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ORIGINAL CONTRIBUTION

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Oviposition and olfactometry response of codling moth (*Cydia pomonella*) to quince (*Cydonia oblonga*) cultivars

María Pía Gómez[1,2] | Flavia Jofré Barud[1,2] | Ariel Díaz[3] | María Liza López[1,2]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Estación Experimental Agropecuaria INTA San Juan, San Juan, Argentina
[3]Departamento de Agronomía, Facultad de Ingeniería-Universidad Nacional de San Juan, San Juan, Argentina

Correspondence
María Liza López, Estación Experimental Agropecuaria INTA-San Juan- Calle 11 y Vidart s/n -Pocito, San Juan, Argentina.
Email: lopez.liza@inta.gob.ar

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Abstract

Quince (*Cydonia oblonga* Miller) is one of the common hosts of the codling moth, *Cydia pomonella* L. Knowledge of the chemical ecology of codling moth is based on its behaviour against apples and pears but not quince. Here, we present a laboratory study on olfactometry and oviposition behavior preference of mated females and neonate larvae of codling moth against four quince cultivars. Oviposition of mated females was evaluated on dual-choice test, whereas olfactometry response was evaluated in a Y-tube olfactometer. Neonate larvae were evaluated in a dual-choice test between pieces of fruit of quince cultivars in pairwise comparisons. In the Y-tube olfactometer assay, mated females always responded positively to the fruit-scented arm to quince fruit volatiles. Smyrna and Champion cultivars showed significant differences in the time that mated females spent in the fruit-scented arm. In the oviposition assay, all pairwise comparisons showed significant differences in the number of the eggs laid, with Smyrna and Champion being the cultivars most preferred. No significant differences were detected in dual choice of neonate larvae. All comparisons elicited the behaviour of contacting the fruit source. Based on the potential emission of recognized kairomones and the results found here, cultivars Smyrna and Champion are suggested as cultivars most susceptible to codling moth infestation.

KEYWORDS
behaviour, codling moth, larvae, olfactometry, oviposition, quince

1 | INTRODUCTION

Cydia pomonella L. (Tortricidae), commonly known as codling moth, is the most important pest of apple (*Malus domestica* Borkh.), pear (*Pyrus communis* L.), quince (*Cydonia oblonga* Mill.) and walnuts (*Juglans regia* L.; Cossentine & Vincent, 2013; Welter, 2009). Codling moth is a cosmopolitan and quarantine pest of great economic importance worldwide (Ju et al., 2021; Knight et al., 2019; Pajač et al., 2011). Fruits infested or damaged by codling moth lose commercial value and are not suitable for cold storage, being destined for fresh consumption in the local market or, for the case of apple, to the juice or cider industry (Bosch et al., 2018; Horgan, 2006).

Host-produced volatiles, known as kairomones, are remotely sensed signals that allow insects to locate their hosts. Host location is crucial for a phytophagous (herbivorous) insect to meet its nutritional requirements and to find suitable oviposition sites (Bruce et al., 2005). Host location is a process that includes distance recognition and contact with the potential host where olfactory, visual and gustatory signals are involved (Bruce et al., 2005). This process is mediated by integration, within the insect central nervous system (CNS), of numerous sensory inputs, including

Pía Gómez and Barud are first shared authors.

olfactory or gustatory semiochemical cues, and physical information such as plant colour, shape and texture. It also includes active avoidance of nonhost odours and even nutritionally unsuitable hosts (Bruce & Pickett, 2011). For codling moth, the females choose the site for oviposition and the neonate larvae choose the specific fruit to enter inside (Hughes et al., 2003; Landolt et al., 2000), so both instars have the sensorial system to detect volatiles signals from the host. Females of codling moth are capable of laying between 80 and 100 eggs and generally do so in isolation, but at high population density, they can lay eggs in groups (Jackson, 1979; Wearing, 2016). Regarding larvae, most of them bore directly into the fruit, which takes approximately a few hours (Hughes et al., 2003; Welter, 2009). Host preference behaviour of codling moth is well known for apples, walnut and pears (Joshi et al., 2015; Knight & Light, 2001; Witzgall et al., 2008). Female may discriminate among apple and walnut cultivars for oviposition (Shelton & Anderson, 1990). The fruit size, the chemical composition and the maturity level of fruits are known to affect oviposition preferences (Bezemer & Mills, 2001; Olson, 1977; Shelton & Anderson, 1990). In contrast, to our knowledge, no information is available on the response of codling moth to quince, either females or larvae. In this study, we evaluated, in laboratory, the response of female of codling moth to quince volatiles, the oviposition preference and the choice of neonate larvae against four different cultivars of quince.

