

## Olfactory responses and feeding preferences of *Copitarsia uncilata* (Lepidoptera: Noctuidae) to four aromatic herb species

Preferencias alimenticias y respuestas de olfato de *Copitarsia uncilata* (Lepidoptera: Noctuidae) a cuatro especies de hierbas aromáticas

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**Abstract:** Colombian aromatic herbs have great potential as an export commodity. The genus *Copitarsia* is considered as an economic and a quarantine pest attacking them. In herbivore insects, host plant choice is made by adults and influenced by host plant quality. There were performed olfactory and feeding tests by using four-arm olfactometer and offering four different aromatic herbs (basil, mint, rosemary, or thyme) to determine the host selection behavior of *Copitarsia uncilata* Burgos and Leiva. Parameters, such as adult choice, larval weight, and time spent by larva on particular herb were measured. The preferences of adults and immature of *C. uncilata* varied significantly among the herbs in olfactory and larva feeding tests. The adults showed significantly higher responses to essential oils of basil and rosemary. Higher weight of larva was recorded on mint and basil. Further studies on larval development and longevity of adults on different herbs are necessary.

**Key words:** Quarantine pests, olfactory perception, aromatic herbs, pest ethology.

**Resumen:** Las hierbas aromáticas en Colombia tienen un gran potencial como producto de exportación. En ellas, el género *Copitarsia* es considerado como una plaga de importancia económica y cuarentenaria. Los adultos de insectos herbívoros toman la decisión de escogencia de la planta hospedera. Esta decisión es influenciada por la calidad del hospedero. Se realizaron ensayos de pruebas olfatorias y alimenticias usando un olfatómetro de cuatro brazos ofreciendo cuatro hierbas aromáticas (albahaca, menta, romero y tomillo) para determinar el comportamiento alimenticio de *Copitarsia uncilata* Burgos y Leiva. Se midieron parámetros como la escogencia de hospederos por los adultos, el peso de larvas y duración de asociación con cada una de las cuatro hierbas. Las preferencias de adultos e inmaduros hacia las hierbas variaron tanto en los ensayos de olfatometría con adultos como en las pruebas alimenticias con larvas en forma significativa. Los adultos mostraron una preferencia significativa hacia los aceites esenciales de albahaca y romero. Se registró un peso mayor entre larvas cuando comieron menta y albahaca. Futuros estudios sobre el desarrollo larval y la longevidad de adultos son necesarios.

**Palabras clave:** Plaga cuarentenaria, percepción de olfato, hierbas aromáticas, etiología de plagas.

### Introduction

In Colombia, aromatic herbs have become an important option for agricultural production with potential for exportation and possibilities to compete on international markets such as in North America and the European Union (Fajardo and Sánchez 2007). Eighty percent of the exported aromatic herbs are fresh (Forero 2005). Among the species allowed to be imported into the USA, basil (*Ocimum basilicum* Linnaeus), mint (*Mentha spicata* Linnaeus), rosemary (*Rosmarinus officinalis* Linnaeus), and thyme (*Thymus vulgaris* Linnaeus) represent the major contingent (*ibid.*).

The insect pests that are present on these herbs belong to the four orders; Diptera, Hemiptera, Lepidoptera, and Thysanoptera (*ibid.*). Among these, the families Agromyzidae, Aleyrodidae, Aphididae, Noctuidae, and Thripidae are regularly intercepted by USDA-APHIS-PPQ (Lenis 2007 cited by Patiño 2014). Noctuid moths of the genus *Copitarsia*, subfamily Cucullinae, are frequently intercepted at US border ports on produce and cut flowers from Mexico, Central America, and South America (Venette and Gould 2006). The members of *Copitarsia* spp. are considered a

quarantine pest as these cause significant losses. Especially, *Copitarsia* spp. spreads via commodities contained with eggs or larva. Majorities have been originated in Colombia followed by Mexico and Ecuador (*ibid.*).

The genus *Copitarsia* spp. comprises 21 species. Three of those species are grouped in the *Copitarsia* complex *turbata* (Herrich-Schaeffer); the other 18 species belong to the *Copitarsia* complex *naenoides* (Butler) (Angulo and Olivares 2003). A very cryptic feature defines the difference between these two groups: the uncus vertex. The *Copitarsia* complex *turbata* is characterized by an uncus vertex present in a dorsally and in a plain manner, without overlapped or noticeable structure. The *Copitarsia* complex *naenoides* has two longitudinal plaques with recurved indentations (*ibid.*). Simmons and Pogue (2004) redescribed *C. decoloradecolora* Guenée giving *C. turbata* as synonym.

