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# Attraction and sexual call in *Prodiplosis longifila* (Diptera: Cecidomyiidae): Evidence of a sexual pheromone

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## ABSTRACT

**Introduction:** *Prodiplosis longifila* Gagné is a key pest of tomato in Colombia, Ecuador, and Peru. Using a sex pheromone could be an efficient alternative for its monitoring and control, but its presence is unknown.

**Objective:** To determine whether virgin females perform sexual calling and whether confined virgin females attract males.

**Methods:** In the laboratory, 233 individual virgin females were observed between 5:00-20:30 h using a 60X magnifying glass, and the sexual call was determined by extrusion of the ovipositor. Ten virgin females were confined in 43 $cm^3$ containers with tulle lids and coated with petroleum jelly to trap males (n = 16). Both containers were placed equidistantly on the upper inner side of a metal cage with tulle (2 400 $cm^3$) where 10 males were released. The experiment was repeated with 20 confined females (n = 20). In both experiments, the number of attracted males was counted 12 hours later. In tomato crops, two Jackson traps were impregnated with odorless glue from which hung a 43 $cm^3$ plastic container with tulle containing 19-34 virgin females (n = 15). The control containers had no females. Adult resting sites in the field were searched for.

**Results:** *P. longifila* females performed sexual calling during the scotophase and that containers with females attracted more males than the control. In the field, the attraction was greatest 12 hours after the experiment was set up. At field *P. longifila* adults are active during the scotophase and rest during the photophase mainly on certain plants.

**Conclusions:** The results indicate that virgin females of *P. longifila* perform sexual calling and strongly suggest that females release a sex pheromone.

**Key words:** sexual behavior; tomato; resting plants; *Solanum lycopersicum*; key pest.

**RESUMEN**

**Atracción y llamado sexual en *Prodiplosis longifila* (Diptera: Cecidomyiidae): Evidencia de una feromona sexual**

**Introducción:** *Prodiplosis longifila* Gagné es una plaga clave del tomate en Colombia, Ecuador y Perú. El uso de una feromona sexual podría ser una alternativa eficiente para su monitoreo y control, pero se desconoce su presencia.

**Objetivo:** Determinar si las hembras vírgenes realizan llamados sexuales y si las hembras vírgenes confinadas atraen a los machos.

**Métodos:** En el laboratorio se observaron 233 hembras vírgenes individualizadas entre las 5:00-20:30 h utilizando una lupa de 60X y se determinó el llamado sexual por extrusión del ovipositor. Diez hembras vírgenes fueron confinadas en recipientes de 43 $cm^3$ con tapas de tul y recubiertos de vaselina para atrapar machos (n = 16). Ambos recipientes se colocaron equidistantes en la parte superior interna de una jaula metálica con tul (2 400 $cm^3$) donde se liberaron 10 machos. El experimento se repitió con 20 hembras confinadas (n = 20). En ambos experimentos, el número de machos atraídos se contó 12 horas después. En cultivos de tomate, se impregnaron dos trampas Jackson con un pegante inodoro de las que colgaba un recipiente de plástico de 43 $cm^3$ con tul que contenía 19-34 hembras vírgenes (n = 15). Los recipientes control no contenían hembras. Se buscaron sitios de reposo de los adultos en campo.

**Resultados:** Las hembras de *P. longifila* realizaban llamados sexuales durante la escotofase y los recipientes con hembras atraían a más machos que el control. En el campo, la atracción fue mayor 12 horas después del inicio del experimento. En campo, los adultos son activos durante la escotofase y descansan durante la fotofase principalmente en ciertas plantas.

**Conclusiones:** Los resultados indican que las hembras vírgenes de *P. longifila* hacen llamado sexual y sugieren fuertemente que las hembras liberan una feromona sexual.

