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Variation in the capture of *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) males using commercial sex pheromones on three solanaceous hosts

Variación en la captura de machos de *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) usando feromonas sexuales comerciales en tres hospederos solanáceos

Variação na captura de machos de *Neoleucinodes elegantalis* Guenée (Lepidoptera: Crambidae) usando feromônios sexuais comerciais em três hospedeiros solanáceos

Ana Elizabeth Díaz-Montilla,[*1] Harold Suárez-Barón,[2] Gerardo Gallego-Sánchez,[3] William Fernando Viera-Arroyo,[4] Clara Inés Saldamando-Benjumea[5]

[1] Investigadora PhD, Corporación Colombiana de Investigación Agropecuaria (Corpoica), Centro de Investigación Obonuco. Pasto, Colombia. Correo: aediaz@corpoica.org.co. orcid.org/0000-0002-7902-2549
[2] Profesor de Biología Celular y Molecular, Universidad de Antioquia, Facultad de Ciencias Exactas y Naturales, Instituto de Biología. Medellín, Colombia. Correo: harold.suarez@udea.edu.co. orcid.org/0000-0002-4109-9305
[3] Jefe de Laboratorio de Biotecnología, Centro Internacional de Agricultura Tropical (CIAT). Cali, Colombia. Correo: g.gallego@cgiar.org
[4] Coordinador Nacional de I+D Fruticultura, Instituto Nacional de Investigaciones Agropecuarias (Iniap). Quito, Ecuador. Correo: william.viera@iniap.gob.ec. orcid.org/0000-0003-4472-4126
[5] Profesor titular, Universidad Nacional de Colombia sede Medellín, Escuela de Biociencias. Medellín, Colombia. Correo: cisaldam@unal.edu.co. orcid.org/0000-0001 9368-8200

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* Autor de correspondencia

Resumen

Neoleucinodes elegantalis Guenée (Lepidoptera: Crambidae) representa la plaga más perjudicial de la familia Solanaceae. Estudios actuales demostraron que la especie se diferenció en cuatro razas según la variación de los órganos genitales femeninos, la morfometría del ala y la secuencia del gen mitocondrial Citocromo Oxidasa1 (CO1). Se cuantificó el número de machos capturados en Colombia y Ecuador empleando trampas cebadas con dos feromonas sexuales: Neolegantol® y P228 sintetizadas a partir de la feromona natural de hembras recolectadas en plantaciones de *Solanum lycopersicum* L. en Venezuela. En Colombia, el número de machos capturados fue significativamente mayor con Neolegantol® que con P228, y este número fue significativamente mayor en *S. lycopersicum*, seguido por *S. quitoense* y *S. betaceum*. La red haplotípica obtenida con el gen CO1 produjo dos grupos principales: un clúster conformado por especímenes de *S. lycopersicum* y *S. quitoense* (ambas hospederas dieron origen a hembras con genital mediano) y otro de *S. betaceum* (con genitales femeninos de gran tamaño). El Neolegantol® también fue probado en Ecuador, pero las capturas fueron insignificantes. Los resultados sugieren que la composición o la concentración de la feromona, los biotipos hospederos y la ubicación geográfica son factores relevantes para monitorear las poblaciones de *N. elegantalis*. Otros estudios de la especie deben encaminarse a determinar la composición y concentración de la feromona entre los cuatro biotipos.

Palabras clave: ADN mitocondrial, cebo para trampas, plagas de plantas, plantas huéspedes, variación genética

Abstract

Neoleucinodes elegantalis Guenée (Lepidoptera: Crambidae) represents the most damaging pest of the Solanaceae family. Current studies have demonstrated that the species has differentiated into four races according to variations in female genitalia, wing morphometrics and sequencing of the Cytochrome Oxydase 1 (CO1) mitochondrial gene. The number of males captured in Colombia and Ecuador were registered using traps baited with two sex pheromone: Neolegantol® and P228. These pheromones were synthesized using natural female pheromones collected in *Solanum lycopersicum* L. plantations in Venezuela. In Colombia, the number of male catches was significantly higher for Neolegantol® than for P228 and this number was significantly higher on *S. lycopersicum* followed by *S. quitoense* and *S. betaceum*. The haplotype net obtained with the CO1 gene produced two main clusters: one cluster was comprised by specimens from *S. lycopersicum* and *S. quitoense* plants (both with medium sized female genitalia) and the other cluster by specimens from *S. betaceum* (large sized genitalia). The Neolegantol® pheromone was also tested in Ecuador, however, insignificant number of males were attracted. Results suggest that pheromone composition or concentration, host biotypes and geographic location are relevant to monitor populations of *N. elegantalis*. Further studies of the species should concentrate on establishing the pheromone composition and concentration among the four biotypes.