*Copitarsia* spp. occur along the western edge of South and Central America from the southern tip of Argentina through central Mexico attacking a wide range of crops, such as *Allium*, *Alstroemeria*, *Brassica*, *Mentha*, *Rosa*, *Asparagus*, strawberry, spinach, and many others (Castillo and Angulo 1991; Angulo and Olivares 2003; Gould *et al.* 2013).

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For pest insects, locating suitable host plants is a formidable task requiring a sophisticated detection mechanism. The host selection process can be seen as a continuum between two extremes, namely, insects ‘choosing’ their host from a distance using olfactory and visual cues, and insects ‘selecting’ their host only after contact, when gustatory cues are also employed (Bruce *et al.* 2005). Host selection is crucial for the survival of herbivorous insects, and this decision is often based on the suitability of a plant for feeding and oviposition (*ibid.*). This selection goes together with host plant quality, which depends on several factors including the nutritional value (Scheirs *et al.* 2003), defensive chemicals (Dicke and van Poecke 2002), physical barriers, such as trichome distribution and density (Kennedy 2003), and associated natural enemies (Dicke and van Loon 2000; Scheirs and de Bruyn 2002). Odors from host plants play a vital role in the attraction of insect herbivores (Guerin and Visser 1980) allowing herbivores locating a host plant from a distance (Bernays and Chapman 1994). Plant volatiles are detected by specifically tuned and highly sensitive olfactory receptor neurons (Bruce and Pickett 2011).

These allelochemical interactions between Lepidoptera and plants have been extensively studied. Pavela (2011) pointed out the importance of allelochemicals in the defense system of plants against herbivores. These compounds can be toxic, repellent, attractive or nutritious to insects. They play an important role as stimulants or inhibitors of alimentionation, as part of host plant resistance, or as communication vehicle between competitors, parasitoids and predators (Chapman 1994). Plants liberate and produce many volatiles, specific for each plant and each insect (Torres 2009). Hilker and Meiners (2002) claimed that these compounds are basic for host recognition and acceptance by insects.

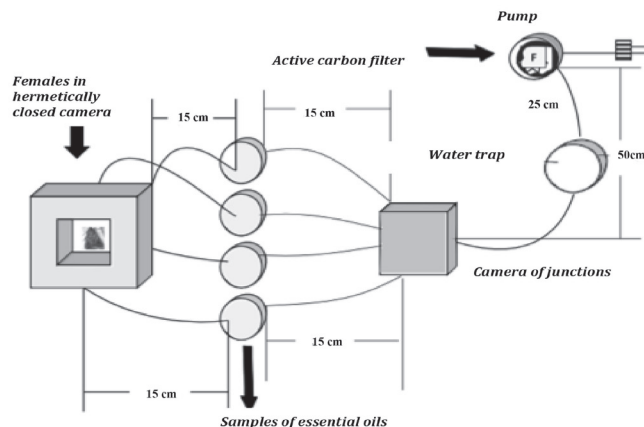
In order to understand better host preferences, various workers have conducted direct and indirect preference tests. Bado *et al.* (2001) offered three Solanaceae species to larvae of the chrysomelid *Lema bilineata* (Germar) in free choice and non-choice tests. The authors measured the consumed leaf area as direct preference tests and the time insects stayed on the different food types as indirect preference tests.

Olfactory experiments complete the above mentioned direct and indirect preference tests. They are mostly used for studies on the perception of plant emitted volatile compounds which insects use for orientation (Chapman 1994). McIndoo (1926) developed one of the earliest olfactometers. He released beetles in Y-glass-tubes testing the attractiveness of odours from different host plants. These type “Y” olfactometers are still the most used approach where the insects have the option to choose between two different odours. Later, Pettersson (1970) designed a four-armed olfactometer. Vet *et al.* (1983) constructed an olfactometer where insects are introduced into a chamber connected to four arms with their respective odour source. The airflow in each arm can be regulated separately avoiding air turbulences as it happens at the junctions in Y-tubes when mixing the offered odours. The Y-tube olfactometer is used for measuring chemotactic or olfactory responses by insects within short distances or in situations where chemical signals are diffused into space without being transported by an airstream (López-Avila and Rincón 2006). There is also an olfactometer designed as a tunnel for insects that have excellent flight capacities towards different odour sources. However, their disadvantage is that only one odour can be tested at the same time (*ibid.*).