2 | MATERIALS AND METHODS

2.1 | Insects

Adults were obtained from pupae collected with corrugated cardboard bands delivered by the National Program for the Control and Eradication of Codling moth executed by the 'Dirección de Sanidad Vegetal Animal y Alimentos' from the government of San Juan, Argentina. The cardboard bands were placed during February and March the time of year when the last generation of the pest is active in orchards with Champion cultivar implanted. The cardboard bands were fixed to the plant in quince orchards in the Tulum Valley and in the San José de Jáchal Valley, San Juan, Argentina. The cardboard bands were placed covering the diameter of the trunks and main branches of the quince trees. During winter, this provides a shelter site for fifth instar larvae to enter diapause. Larvae were stored at 5°C for approximately 60–90 days, and they were extracted sequentially in small groups every 2 days, placing them on tissue paper rolls, thus providing a suitable place for pupation and subsequent emergence of adults. This allowed the gradual emergence of adults and also provided enough time for laboratory assays. Conditions for breaking diapause were 25 ± 2°C and 50 ± 5% RH. Around 1200 adult insects were recovered. Neonate larvae were obtained from eggs laid by mated females on the surface of acrylic oviposition chambers (45 cm × 30 cm × 30 cm). Neonate larvae were manipulated with a fine camel pencil brush. Adults were provided with sweetened water with honey.

2.2 | Quince cultivars

Fruits were collected from a small orchard of *Cydonia oblonga* approximately 30 years old, implanted in the facilities of the Estación Experimental Agropecuaria INTA San Juan, Argentina.

The orchard consists of three rows of 30 plants each with a North–South orientation. The planting frame is 4 m between plants and 5 m between rows. The orchard is made up of cultivars implanted in linear groups of six to eight plants each. Among the available hosts, four cultivars were selected considering their productive potentials. The cultivars chosen were Portugal, Smyrna, INTA 147 and Champion. Four central plants were selected for each cultivar, leaving two plants on each side to avoid interference due to contiguity between cultivars. After fruit set, two branches per plant were protected with nonwoven bag covers (100% polypropylene, 70 cm × 80 cm) to prevent codling moth attack. At the fruit ripening stage, a combined sample of 2 kg of fruits per cultivar was collected from the two previously identified branches of the four plants chosen for each cultivar (considered as treatments). An imaginary division was made of each tree into three equal parts, and fruit was harvested from the middle segment. In order to have fruit with a similar exposition to heliophany, only fruits oriented towards the East were harvested. No pesticide treatment was applied to the orchard.

2.3 | Olfactometer response of mated females to fruit volatiles

Assays were performed with mated females of codling moth. Mated females were obtained by placing five newly emerged females together one male in acrylic boxes. When eggs were observed, all females were considered to be mated. The bioassays were performed under laboratory conditions of 24 ± 2°C and 50 ± 5% RH. For the bioassay, a Y-shaped glass olfactometer was used, based on the protocol proposed by López et al. (2011), with modifications. The glass Y-olfactometer (5 cm id, stem 16 cm, branches 10 cm) terminated in threaded-glass joints, and Teflon screw caps were connected to two separate glass vials (250 mL) with Teflon tubing. A tripod held the Y-tube in an inclining position (angle 25° between Y-tube and horizontal plane). An electric pump was used to pump air into the Y-tube olfactometer. The air stream, moistened and prefiltered through activated charcoal, was blown into the Y-tube olfactometer at 120 mL/min with a flow meter (Supelco). One piece of fresh quince with skin (5 mm thick, 30 mm long and 10 mm wide approximately), obtained from the protected fruits of the experimental design, was placed in one of the vials, while nothing was placed in the opposite vial (control). One individual was used at a time up to a total of 20 individuals

TABLE 1 Time (s) that mated females of *Cydia pomonella* took to respond to fruit volatiles of different quince cultivars in the Y-tube olfactometer (Mean, Standard Deviation, Median, Kruskal–Wallis *H*-Statistic and *p*-value).

Cultivar	*N*	Mean	SD	Median	*H*	*p*	% of response
Champion	20	202.86	182.61	150	3.37	0.3337	70
Portugal	20	170	145.34	150			70
INTA 147	20	133.75	135.05	90			80
Smyrna	20	96.88	124.24	45			80

TABLE 2 Frequency of distribution of mated females for the Initial choice between clean air (control) and quince fruit volatiles in the Y-tube olfactometer, chi-square values adjusted for frequencies <5 (MV-G2), degrees of freedom (df) and *p*-value.