Key words: Mitochondrial DNA, Trapping baits, Pests of plants, Host plants, Genetic variation

Resumo

Neoleucinodes elegantalis Guenée (Lepidoptera: Crambidae) representa a praga mais prejudicial da família Solanaceae. Estudos recentes demonstram que a espécie se diferenciou em quatro raças segundo a variação dos órgãos genitais femininos, a morfometria da asa e a sequência do gene mitocondrial Citocromo Oxidasa 1 (CO1). Quantificou-se o número de machos capturados na Colômbia e no Equador empregando armadilha do tipo isca com dois feromônios sexuais: Neolegantol® e P228 sintetizados a partir do feromônio natural de fêmeas coletadas em plantações de *Solanum lycopersicum* L. na Venezuela. Na Colômbia, o número de machos capturados foi significativamente maior com Neolegantol® que com P228, e esse número foi significativamente maior em *S. lycopersicum*, seguido por *S. quitoense* e *S. betaceum*. A rede haplotípica obtida com o gene CO1 produziu dois grupos principais: um cluster conformado por espécimenes de *S. lycopersicum* e *S. quitoense* (ambas as hospedeiras deram origem a fêmeas com genital médio) e outro de *S. betaceum* (com genitais femininos de grande tamanho). O Neolegantol® também foi testado no Equador, mas as capturas foram insignificantes. Os resultados sugerem que a composição ou a concentração do feromônio, os biótipos hospedeiros e a localização geográfica são fatores relevantes para monitorar as populações de *N. elegantalis*. Outros estudos da espécie devem buscar determinar a composição e a concentração do feromônio entre os quatro biótipos.

Palavras chaves: DNA mitocondrial, isca para armadilhas, praga das plantas, planta hospedeira, variação genética

Introduction

In the insect order Lepidoptera and particularly in moths, female and male communication occurs through pheromones that are usually released by females at night and at long distances to ensure encounters with conspecific males (Löfstedt, 1993; Jurenka, 2004). Moth pheromones usually consist of a blend of two or more components of even-numbered C10-C18 straight-chain, unsaturated derivatives of fatty acids, with the carbonyl carbon modified to form an oxygen-containing functional group (alcohol, aldehyde, or acetate ester) (Groot et al., 2008; Löfstedt, 1993). Once the composition and concentration of these pheromones are established, they are synthetized under laboratory conditions to then be used in the field to monitor and control pest population movements (Jaffe, Mirás, & Cabrera, 2007), as they have previously been tested in mate choice experiments or electroantennograms to study male responses (Badji, Eiras, Cabrera, & Jaffe, 2003). In moths, pheromones display interspecies differences between closely related species or intraspecific differences when races or biotypes with reduced gene flow have evolved (Groot et al., 2008; Olsson et al., 2010).

According to Jaffe et al. (2007), the species *Neoleucinodes elegantalis* Guenée produces sex pheromones that display a typical example of sexual selection, as mate selection and attraction is modulated directly or indirectly by pheromones produced by females. These same authors also found that males of *N. elegantalis* chose a female depending on the component blend of her sex pheromone. Moreover, these males preferred heavier females for mating since females that emitted the preferred blends were heavier and had larger wings. Jaffe et al. (2007) also observed that heavier males were more likely to initiate flight sooner and also to copulate, and thus, gene quality was defined by body size in both sexes. These studies were performed with males and females collected from tomato (*Solanum lycopersicum* L.) plants, but these types of experiments have never been tested in populations of *N. elegantalis* collected from other host plants.