This study thus evaluated the olfactory response and feeding preferences bioassays of *C. uncinata* on four aromatic herbs, basil, mint, rosemary, and thyme.

## Materials and methods

The experiments were conducted in the Faculty of Agricultural Science of the National University in Bogotá (2.640 masl), Colombia, during 2012 and 2013. Laboratory maintained larvae of *C. uncinata* were used in this study. In order to rear *C. uncinata* in our laboratory, we recollected eggs, larvae, and pupae at the installations of the *Alstroemeria aurea* grower “Flores de Conchita Ltd.” in Bojacá (Cundinamarca, Colombia). The colonies were started with one couple of male and female adults. The female laid 350 viable eggs. These eggs hatched after three or four days; the larvae passed through five instars. All larvae of *C. uncinata* were taken from the colonies, which were maintained at 12:12-hours light/dark (natural photoperiod) at  $21.1 \pm 1.7$  °C and  $50.7 \pm 7.5$  % relative humidity according to the average of the environmental conditions in the laboratory, which were similar to those where the insects and plants were recollected. Larvae were individualized in plastic cups (diameter top/bottom: 3.52/2.22 inches, height: 4.3 inches/16 ounces) in order to avoid cannibalism and fed with buds of Peruvian lily (*Alstroemeria aurea* Linnaeus). After 25-35 days the larvae pupated and were separated according to their sex following the descriptions by Pogue and Simmons (2008): as in most insects the openings of the female genitals are located in the eighth and ninth segment, those of the male only in the ninth. The separated pupae were confined in new cups of the same size as mentioned before, filled up to a quarter with vermiculite or sawdust. After 6 to 10 days they developed into adults. These were paired and each pair was confined in a separate crystal bonbonniere. The adults were fed with honey, water, and sucrose solution (1:5:1 ratio) until they died within six to eight days. The complete life cycle oscillated between 45 and 52 days. The colonies were held at  $21.1 \pm 1.7$  °C,  $50.7 \pm 7.5$  % RH, and 12D:12L-hours (natural photoperiod). The age of the harvested herbs was as follows: basil (“Nufar”) at 90-120 days, mint (“Hierbabuena”) at 90 days, rosemary (“Romero común”) at 180 days, thyme (“Tomillo limonero”) at 90 days.



**Figure 1.** Overall illustration of design and process of olfactometer bioassay.



Figure 2. Arrangement of experimental units.

**Identification of the insect.** At the beginning of the experiment we believed we were working with *Copitarsia decolora*, however, an extend analysis of male genitalia using keys developed by Burgos *et al.* (2010) and Quimbayo *et al.* (2010) revealed that the analyzed specimens belonged to *C. uncinata* Burgos and Leiva. Tania Olivares and Andrés Angulo, noctuid specialists at the University of Concepción, Chile, confirmed our findings in November 2012.

**Olfactory response experiment.** It was designed and elaborated an olfactometer with four arms based on López-Ávila and Rincón (2006) and Pacheco *et al.* (2012). A pump equipped with an activated charcoal filter generated the airflow that entered the olfactometer after having passed a water trap in order to avoid the entrance of odours into the system. Then, the air reached the recipients filled with 0.5 ml essential oil (EO). From there the air diffused the aromatic odours through 6/8 inches' tubes to the hermetical camera where six gravid females were confined (Fig. 1). The females could fly or walk towards the exit of the emanations. The olfactive perception of *C. uncinata* was assessed using the Randomized Complete Block Design (RCBD) with four treatments (the aromatic host plants) and nine repetitions (GLM procedure of SAS 2009). The natural EO's were acquired from Green Andina Colombia Ltda. They were processed without any organic solvents in order to obtain more reliable results.

The variable "number of females" was computed based on the studies of Cerda *et al.* (1996), Narayandas *et al.* (2006) and Saïd *et al.* (2006): The orientation of the gravid

females was determined by counting the females that headed to one of the olfactometer arms with its specific odour. The experimental unit was revised during 10 minutes every 60 minutes during 12 hours (8 am to 8 pm). Based on these data we calculated the relative distribution of females by: (number of gravid females in one arm / all gravid females) x 100. Additionally, we determined the time that females remained in the olfactometer arms.