**Palabras clave:** comportamiento sexual; tomate; plantas de reposo; *Solanum lycopersicum*; plaga clave.

## INTRODUCTION

The bud midge *Prodiplosis longifila* Gagné (1986) is a key pest of tomato (*Solanum lycopersicum* L.) in Colombia, Ecuador, and Peru. The adult is small, measuring < 1cm, the female differs from the male by the shape of its antennae (Gagné, 1994) and because it has a long ovipositor that remains inside the abdomen (Gagné, 1986). Larvae feed by sucking the juices of leaf buds, flowers (ovaries and stamens) and small fruits (Hernandez et al., 2015) and because of this, the tissues become brown only after the larvae drop to the soil (Hernandez et al., 2015). Economic losses of more than 50 % have been estimated (Geraud-Pouey et al., 2022; Hernandez-Mahecha et al., 2018). The main and recurrent method of control of *P. longifila* is the spraying of synthetic insecticides without prior knowledge of the population level of the pest, which has led to its ineffectiveness (Díaz-Silva, 2011; Duque et al., 2018; Valarezo et al., 2003) and to the current use of insecticide mixtures that put human health at risk. In Colombia, the overuse of insecticides on tomatoes has led to the production of fruit with traces of insecticides above the permitted levels (Bojacá et al., 2013). In addition to the risk to human health, it is necessary to consider the rational use of insecticide application because, among others, biological control agents can be extremely sensitive to exposure to chemical synthesis products (Ricupero et al., 2020). In fact, *P. longifila* is considered a pest induced by the high application of insecticides against other tomato pests which most likely negatively affected its biological control (Geraud-Pouey et al., 2022) and parasitoid wasps such as *Synopeas varipes* Harrignton 1900 and *Synopeas reticulatifrons* Buhl 2011 only appear at the end of the crop cycle when farmers stop insecticide sprays (Hernandez-Mahecha et al., 2018). It is necessary to develop more specific monitoring and control alternatives for this Cecidomyiid, such as the use of sex pheromones. Precisely, the chemical composition of sex pheromones has been studied in at least 17 species of cecidomids, some of which are already commercially produced and used to monitor populations of pest species (Hall et al., 2012; Xu et al., 2020). Among the cecidomid species monitored with sex pheromones are *Contarinia nasturtii* (Kieffer, 1888) (Hillbur et al., 2005), *Dasineura mali* (Kieffer, 1904) (Cross & Hall, 2007; Liu et al., 2009), *Dasineura oxycoccana* (Johnson, 1899) (Fitzpatrick et al., 2013), *Dasineura plicatrix* (Loew, 1850) and *Mayetiola destructor* (Say, 1817) (Knutson et al., 2017). The emission of sex pheromones is associated with morphological and behavioral characteristics of the cecidomid. Although the presence of a sex pheromone in *P. longifila* is unknown, two morphological characteristics suggest it. First, the presence of sensoria called circumfila that girdle the flagellomeres and form loops on the antenna of males (Hernandez-Mahecha et al., 2018; Gagné, 1994), and second the presence of a long telescopic ovipositor that is typical of insects that reproduce with emission of a pheromone (Hall et al., 2012; Van Lenteren et al., 2002; Zhang et al., 2021). Adults are only active in the dark and during the light hours rest on some plants (Díaz-Silva, 2011). Based on these morphological characteristics and behavior suggesting the importance of chemical communication mediated by a sex pheromone for mating, the objective of this research was to establish whether *P. longifila* females performed sex calling to attract males and to determine whether virgin females confined in laboratory and field conditions attracted males. Adult resting places were determined.

## MATERIAL AND METHODS

**Insects:** Leaflets of *Solanum lycopersicum* L. tomato with *P. longifila* larvae were collected from commercial crops located in several localities of Valle del Cauca, Colombia (Pradera 3°25”9’ N, 76°14”34’ W; Rozo 3°36”53’ N, 76°23”10’ W; Santa Elena 3°39”25’ N, 76°14”21’ W, Trujillo 4°12”41’ N, 76°19”13’ W). Larvae were transported in plastic boxes with wet paper to the Entomology laboratory of the Universidad Nacional de Colombia sede Palmira (3°30”42’ N, 76°18”28’ W) and maintained at average conditions of 24.1 °C ± 0.36 SE, 72.9 % RH ± 0.7 SE and photoperiod of 12L:12D to continue their development.