So far, five pheromone components have been identified in this species from populations collected on tomato plants, as follows: 1) (E)-11-hexadecenol (*E*11–16: OH), 2) (*Z*)-11-hexadecenol (*Z*11–16: OH), 3) (*E*)-11-hexadecenal, 4) (*E*)-11-hexadecenyl acetate, and 5) (*Z*)-3, (*Z*)-6, (*Z*)-9-tricosatriene (Z3, Z6, Z9-23: Hy). According to Cabrera et al. (2001), in electroantennogram (EAG) recordings synthetic *E*11–16: OH elicited stronger antennal responses in males at low doses than other pheromone components, and when evaluated under field conditions it demonstrated ability to attract males compared to other components as Z11–16: OH, as this last component inhibited male attraction in the wild. In addition, low concentrations of (Z3, Z6, Z9)-tricosatriene enhances male attraction but high concentrations produces the opposite response. Also, tricosatriene fails to attract males in the wild when it is presented in absence of the other components mentioned above.

Moreover, Díaz, González, Solis, & Saldamando-Benjumea (2015) evidenced the evolution of four host races in *N. elegantalis* since female genitalia size varied according to the host´s fruit size. These races were divides as follow: 1) *Solanum acerifolium* Dunal; 2) *S. quitoense* Lam., *S. lycopersicum* L., *S. hirtum* Vahl, and *Capsicum annuum* L.; 3) *Solanum atropurpureum* Schrank; 4) *S. melongena* L., *S. crinitum* Lam., and *S. betaceum* Cav. populations. This study was carried out in Colombia from larvae (n=2553) collected in the departments of Antioquia, Bolívar, Boyacá, Caldas, Cauca, César, Córdoba, Cundinamarca, Huila, Magdalena, Nariño, Norte de Santander, Quindío, Risaralda, Santander, Sucre, Tolima, and Valle del Cauca, and was based on 547 females from which six genitalia morphological characters were considered. In addition, a separate study based on sequencing the mitochondrial

Cytochrome Oxidase I (CO1) gene in 103 individuals of *N. elegantalis* collected in the same Solanaceae hosts estimated four main haplotypes and population structuring in this species from Colombia as *Fst* = 0.56 (p<0.0001) (Díaz-Montilla, Suárez-Barón, Gallego-Sánchez, Saldamando-Benjumea, & Tohme, 2013). Furthermore, another study conducted by Obando (2011) based on a wing morphometric approach on both males and females demonstrated that *N. elegantalis* could be separated into four races according to wing size and shape differentiation, and host fruit size.

Taking into consideration the above mentioned, the aim of this study was to determine differences in the number of male catches using as bait two commercial pheromones (Neolegantol® and P228) in Colombia. Moreover, to analyze the number of *N. elegantalis* male catches with Neolegantol® on *S. quitoense*, *S. betaceum*, and *S. lycopersicum* in Colombia, and on *S. quitoense*, *S. betaceum*, *S. lycopersicum* and *Capsicum annuum* in Ecuador. Furthermore, this study estimated the level of genetic differentiation in males of *N. elegantalis* in Colombia by using DNA sequences of the Cytochrome Oxidase 1 (CO1) gene. The results obtained in this work are useful to improve the species´ management programs, as this is so far the only study that has considered the existence of four biotypes or races.

Materials and methods

Male capture comparisons between the commercial pheromones Neolegantol® and P228 in Colombia

Male capture efficiency was evaluated using as bait two commercial pheromones: Neolegantol® (synthetized and commercialized by Agroecológica Platom C.A., Caracas, Venezuela) and P228 (synthetized and commercialized by ChemTica Internacional, S.A. San José, Costa Rica). Neolegantol® is composed by (E)-11-hexadecenol (E11–16: OH) + 5% tricosatriene (Z3, Z6, Z9-23: Hy) whereas the only pheromone component of P228 is (E) 11-Hexadecenol. Trap design was based on a BM94 trap (Mirás, Issa, & Klaus, 1997) with soapy water. These two pheromone baits were distributed in a *S. quitoense* orchard with 2396 plants (2.2 ha approximately) planted at a distance of 3 x 3 m from each other. This orchard is located in a premontane very wet forest (vwf-PM) in the locality of Palo Blanco (05°14'58.2" N, 075°46'07.8" W, 1846 m.a.s.l.) in Anserma (Caldas, Colombia). Four traps were alternately placed on each of the four border lines of the crop, two with the pheromone Neolegantol® and the other two with P228. A total of 16 traps were analyzed for this crop. The number of male catches was weekly quantified during November 2012 on each trap. The level of infestation in this orchard by *N. elegantalis* was on average 28.5%. Note that the P228 pheromones was only evaluated in this site and crop species.

