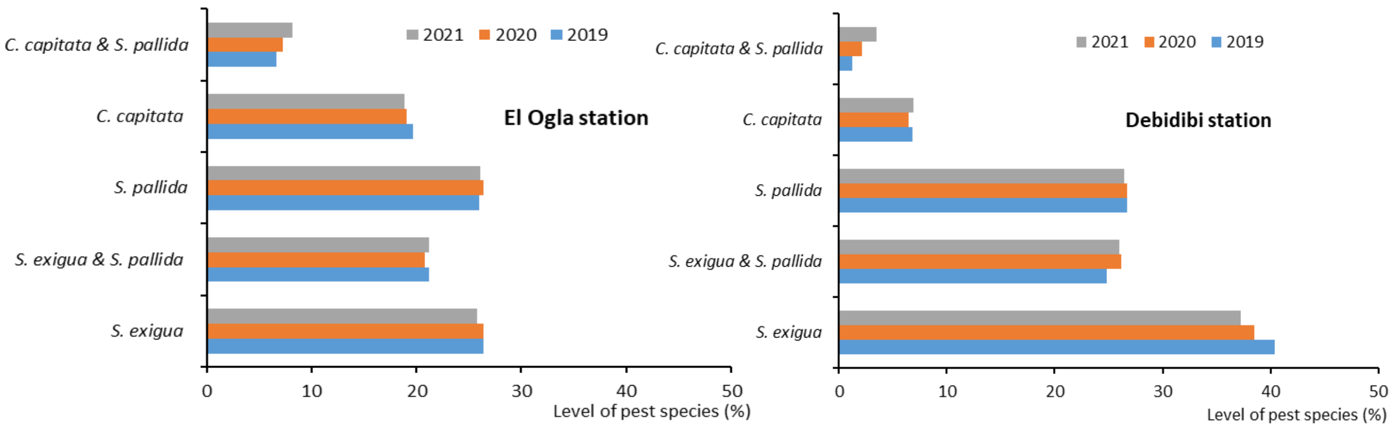
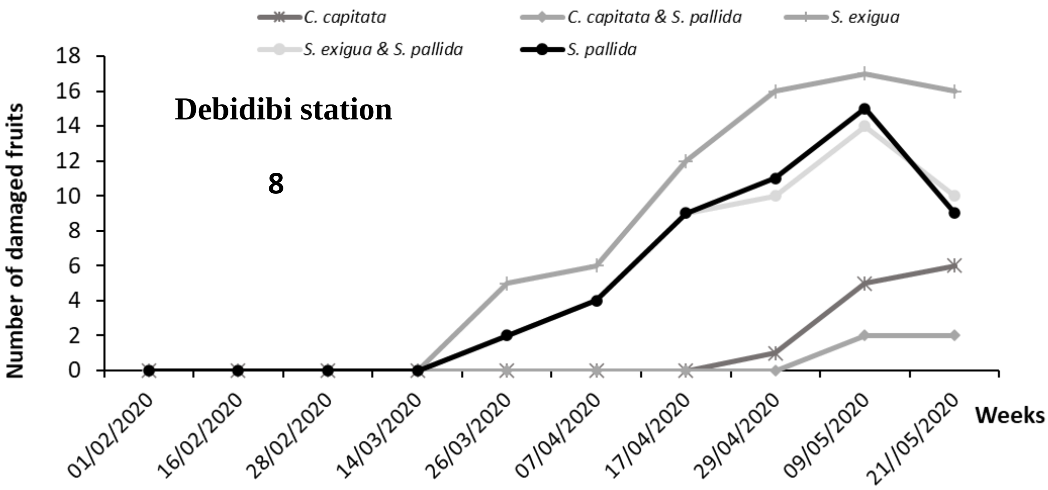


Fig. 7. Percentage of pest species damaging pepper fruits, either in association or alone, detected at El Ogla and Debidibi stations.

The monitoring of infestation in Debidibi and El Ogla stations during 2020, demonstrated that infestation initiates in March and revealed the prevalence of *S. pallida* and *S. exigua* (Fig. 8 and 9). The association of *C. capitata* and *S. pallida* present the lower infestation in the two station that didn't exceeded two and five fruits in Debidibi and El Ogla station respectively. The alone infestation of *C. capitata* on pepper is lower than its association with *S. pallida*.