Pupae were maintained in moistened clay soil according to the method suggested by Geraud-Pouey et al., (2022). Soil was obtained from an abandoned cacao (*Theobroma cacao* L.) crop that was dried for 48 h at 200 ˚C and sifted with a 60-inch sieve to obtain a fine substrate. The emerged adults were continuously sexed soon after emergence to avoid mating according to the type of antennae and genitalia (Gagné, 1986; Gagné, 1994) through a Nikon SMZ 745 stereoscope and red light produced by three 60 W glass bulbs painted red so as not to disturb the insects. Although *P. longifila* is the only species of Cecidomyiidae present on *S. lycopersicum* in Colombia (Velasco-Cuervo et al., 2016), 5 adults/night were routinely sampled to corroborate species identity according to taxonomic keys (Gagné, 1986; Gagné, 1994).

Laboratory experiments were conducted in the Entomology Laboratory and field experiments were conducted in a commercial tomato crop during flowering and fructification, located in the village of Cedrales, municipality of Trujillo, Valle del Cauca, Colombia (4°13’35” N, 76°19’24” W) at 1 596 m.a.s.l., annual average of 21.5 °C and 78.1 % RH.

**Calling behavior:** To determine the presence and time of the sexual call, 233 newly emerged virgin females were placed in individual in 1.5 oz plastic cups and observed every 30 minutes between 5:00-20:30 h. To avoid disturbing the insects, observations were made with 60X Walfront magnifying glass with LED light. Calling females extruded partially the ovipositor (only the final part of the ovipositor is shown accompanied by contractions and circular movements of the ovipositor) or totally (ovipositor completely visible). Each observation by using LED lights lasted 20 seconds because previous observations determined that this lasts from 10 to 15 seconds. Females were observed until death. The number of females extruding the ovipositor (partial or total) at each observation time was plotted.

**Attraction of males to confined females of *Prodiplosis longifila* under laboratory conditions:** The experiments were conducted with males and females of *P. longifila* emerged on the same day. The adults emerged from 15:00 h and around 18:00 h the required number of females and males for the experiment was completed. The experiment started between 19:00-20:00 h.

Virgin females were confined in a 43 $cm^3$ plastic container with a lid lined with synthetic tulle mesh allowing air flow and covered on the outside with petroleum jelly to trap attracted males (treatment); a similar container, but without females, was the experimental control. A control and a treatment container arranged upside down were velcroed equidistantly to the inside top face of a tulle-lined aluminum cage (10 cm length x 12 cm width x 20 cm height). Inside the cage, groups of newly emerged virgin males were released and had free choice to fly to the treatment or the control. In the first group of experiments (n = 16) 20 females were confined and 10 males were released (ratio 1 male: 2 females). In the second group of experiments (n = 20), 10 females were confined, and 10 males were released (ratio 1 male: 1 female). Both males and females were used only once. During the experiment, the cages were kept inside the laboratory covered with a black cloth to reduce the influence of any artificial light. Twelve hours later, the number of males attracted and attached to the treatment and control containers were counted. The treatment and control containers were used only once, and the aluminum cages were washed with water and cleaned with alcohol between experiments.

**Attraction of males to confined females of *Prodiplosis longifila* under field conditions:** The experiment was conducted on a commercial crop of tomato *S. lycopersicum* Libertador variety planted in a semi-covered area of 1.3 ha with distances of 0.40 m between plants and 1.4 m between rows with approximately 21 000 plants in the phenological stage of flowering and fruiting. Two Jackson traps (n = 15) made of cardboard (SÁFER®) were installed in one furrow of the crop at a height of 1 m from the ground, which corresponds approximately to the middle part of the plants, separated by 2 m, and protected from the rain with a zinc roof covered with PVC. In the center of each trap, a 43 $cm^3$ plastic container with a lid lined with synthetic tulle mesh to allow air flow was placed face up. Between 19-34 newly emerged virgin *P. longifila* females were confined in the treatment Jackson trap container. The control trap container was empty. The confined females were from the biological development of larvae collected in the experimental culture. All surfaces of the Jackson traps and jars were impregnated with Sáfertac® (SÁFER) odorless glue to trap attracted males. Confined females were fed by offering the insects balls of cotton soaked in 10 % sugar solution to prolong their longevity. Treatment and control traps were installed between 18:00-19:00 hours and 12, 24 and 36 hours after installation the number of males attracted to each trap was counted with the aid of a 60X magnifying glass (Warrant). Each group of *P. longifila* females was used only once (n= 15). Captured males were removed after each evaluation.