Comparisons of the Neolegantol® efficiency between Colombia and Ecuador

The efficiency of the pheromone Neolegantol® to attract males of *N. elegantalis* was evaluated and compared in Colombia and Ecuador.

In Colombia, from 2009 to 2010, attraction to the sexual pheromone Neolegantol® was assessed during a twelve months period using BM-94 traps with soapy water. These traps were placed in commercial *S. quitoense*, *S. betaceum* and *S. lycopersicum* crops and in four wild habitats (disturbed primary forests). The crops and the wild habitats were located in three of Holdridge´s life zones: Premontane wet forest (wf-PM), premontane very wet forest (vwf-PM) and lower montane very wet forest (vwf-LM) (Table 1). Management of all crops was performed by the land owner that applied the most commonly used insecticides in the area.

Tabla 1. Location information and host crops where the efficiency of the pheromone Neolegantol® was tested in Colombia and Ecuador

Country	Location	Elevation (m.a.s.l.)	Life zone	Crop
Colombia	Santa Rosa/Risaralda	1,467	vwf-PM	*S. quitoense*
	Chinchiná/Caldas	1,522	wf-PM	
	Jardín/Antioquia	1,695	vwf-PM	
		1,845	vwf-PM	
		2,066	vwf-LM	
	Manizales/Caldas	1,743	vwf-PM	*S. betaceum*
	Jardín/Antioquia	1,945	vwf-PM	
	Santo Domingo/Antioquia	2,048	vwf-LM	
	Peñol/Antioquia	2,155	wf-PM	
	Manizales/Caldas	1,261	vwf-PM	*S. lycopersicum*
	Santa Rosa/Risaralda	1,679	vwf-PM	
	Peñol/Antioquia	2,186	wf-PM	
	Manizales/Caldas	1,800	vwf-PM	Wild habitat
	Jardín/Antioquia	2,018	vwf-LM	
	Santo Domingo/Antioquia	2,055	vwf-LM	
	Peñol/Antioquia	2,100	wf-PM	
Ecuador	Los Bancos/ Pichincha	681	vwf-PM	*S. quitoense*
	Awayaku/ Napo	735		
	Río Negro/Tungurahua	1,242		
	Río Verde/ Carchi	814	df-PM	
	Guagua Sumaco/Napo	1,053	wf-T	
	El Chaco/Napo	1,607	wf-PM	
	Río Negro/Tungurahua	1,497	vwf-PM	*S. betaceum*
	El Chaco/Napo	1,988	wf-PM	
	Río Negro/Tungurahua	1,232	vwf-PM	*S. lycopersicum*
	El Chaco/Napo	1,588	wf-PM	
	Río Negro/Tungurahua	1,553	vwf-PM	*C. annuum*
	El Chaco/Napo	1,678	wf-PM	
	Los Bancos/Pichincha	881	vwf-PM	Wild habitat
	Río Negro/Tungurahua	1,242		
	El Chaco/Napo	1,607	wf-PM	

Source: Own elaboration including information on Holdrige´s life zones

Four traps were distributed on the border lines of each crop opposite to the direction of the wind. These were hanged on wood or guadua (*Guadua angustifolia* Kunth, Poaceae) fences on each host plant at canopy level. In wild habitats (disturbed primary forests), traps were located 2 m from the ground. Blank traps (controls without pheromones) were also set up in all experiments, although no males were caught on them, and therefore, these were omitted in the statistical analyses. Approximately 48 samples were taken during the twelve months the experiment lasted. Traps were monitored weekly. The number of captured males of *N. elegantalis* and other Lepidoptera males that were attracted to the traps were recorded and identified to family level using the taxonomic keys published by Kristensen (1999). The soapy water was replaced with a new batch every week after the collected moths were quantified in the traps (unscented soap was used in the traps to ensure catching was produced only by the pheromones), and pheromones were changed every 45 days. Additionally, precipitation records were provided by Ideam-Colombia and by the Center for Agriculture Services, LAN, Syngenta S.A., Bogotá. Correlations between precipitation data and number of captured males were performed, but these two variables did not show significant values (data not shown).