	Y-tube olfactomer arm					
Cultivar	Control	Fruit	MV-G2	df	*p*	Nonresponding
Champion	4	10	2.66	1	0.1031	6
Portugal	7	7	0.00	1	>0.9999	6
INTA 147	4	12	4.19	1	0.0408	4
Smyrna	4	12	4.19	1	0.0408	4

for each cultivar under study. Each mated female was placed on the main stem of the Y-tube olfactometer during 2 min with no airflow, which allowed the females to acclimatise to the olfactometer. The pump was then activated to provide the air stream for another 8 min, setting a maximum time of 10 min for each tested female. During this period, the time, in seconds, that each female remained in the treatment arms or in the stem itself (longest branch of the Y-tube) was recorded and was called permanence time. The time it took the female to respond (latency) was also recorded as well as the choice at the beginning of the assay and at the end of it (initial and final choice). Assays were carried out from 5:00 PM to 8:00 PM under a 60 W red light bulb placed centrally over the joint of the two arms to provide illumination given the crepuscular activity of the codling moth. Every five insects, the entire olfactometer was rotated 180° to avoid position effects. For each female, the fruit sample was changed to avoid potential interference due to the rapid oxidation of the fruit. Latency was analysed by Kruskal–Wallis test. Taking into account that permanence time data are not statistically independent, the Wilcoxon sign test was used for paired samples. Initial and final choice data were analysed with a chi-square goodness-of-fit test. SPSS 15.0 (SPSS Inc.) software was used.

2.4 | Oviposition preference of codling moth for quince cultivars

An oviposition preference bioassay was carried out offering mated females two quince fruits of different cultivar, making pairwise comparisons of the four cultivars under study.

Five mated females were placed into 3-L glass jars placed in a horizontal position and closed with a nylon mesh. Inside each jar, a 5 cm×8 cm acetate card was placed at each end of the jar as an oviposition substrate, on which a fruit of each quince cultivar was placed. The number of eggs per card was counted after 12 to 15 days, since this is the average life time of the adults under field conditions. Each pairwise comparison was repeated four times. Data were analysed by a chi-square goodness-of-fit test. SPSS 15.0 (SPSS Inc.) software was used.

2.5 | Neonate larvae preference bioassay

The preference of neonate larvae was carried out according to Andreadis et al. (2014) with modifications. The assay consisted of a double-choice bioassay of pairwise comparisons. A piece of quince fruit (1 cm^3) corresponding to each cultivar was placed at the ends of a Petri dish covered at its base with a circle of filter paper (10 cm in diameter). A neonate larva was placed in the centre of the circle, considering an equidistant length of each piece of fruit. A maximum time of 10 min was given for the choice of the larva. The choice assay was replicated 20 times for each comparison. The first choice made by the neonate larvae was recorded, as well as the time and distances travelled by the larvae. Two undefined behaviours were also analysed, those larvae that did not have any movement were designated as 'Arrestment', and the larvae that did show an exploratory movement but did not make a clear choice (they did not contact the piece of fruit), were designated as 'Neutral Choice'. Data were analysed with a chi-square goodness-of-fit test. SPSS 15.0 (SPSS Inc.) software was used.

3 | RESULTS

No significant differences were found for the latency, the time it takes the mated females to respond to the volatiles of the different quince cultivars. More than 70% of females responded positively in the Y-tube olfactometer within 3 min of time elapsed. A slight trend of faster response time was recorded for the Smyrna cultivar, whereas the highest response time was found for the Champion

Cultivar	Y-tube olfactomer arm Control	Fruit	MV-G2	df	p	Nonresponding
Champion	3	11	4.86	1	0.0275	6
Portugal	3	10	3.98	1	0.0461	7
INTA 147	4	12	4.19	1	0.0408	4
Smyrna	3	13	6.74	1	0.0094	4

TABLE 3 Frequency of distribution of mated females for Final choice between clean air (control) and quince fruit volatiles in the Y-tube olfactometer, chi-square values adjusted for frequencies <5 (MV-G2), degrees of freedom (df) and *p*-value.