To know the population density of *P. longifila* in the crop that would guarantee the presence of males in the crop, the larval population of *P. longifila* was sampled and counted weekly based on what was suggested by Duque et al., (2018). Three crop furrows were randomly selected, and 10 leaf buds were randomly taken from each furrow (30 leaf buds/week/crop). Using a 60X magnifying glass, the number of *longifila* larvae was counted and the average number of larvae per leaf shoot in the crop was determined. Since the development time between the first instar larva and the adult *P. longifila* in tomato is 14 days (Duque et al., 2018), continuous emergence of adults in the crop was expected. Additionally, 15 nocturnal visits were made to the crop to observe and confirm the presence of adults, and five individuals were randomly collected/night to confirm their taxonomic identity in the laboratory.

**Resting sites of *Prodiplosis longifila* adults in the field:** Daytime (between 8:00-17:00 h) and nighttime (between 18:00-19:00 h) searches for adults of *P. longifila* were conducted on uncultivated plants growing spontaneously in the surroundings of the tomato crop. Flashlights with LED light were used at night. The plants with adults were pressed and transferred to the Josep Cuatrecasas y Arumí Herbarium of the Universidad Nacional de Colombia sede Palmira for taxonomic identification.

**Statistical analysis:** The number of males was expressed as mean ± standard error (SE). For the analysis of attraction in the laboratory, the Analysis of Variance (ANOVA) analysis indicated a significative effect of the treatment (presence/absence of females) ($F = 48.15$, df (1,68), $p < 0.05$). Using the residual values of ANOVA there were tested normality and homogeneity of variances assumptions. Normality distribution was indicated by the Anderson-Darling test ($AD = 0.617$, $p = 0.105$). Homogeneity of variances was tested with Bartlett's test (Barlett´s test statistic = 11.24, $p = 0.010$) (SAS 9.2, 2008). Because the variances were not homogeneous and the values from numerical counts of attracted males were very small, the variable number of males was transformed with $\sqrt{(x+0.5)}$. With the transformed data, Levene's test confirmed the assumption of equal variances. For attraction experiments under laboratory conditions the number of males attracted between control and treatment, and between female: male ratios of 1:1 and 2:1 and their interaction were compared through an ANOVA. Means were compared with Tukey's test $p < 0.05$. For experiments at field conditions the number of males attracted between control and treatment at 12 h, 24 h and 36 h was compared. The assumption of homogeneity of variances was not met for the 24 h male field attraction experiments ($F = 0.10$, $p < 0.05$) but the assumption of normality was met (Ryan Joiner test, $RJ = 0.976$, $p > 0.100$), therefore Welch's non-parametric test and Games-Howell mean comparison test with $p <0.05$ were used. No normality at 24 h and 36h was indicated by the Ryan Joiner test ($RJ = 0.908$, $p <0.010$ and $RJ = 0.885$, $p < 0.010$ respectively). The assumption of homogeneity of variances was met for the 24h male field attraction experiments (Levene test, $p = 0.134$); for the 36 h experiment it was proposed homogeneity of variances because the control was cero (no insects were attracted to the females). Number of males attracted between control and treatment at 24 h and 36 h were compared using one-way ANOVA. Means were compared with Tukey's test $p < 0.05$.