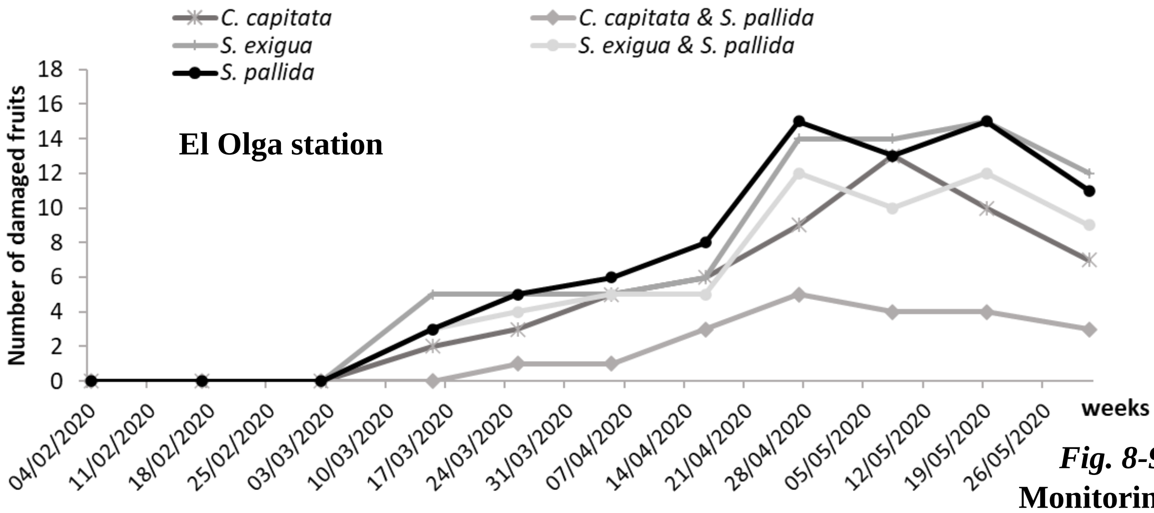


***Fig. 8-9.* Monitoring of *C. capitata, S. exigua* and *S. pallida* on pepper fruits in (8) Debidibi and (9) El Ogla stations during 2020**

4. Discussion

The present study contributes to the identification of three Diptera species colonizing pepper greenhouses in the stations of El Ogla and Debidibi in Algeria: *Scaptomyza pallida*, *Scaptomyza unipunctum* (Drosophilidae) and *Scatella tenuicosta* (Ephydridae). *Scaptomyza pallida* is reported in Algeria [15]. However, *S. unipunctum* and *S. tenuicosta* are a newly recorded in Algeria, both as species and as pest on pepper greenhouse. These Diptera species are widespread and especially in the Palearctic region. For example, *S. pallida* is very common in Japan, Korea and USA [16], in Malta [2] and in Russia [17]. It was detected for the first time in Azerbaijan [18], Georgia [19] and Kamchatka [20]. Concerning *S. unipunctum*, it was found in Palearctic region ([21], [17]) and recorded for the first time in Kamchatka [20].

Scatella tenuicosta is distributed across several regions, including Great Britain, the Afrotropical Region (Cape Verde Islands), the Nearctic Region (Greenland, United States: Delaware, District of Columbia, Maryland, Oregon, Virginia, West Virginia), and the Palaearctic Region (Austria, Azores, Bulgaria, Canary Islands, Cyprus, Denmark, Egypt, Finland, France, Germany, Greece, Hungary, Iceland, Norway, Russia (European Territory), Spain, Sweden, Switzerland, Tunisia, Turkey, Yugoslavia, UAE) [5]. This small fly, characterized by faint white wing spots, is a widespread commensal fly frequently found in greenhouses, particularly in hydroponic systems where it feeds on algae. Although it does not cause direct damage, it depreciates vegetable leaves with its excrement and can transmit pathogenic fungi [22].

Unfortunately, limited data are available on *S. pallida* and *S. unipunctum*. Generally, *Scaptomyza pallida* is commonly found feeding on decomposing organic matter. Most *Scaptomyza* species are known as leafminer pests of cruciferous crops, typically not causing damage to the fruits. However, *S. pallida* can attack fruits previously colonized by other insects, such as in canola fields [23]. Our results confirm this, demonstrating that *S. pallida* can coexist with *S. exigua* (21.06% and 25.26% respectively in El Ogla and Debidibi stations). Nonetheless, *S. pallida* was found to be the only damaging insect on pepper, with percentages of 26.7% and 26.15% respectively in Debidibi and El Ogla stations. Other authors suggest that *S. pallida* is not an herbivorous insect [24]. Additionally, Nakonechnaya et al. [25] reported that *S. pallida* can be considered pollinators of *A. manshuriensis*, as they can transfer up to 2500–4000 pollen grains on their bodies and visit many plants such as Bellis, Euphorbia, Reichardia, and various Umbelliferae [2].To the best of our knowledge, no studies have been conducted on the dynamic population of *S. pallida* and its association with other pest insects. Our results demonstrate a decrease in the duration of the larval stage and pupation period with an increase in temperature by 3.1°C. Additionally, the lifespan of

second-generation adults is shorter than that of the first generation, and there is a higher percentage of females compared to males, which affects the male-to-female ratio (0.44).
These findings are invaluable for controlling insect pests in pepper crops cultivated under greenhouse conditions. With this information, it becomes easier to determine the optimal timing and frequency of interventions to effectively manage *S. pallida*.

In the future it will be interesting if a study is carried on the impact of *S. pallida* association with other insect pests and the control measures must be undertaken. Especially with *C. capitata* as a new pest on pepper, this is what he pointed out [26].

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