In Ecuador, attraction to the sexual pheromone Neolegantol® was studied from the end of December 2011 until March 2012. Two samplings were performed per site following the same procedure used in Colombia. Four traps with and without the pheromone (control) were used in all crops and in the wild habitats (disturbed primary forests) considered. Traps were checked every 15 days. Samplings from four Solanaceae crop species were analyzed: *S. quitoense*, *S. betaceum*, *S. lycopersicum*, *Capsicum annuum* and in three wild habitats (disturbed primary forests). These fields were located in four of Holdridge´s life zones: Premontane wet forest (wf-PM), Premontane very wet forest (vwf-PM), Tropical wet forest (wf-T) and Premontane dry forest (df-PM) (Table 1).

Genotyping of the sampled males from Colombia

Genotyping was only carried out in 47 males sampled on *S. quitoense*, *S. betaceum* and *S. lycopersicum* crops located at different elevations and at different life zones according to Holdridge in Colombia. This analysis was performed by sequencing the mitochondrial gene Cytochrome Oxidase 1 (CO1) previously standardized by Díaz-Montilla et al. (2013). Information on DNA preparation and amplification is given by these same authors.

Data analysis

The Shapiro-Wilk test for normality and the Levene tests for homogeneity of variance were used for the mean number comparisons as these demonstrated that all data were non-parametric. Therefore, the mean number of males captured with Neolegantol® vs. P228 was tested on *S. quitoense* plants employing a Mann Whitney test (Sokal & Rolhf, 1995). This analysis was only made in Colombia because no males were caught in Ecuador. On the other hand, the mean number of male´s caught with these two baits was analyzed using a Welch F-test in *S. lycopersicum*, *S. quitoense* and *S. betaceum* crops only in Colombia. This test generates reliable Anova results when data are not normally distributed and the variances are not homogeneous (Sokal & Rohlf, 1995). Also, a Chi-square test was carried out to establish the interaction between hosts and elevation. All statistical analyzes were carried out using the software package Past 3.1 (Hammer, Harper, & Ryan, 2001).

Sequence analyses

The sequences obtained with the data from Colombia were edited with Bioedit (Hall, 1999) and aligned with the algorithm Clustal W (Larkin et al., 2007). The estimations for nucleotide polymorphism, nucleotide divergence, segregating sites, number of polymorphic sites and number of haplotypes, as well as the Tajima test were obtained using the DNAsp V5 software (Librado & Rozas, 2009). The Tajima test was used to establish if *N. elegantalis* populations are expanding or contracting, or if their population size is constant (Librado & Rozas, 2009). Additionally, a network tree was obtained for all regions in Colombia using Network 4.6.

Results

Male capture comparisons between the commercial pheromones Neolegantol® and P228 used as baits on *S. quitoense*

In Colombia, the number of *N. elegantalis* male catches on *S. quitoense* was significantly different for the two commercial pheromones (H = 13.05, P = 0.0002472) based on the results provided by the Mann Whitney test. The mean number of male catches obtained using Neolegantol® traps was μ = 12.81+/-2.47 whereas this number was much lower for P228 with μ = 2.71+/-0.69.