## RESULTS

**Calling behavior:** The emergence of adults occurred after 15:00 h. Adults were not highly active for about 20 min after emergence, which allowed them to be sexed more easily. Females of *P. longifila* performed sexual calling through complete (dark bar) or partial extrusion (pale bar) of the ovipositor mainly in the early morning (5:00-7:30 h), in the afternoon (16:30-18:00 h) and in the evening (18:30-20:30 h) (Fig. 1). There was a higher frequency of complete ovipositor extrusion (387 times) versus partial ovipositor extrusion (242 times) (Fig. 1). Females showed the ovipositor at least once up to 35 hours after emergence, however, after this time and up to 96 hours of follow-up (day 4) no ovipositor extrusion was evident. It was found that 95 % of the females evaluated showed complete or partial ovipositor for the first time during the first 4 hours after emergence. Ovipositor extrusion occurred mainly during scotophase.

**Attraction of males to confined females of *Prodiplosis longifila* under laboratory conditions:** A higher average number of *P. longifila* males were attracted to the container with virgin females than to the control in the 20 females :10 males densities (Fig. 2).

Similarly in the 10 females:10 males densities more males were attracted to the treatment than to the control (Fig. 3). The number of males attracted was significantly different from the control for both ratios (ANOVA, $F = 47.82$, df = 1.68, $p < 0.05$; Tukey $p < 0.05$). However,

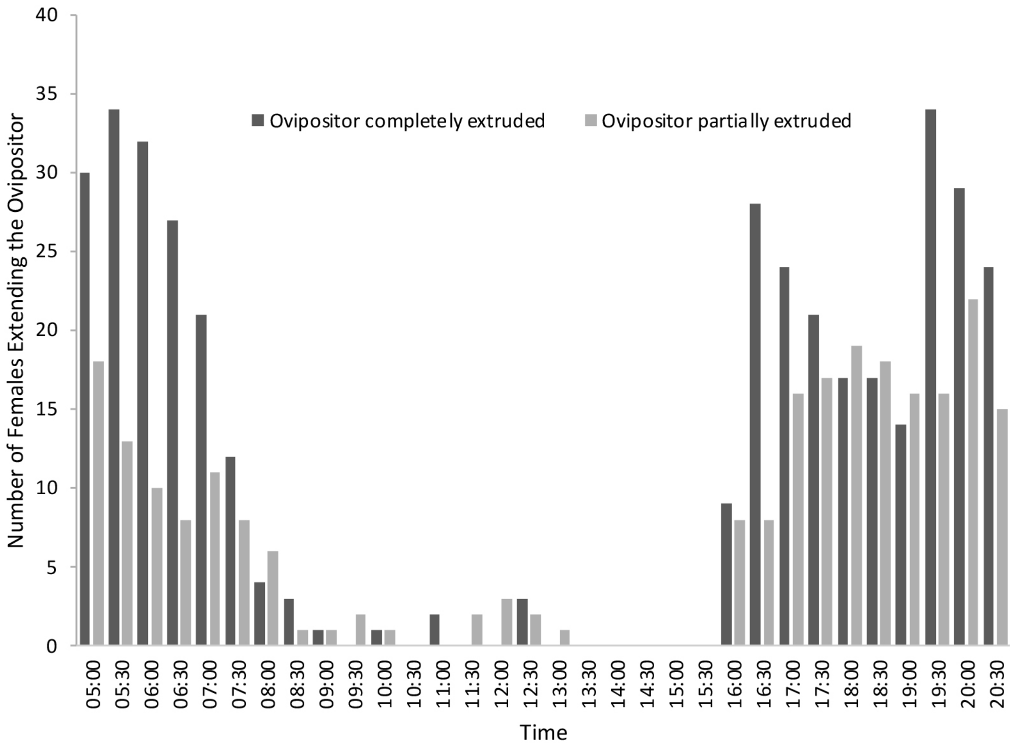


**Fig. 1.** Number of *P. longifila* females (n = 233) with complete and partial extrusion of the ovipositor during four days since emergence. The number above the bar indicates the number of females that performed these behavioral events.