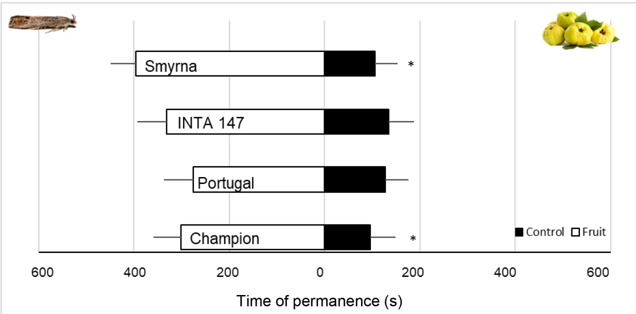

FIGURE 1 Preference of codling moth, *Cydia pomonella*, in a Y-tube olfactometer to volatiles emitted by fruits of quince, *Cydonia oblonga*, cultivars. Each bar represents the mean (±SEM) time of permanence of females for each of the two odour sources. For each comparison, 20 females were tested on independent fruit pieces. An asterisk indicates a significant preference between the control and fruit-scented arm; $p<0.05$ (Wilcoxon sign test). [Colour figure can be viewed at wileyonlinelibrary.com]

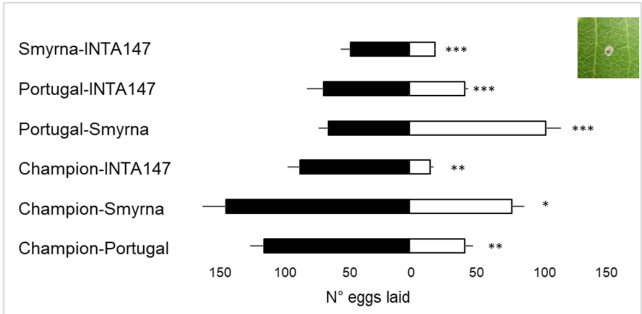

FIGURE 2 Oviposition preference of *Cydia pomonella* females in dual choice assays in pairwise comparisons of four quince, *Cydonia oblonga*, cultivars. Each bar represents the mean (±SEM) number of eggs laid and asterisks indicate significant differences between quince cultivars (χ^2 test: *$p<0.01$, **$p<0.001$, ***$p<0.0001$). [Colour figure can be viewed at wileyonlinelibrary.com]

cultivar (Table 1). Most of the females that showed preference did so for the fruit-scented arm of the Y-tube olfactometer. At the initial choice, significant differences were found for INTA 147 and Smyrna cultivars (Table 2), whereas at the final choice, significant differences were found for all cultivars (Table 3). Regarding the time spent by the females in each arm of the Y-tube olfactometer, the highest values were found in the fruit-scented arm, the females were active about 80% of the time. Significant differences between the control and the fruit-scented arm were found for Champion and Smyrna cultivars (Figure 1).

In the preference for oviposition, all comparisons showed significant differences ($p<0.05$). The highest value of eggs laid was observed in the Champion cultivar compared with the three remaining cultivars. The Smyrna cultivar was the second most preferred since twice as many eggs were found compared with the INTA 147 and Portugal cultivars. INTA 147 cultivar was always the least attractive (Figure 2).

When analysing the distribution frequency of larvae for each pair of comparisons, no significant differences were found in any of the six comparisons (Table 4). Undefined behaviours were observed (Table 5). Larvae exhibited loitering behaviour or remained in the central point of the experimental arena (arrestment). In the Champion vs Portugal comparison, no loitering or arrest behaviours were observed. On the contrary, the largest number of larvae with undefined behaviours were recorded in the comparisons Smyrna vs Portugal and Champion vs Smyrna. In addition, no significant differences were found in the distance travelled by the larvae for any pair of comparisons or between the time it takes to reach the fruit. The larvae travelled an average distance of 3.03 ± 0.06 cm and took 381.17 ± 18.87 s to contact the fruit.

4 | DISCUSSION

Both larvae and adults of the codling moth are well known to use kairomones during host location (Hughes et al., 2003). Responses of mated females exposed to quince volatiles in the Y-tube olfactometer were positive in all cases because females were always attracted by the fruit-scented arm. The most attractive cultivars were Smyrna and Champion. Champion and Smyrna cultivars were more attractive than Portugal and INTA 147 cultivars for oviposition. These results suggest that other than chemical stimuli are important for mated females, since in the Y-tube olfactometer, only chemical stimuli are available, whereas in the oviposition assay visual, tactile and volatile stimuli are accessed by females. Neonate larvae showed no discrimination between fruit cultivars, since no significant differences were found in the choice frequency in pairwise comparisons. It could be possible that quince cultivars emit competing levels of kairomones, which could explain the undefined behaviours and finally the lack of preference for a particular cultivar. Neonate larvae are attracted to apples from 1.5 cm in still air (Sutherland, 1972) and greater distances with airflow (Landolt et al., 1998, 2000). Therefore, the distance we used in the assay is close to those reported to stimulate the larvae movement. Moreover, arrestment was observed in larvae

TABLE 4 Frequency of distribution of neonate larvae for each pair of comparisons of quince cultivars, chi-square values adjusted for frequencies <5 (MV-G2), degrees of freedom (df) and *p*-value.