Comparisons of the Neolegantol® efficiency between Colombia and Ecuador

The F Welch test (F = 21.29; df = 142.3; P<0.0001) followed by the Tukey tests demonstrated significant differences in male catches amongst the three plant hosts, as the highest average of weekly individuals captured per trap was obtained on *S. lycopersicum* (μ = 15.57+/-12.91), followed by *S. quitoense* (μ = 14.92+/-19.02) and the lowest was found on *S. betaceum* (μ = 6.65 +/-6.38). The Wald Chi-square test also showed that in Colombia, the number of captured males was mostly influenced by both the type of host and the elevation (meters above sea level) (Tables 2, 3). The majority of male catches (N = 430.67 adults) occurred in altitudes from 1601 to 1950 m.a.s.l., followed by altitudes from 1200 to 1600 m.a.s.l. (N = 292.25 adults) and then by altitudes from 1951 to 2200 m.a.s.l. (N = 83.50 adults). In S. quitoense the capture occurred mostly when the crops were found at lower elevations (1200-1600 m.a.s.l.) and on *S. betaceum* and *S. lycopersicum* at medium elevations (1601-1950 m.a.s.l.). For the three crops, the lowest male captures occurred at higher elevations (1951-2200 m.a.s.l.) (Table 4). Neolegantol® also attracted species of the families Noctuidae and Pyralidae on *S. quitoense* and *S. betaceum*. In wild habitats, most specimens belonged to the family Geometridae. In Antioquia (Northern Colombia), one *Euleucinodes conifrons* Capps (Lepidoptera, Crambidae) specimen was trapped at El Rayo (06°28'54.7" N, 75°07'68.9" W) at 2008 m.a.s.l. This moth was further identified using male genitalia morphology as well as with the DNA sequencing of the mitochondrial gene CO1 (Díaz-Montilla et al., in press). Males of *N. elegantalis* were also collected with pheromone traps in wild habitats in the departments of Caldas (Manizales, Java, 05°00'58" N, 75°32'28" W) at 1800 m.a.s.l. and in Antioquia (El Jardin, 05°35'13.6" N, 75°47'52.1" W) at 2018 m.a.s.l. Moreover, in Antioquia, a specimen of *N. dissolvens* Dyar was caught in a trap. This specimen was identified using male genitalia morphology. This species has been reported by Capps (1948) in Ecuador, Guiana and Brazil, but this is the first time the species is reported for Colombia.

Table 2. Wald Chi-square test results between the number of *N. elegantalis* males captured with Neolegantol® compared to the elevation, to the host crop and to their interactions

Origin	Type III		
	Wald Chi-square test	df	*P*
(Intersection)	65.374	1	<0.001
Elevation (m.a.s.l.)	23.219	2	<0.001
Host Crop	11.996	2	0.002
Elevation (m.a.s.l.) x Host Crop	36.911	3	<0.001

Source: Hammer et al. (2001)

Table 3. Wald Chi-square test results of the association between the number of male catches with Neolegantol® and the elevation (m.a.s.l.) where the host crop is cultivated

Elevation (m.a.s.l.)	Mean	Error type	Wald confidence interval 95%	
			Below	Top
High (1950-2200)	83.50	54.545	-23.48	190.48
Medium (1601-1950)	430.67	48.821	334.98	526.36
Low (1200-1600)	295.25	63.421	170.95	419.55

Source: Hammer et al. (2001)

Table 4. Wald Chi-square test results of the number of *N. elegantalis* males captured with Neolegantol® and the interaction between elevation (m.a.s.l.) x host crops (Solanaceae)

Elevation (m.a.s.l.)	Crops	Mean	Error type	Wald confidence interval 95%	
				Below	Top
High (1950-2200)	*S. lycopersicum*	89.00	103.566	-113.99	291.99
	S. quitoense	66.00	103.566	-136.99	268.99
	S. betaceum	95.50	73.232	-48.03	239.03
Medium (1601-1950)	*S. lycopersicum*	899.00	103.566	696.01	1101.99
	S. quitoense	286.00	73.232	142.47	429.53
	S. betaceum	107.00	73.232	-36.53	250.53
Low (1200-1600)	*S. lycopersicum*	131.00	103.566	-71.99	333.99
	S. quitoense	459.50	73.232	315.97	603.03

Source: Hammer et al. (2001)

In Ecuador, the only catch occurred on the species *S. quitoense* (N = 7 individuals, 6 males and 1 female) (Table 5). Moreover, an additional individual was collected in a trap without the pheromone (control). These collections were made at the provinces of Tungurahua, Napo, Pichincha and Carchi in premontane very humid forest (vwf-PM), premontane humid forest (wf-PM) and premontane dry forest (df-PM). No other captures were made in Ecuador. However, other studies have reported up to 60 % infestation of *N. elegantalis* on *S. quitoense* in Ecuador (Paredes, Peralta, & Gómez, 2010). In this case, the few number of catches might be related to the lack of attractiveness of the tested pheromone given that in this area of Ecuador, this pest might usually be present causing great damage to crops of *S. quitoense*. Two individuals were trapped in bait traps on *S. betaceum* at Rio Negro (Tungurahua) in premontane very humid forest (vwf-PM). However, no damaged fruits were found on this host or in other provinces of Ecuador. Some fruits that showed drilled symptoms have sporadically been seen on an abandoned orchard at El Chaco and Los Bancos, localities in the provinces of Napo and Pichincha, respectively. Furthermore,