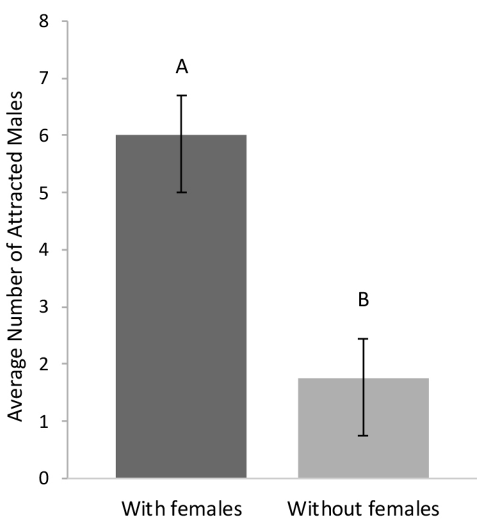


**Fig. 2.** Average number of *P. longifila* males attracted to confined females under laboratory conditions (ratio of attraction of 20 females to 10 males, n = 16), different letters indicate significant differences (Tukey test, $p < 0.05$).

comparison between the attraction proportions showed no significant difference in the number of males attracted (ANOVA, F = 0.00, df = 1.1, p = 0.959; Tukey p > 0.05).

**Attraction of males to confined females of *Prodiplosis longifila* under field conditions:** Field monitoring showed an average population of 12.5 ± 1.3 larvae/ leaf shoot in the tomato lot during the experiment. Twelve hours after confinement of the females, a significant difference was found between the treatment and the control (Welch's test, F = 9.46 df = 1.16.89, p = 0.007; Games-Howell p < 0.05), but no significant difference was found at 24 h (ANOVA, F = 2.38, df = 1.28, p = 0.134; Tukey p > 0.05) or at 36 h (ANOVA, F = 3.50, df = 1.28, p = 0.959; Tukey p > 0.072) (Fig. 4).

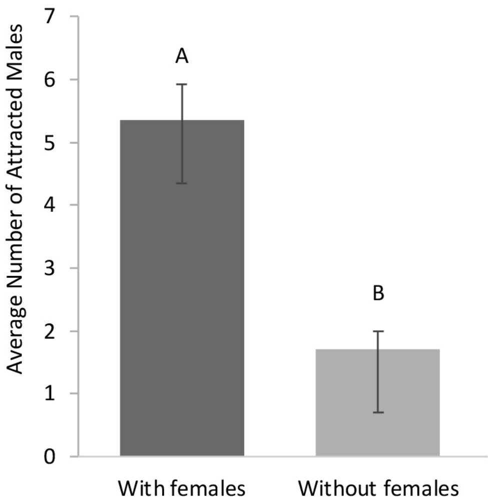


**Fig. 3.** Average number of *P. longifila* males attracted to confined females under laboratory conditions (ratio of attraction of 10 females to 10 males, n= 20), different letters indicate significant differences (Tukey test, $p < 0.05$).

**Resting sites of *Prodiplosis longifila* adults in the field:** No flight activity of *P. longifila* adults was observed during photophase between 7:00 and 16:00 hours; after 16:00 hours adults moved with rapid movement around tomato plants and between the crop furrows. Only adults (not larvae) were found resting on the foliage of spontaneously low-growing plants identified as *Wedelia* sp. and *Spilanthes acmella* Murr, both from the Asteraceae family. Adults were also found in areas with humid and shaded microclimates, for example, under the guadua (*Guadua angustifolia* Kunth) structures where tomato fruits are left to be packed and marketed, and under an abandoned guadua support that was outside the crop where the two species of Asteraceae named above were growing in the middle of pastures. During scotophase the adults remained still and visible on leaf buds and on the upper and lower sides of tomato leaves.

## DISCUSSION

The results of the sexual call of *P. longifila* coincide with those reported for other cecidomid species that release a sex pheromone. Virgin females of *P. longifila* perform sexual calling through total or partial extrusion of the ovipositor accompanied by circular movements of the ovipositor. This has been observed in other cecidomid species that through the extension of the ovipositor emit sex pheromones that are produced by specialized glands present in