**No-choice experiment.** Two types of tests were conducted in order to determine the feeding preferences of *C. uncinata*. With means of the direct "Weight Test" (no-choice test) it was determined the weight increase of each larva feeding on a given host plant. In the "choice test" it was measured the time that the insect was associated with each of the simultaneously offered four host species. In the latter test we classified also as an "indirect" test because only permanence or time on each leaf was recorded (not food ingestion). Morenos Ltd. KISKA in Bogotá provided all plants we deployed for these experiments.

"Weight increase" was determined by weighing the larva before and after a feeding time of two hours on each of the four plants species. Before initiating the test 30 larvae from *Alstroemeria* buds were separated as adaptation period. After a starving period of two hours each larva was weighed and placed individually on a leaf segment (20 grams each) of one of the four aromatic plants. Larvae were weighed every two hours with the precision scale (Precisa®, Mark 520, readability 0.01 g). After 12 hours the increase of the accumulated weight was calculated. This procedure was

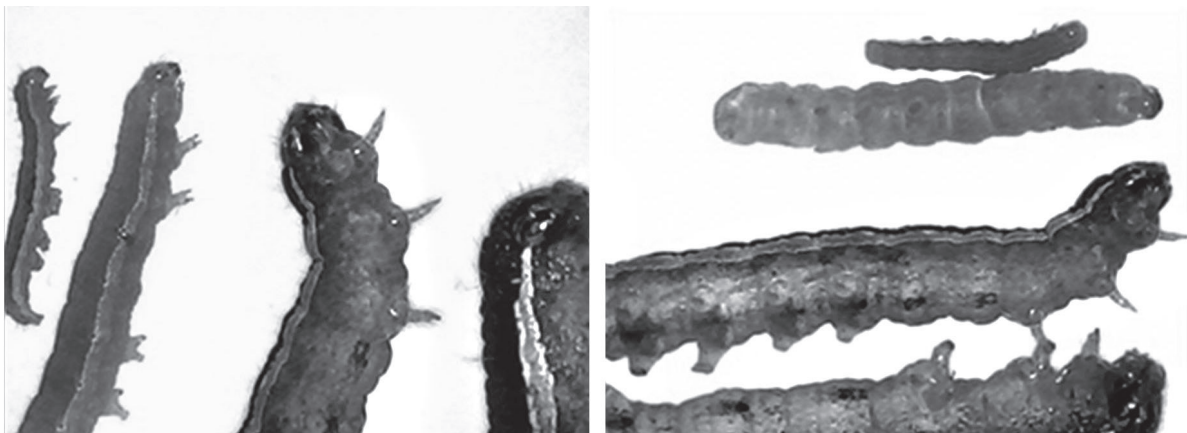
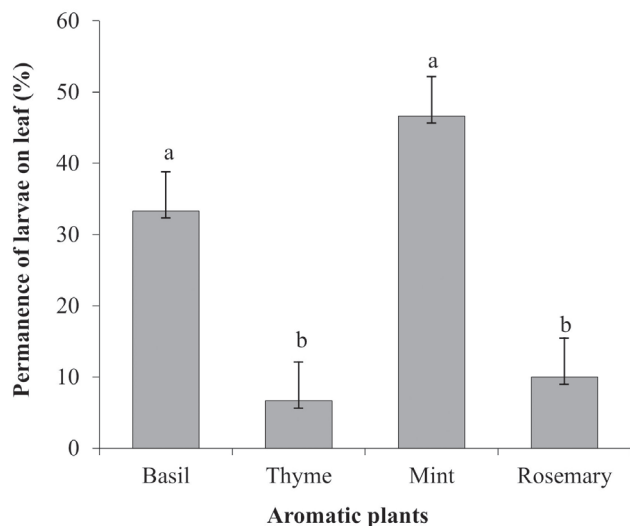


Figure 3. Larval instars 2, 3, 4, and 5 of *Copitarsia uncinata*. This picture may explain why the third instar has the highest proportional weight increase.