no males were collected on tomato (*S. lycopersicum*) and no damages produced by this insect were recorded in this crop. In wild habitats, no males of *N. elegantalis* were attracted to the sex pheromone used. Thus, the pheromone Neolegantol® failed to capture individuals of *N. elegantalis* in these two locations of Ecuador. In field-planted *C. annuum* at Rio Negro (Tungurahua) in a premontane very humid forest (vwf-PM), four specimens were trapped in bait traps. Nevertheless, no damages were observed on the crop´s fruits, and no infestation records were produced by *N. elegantalis* in Ecuador for this host crop. The few catches obtained in Ecuador in the host crops could be due to the low densities of this pest on the specific ecological conditions found in these provinces. Moreover, this could also be due to genetic differences between populations of *N. elegantalis* from Ecuador (Díaz-Montilla et al., in press) Furthermore, 346 Lepidoptera specimens were collected from all traps installed, 14 of these belonging to different families. Traps with pheromone bait caught 76.9 % of all the Lepidoptera specimens collected, while the ones without pheromone bait caught 23.41 %. Most samples were collected at El Chaco (30.06 %), followed by Río Negro (28.03 %), while Guagua Sumaco had the lowest number of captured males (2.02 %). Most of the non-target lepidopteran moth species trapped belonged to Pyralidae (29.77 %), Crambidae (24.28 %) and Noctuidae (19.94 %), and the lowest percentages belonged to Citheroridae, Arctiidae and Saturniidae, with percentages of ca. 0.29 % for these three families.

Table 5. Number of captured males of *N. elegantalis* in the host crops considered in the different provinces and locations sampled in Ecuador (December 2011-March 2012), using traps with and without Neolegantol® bait

Crops Host	Province / Location	Elevation (m.a.s.l.)	Life Zone	*N. elegantalis*	
				Neolegantol®	Control
S. quitoense	Napo/El Chaco	1,607	wf-PM	1	0
	Napo/Aguayacu	735		0	0
	Pichincha/Los Bancos	681	vwf-PM	1	0
	Tungurahua / Rionegro	1,242		3♂ - 1♀	1
	Napo/Guagua Sumaco	1,053	wf-T	0	0
	Carchi/Rio Verde	814	df-PM	1	0
S. betaceum	Napo/El Chaco	1,988	wf-PM	0	0
	Tungurahua / Rionegro	1,497	vwf-PM	2	0
C. annuum	El Chaco/Napo	1,678	wf-PM	0	0
	Tungurahua/ Rionegro	1,533	vwf-PM	4	0
S. lycopersicum	Napo/El Chaco	1,588	wf-PM	0	0
	Tungurahua / Rionegro	1,232	vwf-PM	0	0
wild habitat	Napo/El Chaco	1,607	wf-PM	0	0
	Pichincha/Los Bancos	881	vwf-PM	0	0
	Tungurahua / Rionegro	1,242	vwf-PM	0	0

Source: Own elaboration including information on Holdrige´s life zones, elevation at meters as sea level, Crops Host and Provinces from Colombia and Ecuador

Genotyping *Neoleucinodes elegantalis* in Colombia

DNA extraction and amplification of the mitochondrial gene CO1 produced a 657 bp portion from a total of 47 individuals sampled in Colombia. The DNA polymorphism found for this gene was described by the following parameters: h = 20, π = 0.01335, Hd = 0.940, Sd = 0.015, S = 39, and θ = 0.01387; D Tajima = -0.129, P>0.01. The 20 haplotypes were estimated and their frequencies were: H1 (4/47); H2, H3, H5, H6, H7, H9, H11, H16, H19 and H20 (1/47); H4 H14, H17 and H18 (3/47); H13 (5/47), H12 (6/47). These haplotypes were grouped in two main clusters: the first cluster was comprised by individuals sampled on *S. quitoense* and *S. lycopersicum*, and the second cluster by individuals obtained from *S. betacecum* (Figure 1). In this experiment the sequencing of samples from Ecuador were not determined and thus, haplotype examinations were not made.