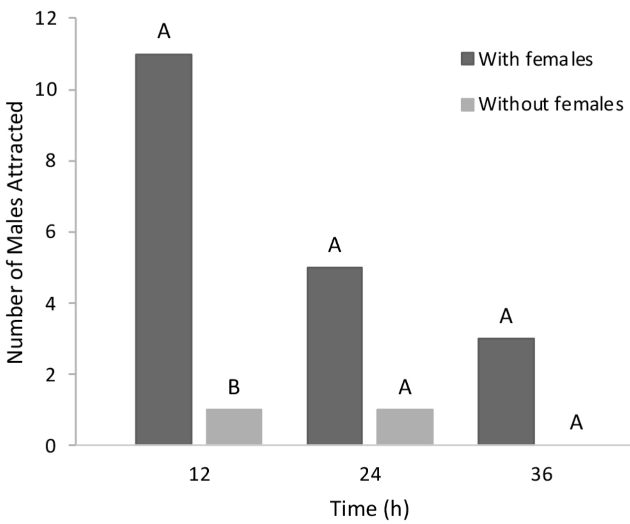


**Fig. 4.** Number of *P. longifila* males attracted to field-confined females and the control 12, 24 and 36 hours after installation (n = 15). Different letters show differences between treatment and control (for 12 h, Games-Howell post Welch's test $p < 0.05$; for 24 h and 36 h, Tukey post one-way ANOVA $p > 0.05$).

their abdomen (Boddum et al., 2010; Hall et al., 2012; Xu et al., 2020) and that with movements and postures facilitate the dispersion and distribution of the pheromones to the exterior (Cade, 1985). For example, virgin females of *Contarinia oregonensis* Foote immediately prior to mating "call" males by spreading and shaking back and forth their ovipositors (Miller & Borden, 1984). During sexual calling the extended ovipositor of *Dasineura brassicae* Winn can even double the length of the female's body (Isidoro et al., 1992) and these telescopic ovipositors such as the one of *P. longifila* are characteristic of females emitting sex pheromones (Riolo et al., 2014). It is not clear if pheromone could be released during the partially ovipositor extrusion observed on *P. longifila.* Emergence of *P. longifila* after 16:00 h and sex calling during the hours of darkness has also been observed in cecidomids that produce a sex pheromone such as *Sitodiplosis mosellana* Géhin (Bruce et al., 2007; Gries et al., 2000). Their females, after emerging in the late afternoon begin to extend their ovipositors, increase sexual calling gradually during the last hours of photophase but call more frequently in scotophase (Pivnick & Labbé, 1992). *Prodiplosis longifila* females called at least once during the first four hours after emergence (95 % of females) suggesting that due to their short longevity (1.1 days ± 0.05) (Duque et al., 2018) their sexual maturity is rapid and mating must occur early, adaptive characteristics of insects with *r*-reproductive strategy (Pianka, 1982). This behavior has also been reported in females of *M. destructor*, which due to their short longevity (Harris & Rose, 1991), immediately after emergence mature oocytes and in the first four hours of the morning extend their ovipositor to emit a sex pheromone that attracts males for copulation (McKay & Hatchett, 1984).

The increased activity of adults of *P. longifila* during the scotophase coincides with our nocturnal observations made in the field which showed that during the day adults are inactive and take refuge in plants or guadua structures but are active during the scotophase. Valarezo et al., (2003) reported that copulation of *P. longifila* occurs during the night and Díaz-Silva (2011) indicated that adults are active during the night. The presence of *P. longifila* adults on plants such as *Wedelia* sp. (Asteraceae) and *Spilanthes acmella* Murr (Asteraceae) opens the possibility of investigating them as trap plants within an integrated management program for *P. longifila* in the tomato agroecosystem. Delgado (1998) reported the presence of *P. longifila* larvae in the Urticaceae *Fleurya aestuans* L. while, in Peru, Díaz-Silva (2011) reports *P. longifila* larvae in two other plant species of the Asteraceae family which are *Cynara scolymus* L. (damage on shoots of seedlings or tender plants) and *Tagetes erecta* L. (scraping on flower buds). It is unknown whether *P. longifila* takes nectar from these plant species, but adult longevity is known to be increased by consuming sugar (Duque et al., 2018) and that after flowering of the tomato crop *P. longifila* infestation increases suggesting the importance of flowers in the ecology of the insect.