Comparison[a]	Fruit 1	Fruit 2	Total	Exp.[b]	Chi^2 (MV-G2)	df	*p*
Champion/INTA 147	11	5	16	8	2.31	1	0.1286
Smyrna/Portugal	9	4	13	6.5	1.83	1	0.179
Champion/Portugal	13	7	20	10	1.83	1	0.1764
Champion/Smyrna	7	8	15	7.5	0.07	1	0.7962
Portugal/INTA 147	8	8	16	8	0.00	1	>0.99
INTA 147/Smyrna	8	9	17	8.5	0.06	1	0.8083

[a]The first cultivar is Fruit 1 and the second is Fruit 2.
[b]Exp. expected frequency value according to random choices.

TABLE 5 Number of larvae with undefined behaviours (Arrestment: they do not show movement, Neutral choice: they move but do not show choice).

Comparison	Arrestment	Neutral choice	Sum of undefined behaviours
Smyrna/Portugal	3	4	7
Champion/Smyrna	2	3	5
Champion/INTA 147	3	1	4
Portugal/INTA 147	1	3	4
INTA 147/Smyrna	2	1	3
Champion/Portugal	0	0	0

exposed to Smyrna vs. Portugal and Champion vs INTA 147, and the maximum number of loitering behaviour was observed for Smyrna vs Portugal, this comparison being the one that presented the highest number of undefined behaviours. While (E, E)-a-farnesene acts as an attractant to neonate larvae, other compounds present are needed to cause arrestment, although candidate compounds have not yet been identified (Hughes et al., 2003). The host location phase is the period when the larvae are most vulnerable and is a critical stage in the lifecycle of these insects, since disruption of this behaviour extends the phase of host location. Bengtsson et al. (2001) found that the preference of codling moth for apple volatiles depends on the perception of terpenoids and esters present in different varieties and phenological moments. In our study, ripening fruits were used for female preference in the Y-tube olfactometer assay and neonate larvae preference, whereas growing fruits were used for the oviposition assay. During phenology, the fruits undergo changes in their chemical composition. Reed and Landolt (2002) argue that females exposed to volatiles of apple fruits in different phenological states, both healthy and damaged, responds preferentially to the Red Delicious apple cultivar since it produces greater attraction due to the emission of kairomone, α-farnesene and, in lesser extent β-caryophyllene. According to López et al. (2022), healthy and infested fruits of Champion and Smyrna cultivars show higher percentages of α-farnesene and pear ester, than INTA 147 and Portugal cultivars, both compounds recognised as kairomones of codling moth. Then, the preference of females for oviposition in Smyrna and Champion cultivars may be related to the higher levels of kairomones emitted by these cultivars.

Knowledge about susceptible varieties or cultivars to pests is a useful tool for producers, since resistant cultivars can be selected and, within them, those with better yields in production parameters. Royo Jordán (2014) found that the Franquette walnut variety causes highest attraction of different pests, especially codling moth, compared with varieties such as Chandler and Lara. Based on the results of the oviposition preference, we suggest that Champion and Smyrna might be more susceptible cultivars to codling moth infestation since they are preferentially selected by females. Since the codling moth synchronizes its population peaks with the crop cycle, further studies on its behaviour throughout the crop cycle of quince cultivars are required. To the best of our knowledge, the present study is the first evaluating the response of codling moth to quince cultivars.

AUTHOR CONTRIBUTIONS

María Pía Gómez: Data curation; formal analysis; investigation; writing – original draft; writing – review and editing. **Flavia Jofré Barud:** Data curation; formal analysis; investigation; writing – original draft; writing – review and editing. **Ariel Díaz:** Conceptualization; data curation; formal analysis; investigation. **María Liza López:** Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicting interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.7643581.

ORCID

María Liza López https://orcid.org/0000-0001-8026-5073

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