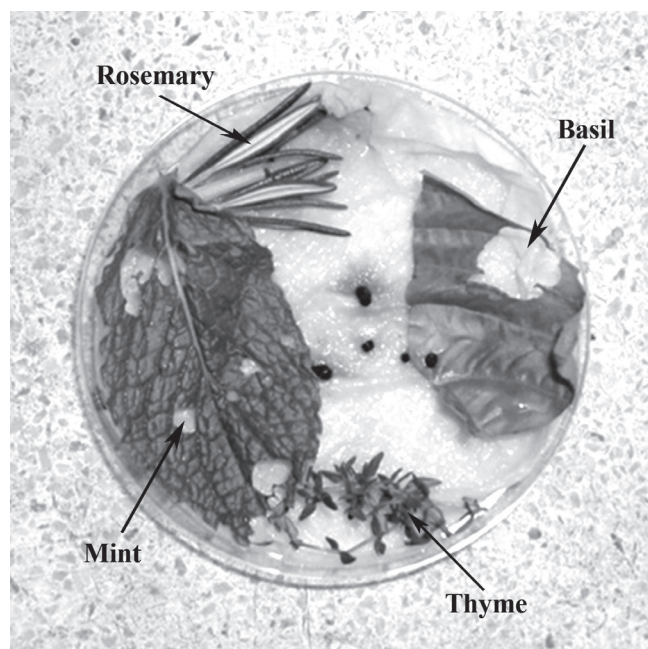
**Figure 4.** Permanence test of *Copitarsia uncinata* (3rd instar) versus four aromatic plants (basil, thyme, mint, and rosemary). Bars represent standard error (SE) or standard deviation (SD). Same letter indicates no significant differences between means ( $F_{2,7} = 87$ ;  $P \leq 0.05$ ; Scheffé test).

repeated with the same individuals using the other three aromatic hosts. A randomized complete block design (RBD) was used with the four aromatic plants as treatments and 30 replicates.

**Choice experiment.** In the “preference” or “choice-test” the frequency of associations between larvae and the aromatic leaves was evaluated. Before starting the experiment larvae were starved as an adaptation for two hours. Then, each larva was placed in the centre of a filter paper disc (Whatman Grade 1,  $\varnothing$  10 cm) in a Petri dish and surrounded by one leaf (20 g) of each aromatic plant (Fig. 2). After this adaptation period a two hours observation was started and recorded the leaves the larva was associated with. The experiment was repeated for 30 times. We considered a larva present on a leaf as 1, absent as 0. These data of the indirect test were basis for the construction of the relative distribution as Bado *et al.* (2001) suggested: (Number of individuals associated with host 1, or 2, or 3, or 4 / total number of larvae on all host plants)  $\times$  100.

In both experiments, 3<sup>rd</sup> instar larvae were used because at this stage their body length and cephalic capsule width grow faster than in any other stage (Fig. 3) (Moreno and Serna 2006). Hence, we assumed that this stage makes it easier to find a correlation between ingestion quantity and body growth. We considered a larva as third instar when the diameter of the cephalic capsule reached 0.84 mm (*ibid.*). was used the capsule width by means of the software ImageJ 1.46 (2012) and using a vernier scale as base.

**Statistical analysis.** Before initiating the statistical analysis, the assumptions of the randomized complete block design (RCBD) were assessed, such as normal distribution or homogeneity of variances. Data of both experiments “Weight increase” and “Preference” test did not fulfill the requirements for normal distribution, even after transformation with square roots or logarithm. For this reason, we deployed the non-parametric Kruskal-Wallis test and the Scheffé-test for



**Figure 5.** Experimental unit of choice test. Larvae of *Copitarsia uncinata* didn't cause any damage to rosemary and thyme, whereas basil and mint leaves showed clear feeding symptoms.

analyses of significance. We conducted a correlation analysis between the treatments “Weight” and “Preference” using the Spearman coefficient of SAS 9.2 (2009).