**Attraction of males to confined females of *Prodiplosis longifila*:** Confined virgin females of *P. longifila* exerted attraction behavior on virgin males most likely mediated by the production of a sex pheromone. Our experiments took place during the scotophase, a period that included the sexual calling phase. Insects in general, including moths, are governed by a circadian clock that allows females to synchronously release pheromones, which in turn will generate an attraction response in males during a set time (Gadenne et al., 2016; Groot, 2014). For *P. longifila,* pheromone release and male attraction at laboratory conditions was tested during a 12 h period using adults up to three hours of emergence. Density variation of confined *P. longifila* females had no significant effect on the number of attracted males. This could be explained on the one hand, because Cecidomyiids have long circumfila sensoria (Zhang et al., 2021) present in *P. longifila* (Gagné, 1994; Hernández et al., 2015) and which structurally possess abundant dendritic neuronal branches around each antennal flagellum node, indicating an olfactory function and a close relationship with female sex pheromone detection (Boddum et al., 2010; Crook & Mordue 1999; Hillbur et al., 2001; Solinas & Nuzzaci, 1987). On the other hand, cecidomid pheromones are highly selective, potent, and efficient (Witzgall et al., 2010), which added to the fact that our attraction experiments under laboratory conditions were conducted at a short distance (20 cm) would favor attraction of males with at least 10 virgin females. For example, in a short distance wind tunnel experiment (horizontal plane ranging from 10 to 35 cm), *M. destructor* males were exposed to 20 ng and 2 ng of their sex pheromone compound (2S)-(E)10-tridecen-2-yl acetate and both doses elicited a significant response in the flight attraction response of males to the compound (Harris & Foster, 1999). Field experiments on the cecidomid *Haplodiplosis marginata* (von Roser), where pheromone dispensers were loaded with the compound nonan-2-yl butanoate at doses of 5 mg and 10 mg, showed that both doses generated a male attraction response (Censier et al., 2016).

Field experiments indicate that a greater number of males were attracted 12 hours after setting up the experiment. During this time, virgin females attracted 57.9 % of the males attracted during the entire experiment, a trend that was similar in the laboratory trials where on average 56.7 % of males flew to females. In this regard, Hodgdon et al., (2019) found that in Brassicaceae crops, 73 % of *C. nasturii* males were caught in Jackson traps with commercial pheromone during the first 5 hours after emergence and Isidoro et al., (1992) reported that 57 % of virgin *D. brassiccae* females performed sexual calling on the first day of life. In the experiments of Hodgdon et al., (2019), from 5 h to 24 h after emergence, the attraction of males decreased significantly, similar to what was found in *P. longifila* in the field, because after 12 h the attraction of virgin females decreased; this result matches our sex call experiment because between 16 h and 35 h after the emergence of *P. longifila* the frequency of sex calling decreased until it disappeared after 35 h after the emergence of adults.

The fact that *P. longifila* females in the field attracted mostly males during a relatively brief time span and at laboratory conditions 95 % of the females showed complete or partial ovipositor for the first time during the first 4 hours after emergence suggest several explanations. First, adaptation to rapid reproduction in short-lived insects such as *P. longifila* (Duque et al., 2018), second, a strong and reliable attraction to the emitted infochemical (Witzgall et al., 2010) and third that females move soon after emergence. In this regard, Valarezo et al., (2003) mention that the insect pupae fall to the ground or adhere to the branches, stems, or leaves of the tomato and that copulation can occur on the same plant where the females emerge and perch so that the males initiate a courtship flight by vividly flapping their wings. For *C. nasurtii* it is suggested that the insect mates near emergence sites because females extended their ovipositors when emerging from the ground (Hodgdon et al., 2019).

The results presented strongly suggest that females of *P. longifila* emit a sex pheromone in glands located in the abdomen and that they produce an attraction response at short distance under laboratory conditions and at long distance under field conditions. It is necessary to detect and identify this pheromone through molecular and chemical techniques to develop an attractant for monitoring and control of this pest in tomato.

**Ethical statement:** the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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