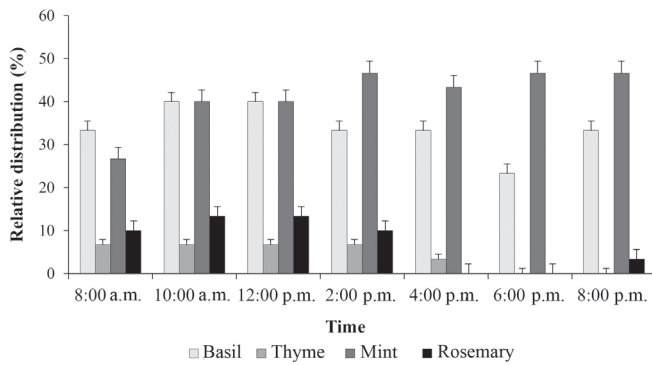
## Results and discussion

The preferences are presented as percentage measuring the time that larvae remained on a leaf. There was a clear preference of the *C. uncinata* larvae to stay longer on mint or basil than on rosemary or thyme (Fig. 4). Time of permanency in association with thyme and rosemary was almost the same ( $F_{2,7} = 87$ ;  $P < 0.05$ , Scheffé-test).

These results are corroborated by our observation that leaves of mint and basil were more consumed by the larvae than thyme and rosemary (Fig. 5). Moon *et al.* (2011) reported that mint oils attract *Lycorma delicatula* (White) (Hemiptera: Fulgoridae). This stands in contrast to Regnault-Roger *et al.* (2012) who stated that the great majority of reviews clearly comment that most aromatic plant species have a repellent and deterrent effect on insects. However, the chemical composition of mint, particularly the presence of the terpenoid carvone, creates an attraction to insects that is superior to others compounds, which can be explained by its predominance (89.7 %) in its EO's (Moon *et al.* 2011). Cohen (2005) argued that the compound, which is a product of the secondary plant metabolism, permits the so-called token-stimuli. This basically stimulates feeding without being involved in the nutritional process. This might be considered as an explanation why *C. uncinata* larvae did prefer mint.

Ferreira and Moore (2011), as well as Moon *et al.* (2011), described that rosemary and thyme have a well-known repellent effect on Culicidae and Cicadidae, respectively. For this reason we assumed that most *C. uncinata* larvae didn't feed on those two aromatics either.

Regarding preferred daytime larvae showed a tendency to remain longer associated with mint after midday (Fig. 6).

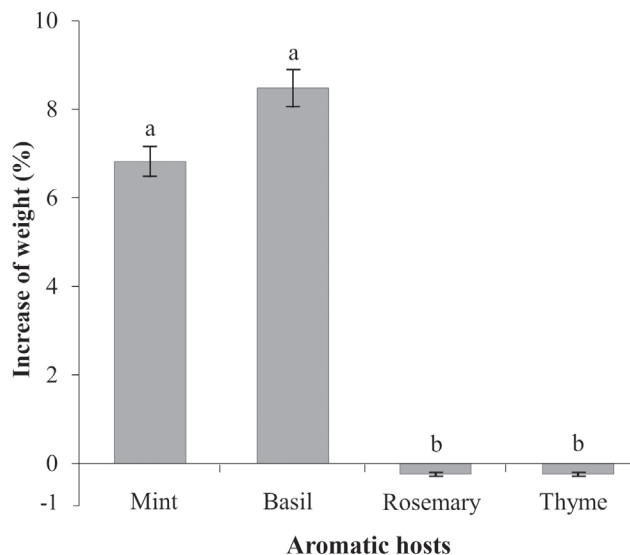


**Figure 6.** Time of permanency of larvae (3rd instar) of *Copitarsia uncinata* on four host plants. Data are presented as percentage of the whole time of permanency during 12 hours.

This may be explained by the increasing temperature. Scriber and Slansky (1981) mentioned that food quality (in terms of water and dry weight) could be affected by temperature. This was corroborated by our observation that basil loses more liquid than mint indicating that latter conserved water with increasing temperatures around midday, which did not occur with basil although the leaves laid on a moist filter paper in a sealed Petri dish.

The weight of larvae increased more when they fed on mint or basil in contrast to thyme and rosemary, where their weight even slightly decreased ( $F_{2,7} = 87$ ;  $P \leq 0.05$ ; Scheffé test) (Fig. 7). The treatments basil and mint were not significantly different, as well as the means of rosemary and thyme. The weight increase correlated with preference ( $r = 0.772$ ;  $P < 0.0001$ ).

**Olfactory test.** Figure 8 shows the preference of gravid females for the four offered EO's in the olfactometer. We didn't observe any significant differences between basil and rosemary; however, there was a clear difference between mint and thyme. Mint was significantly less attractive than basil

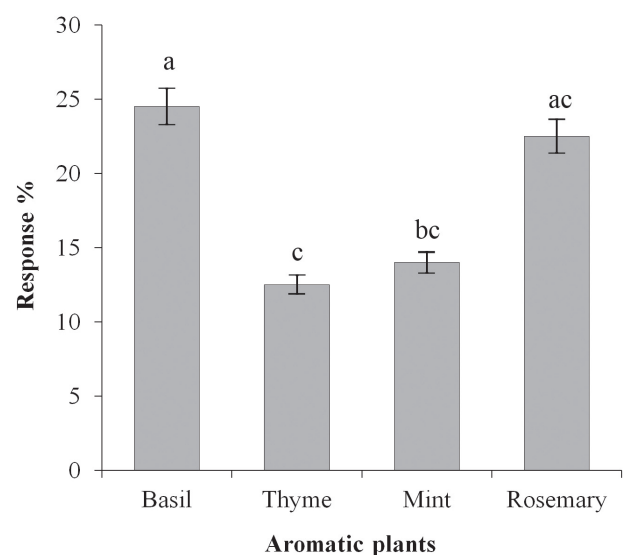


**Figure 7.** Weight increase of larvae feeding on four aromatic hosts, respectively, during 12 hours. Treatments with the same letter are not significantly different ( $F_{2,7} = 87$ ;  $P \leq 0.05$ ; Scheffé-test).

and even less than rosemary, and but was more accepted than thyme. Although the difference between mint and thyme was not significant the result suggested the repellent character of thyme.

This behavior was not expected since the larvae showed a clear preference for basil and mint. The preference-performance hypothesis predicts that oviposition preference should correlate with host suitability for offspring development (=survival offspring and later adult potential fecundity) because females are assumed to maximize their fitness by ovipositing on high quality hosts (Scheirs and de Bruyn 2002; Schowalter 2006). However, some studies reported strong, positive correlations between host preference and offspring performance, many studies found poor correlations (Scheirs and de Bruyn 2002). Petit *et al.* (2015) came to a similar conclusion, although they argued in a different way: Host selection may differ according to the host range given that poly-, oligo-, and monophagous species use different host selection strategies. For example, oligophagous and monophagous species are more efficient when making decision for host selection than polyphagous species (Bernays 2001). This tendency might have affected the females of the polyphagous *Copitarsia* genus by their selection of volatiles. Carrasco *et al.* (2015) hypothesized that innate plant preferences in combination with the current environmental conditions would regulate the final oviposition choice. Nevertheless, the preference hierarchy is a dynamic one, since it can be modified, for instance, by previous experiences, especially in oligophagous and polyphagous species.

Regnault-Roger *et al.* (2012) concluded that the efficacy of ethereal oils and their constituents varies according to the phytochemical profile of the plant extract and the entomological target. The bruchid *A. obtectus* is more sensitive to phenolic monoterpenes and the aphid *Rhopalosiphum padi* (Linnaeus) to methoxylated monoterpenes, whereas *Ceratitis capitata* (Wiedemann) flies respond to both types of compounds. Ethereal oils such those extracted from of thyme, rosemary, and Eucalyptus (L'Heritier) have antifeedant or



**Figure 8.** Response of essential oils by gravid females of *Copitarsia uncinata*. Treatments with the same letter are not significantly different ( $P < 0.005$ ; Tukey-test).

repellent activity. The oil of citronella (*Cymbopogon nardus*, (Linnaeus) Rendle) repels mosquitoes and flies. Garlic (*Allium sativum* Linnaeus) oil is a deterrent to many insect herbivores. These are currently marketed to horticulturists, greenhouses, and home gardens in the United States and the United Kingdom (*ibid.*). Scriber and Slansky (1981) claimed that insect behavior is related to feeding habits and other characteristics, and influenced by a wide range of biochemical variables, morphological mechanisms, the host, and the pest. These authors also mentioned the repellent and antifeedant action of the EO's of thyme and rosemary that exactly corroborated our results.

### Conclusions

Future studies on the establishment of cultural practices focusing on repellent products based on thyme and rosemary as a strategy against *Copitarsia* spp. are required. It should be the aim of these future investigations to include factors like plant material, insect's developmental stage (larval instar, gravid or not gravid females), olfactometer design (number of arms, hermetical camera, air pump), and disposition of treatments (time and space) for the search for reliable answers in order to acquire more knowledge about ethology of this pest insect. Moreover, studies on larval development and longevity of adults on different herbs should be continued.

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