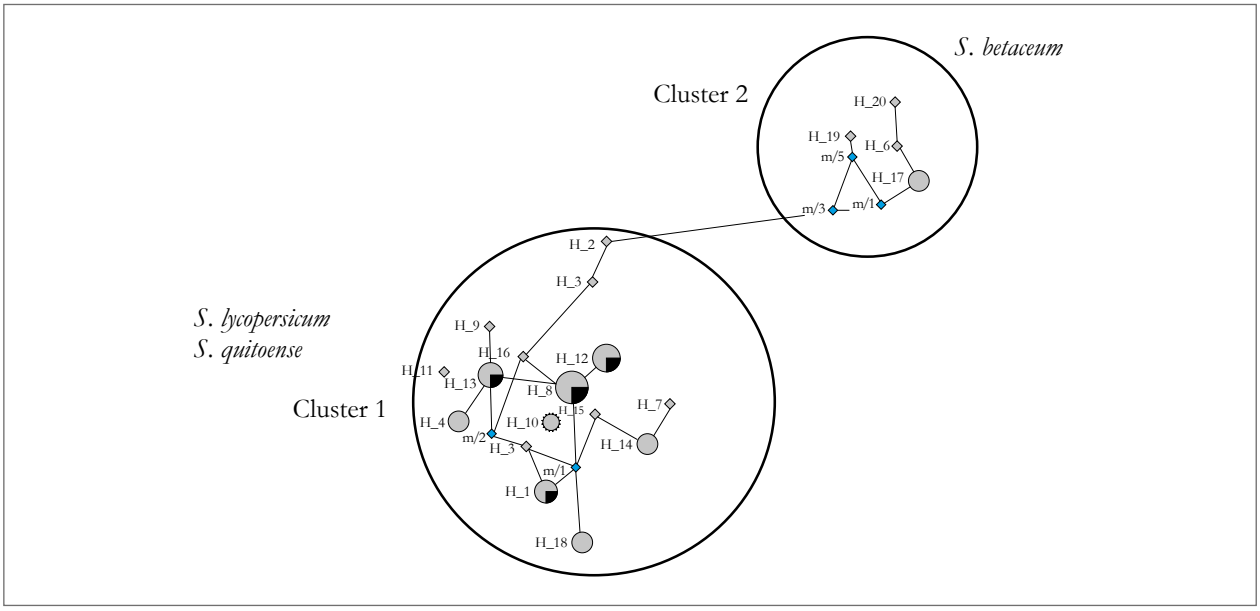

Figure 1. Haplotype network obtained for *N. elegantalis* showing the mutations of 18 haplotypes and the clusters produced per host plant.
Source: Own elaboration

Discussion

Comparisons between the commercial pheromones Neolegantol® and P228 used as bait on *S. quitoense*

In general, in Colombia the pheromone Neolegantol® attracted more *N. elegantalis* males than P228. These differences might be explained by each of the pheromone´s composition. Neolegantol® was created based on a pheromone attraction study made by Jaffe et al. (2007) who found that chemical composition rather than chemical concentrations played a role in male attraction on this species. They evaluated the males of *N. elegantalis* for their attraction to the pheromone in *S. lycopersicum* crops finding that five chemical components can be reduced into two main components: (E11 16OH) + 5% of tricosatriene for the improvement of the number of male captured in nature. This synthetic pheromone significantly attracted 60 times more males than the natural pheromone produced by virgin females of *N. elegantalis* (Jaffe et al., 2007). On the other hand, P228 was synthetized according to a study made by Badji et al. (2003) based on an electroantennograph study showing that males were highly attracted to the component (*E*)-11-hexadecenol (*E*11-16: OH).

Comparisons of the Neolegantol® efficiency between Colombia and Ecuador

Although sampling periods differed between Colombia and Ecuador (in Colombia, collections were made during the whole year and in Ecuador during three months), the number of male catches with the sex pheromone Neoelegantol® was higher in Colombia than in Ecuador. The number of male captures during one month in Solanaceae crops located in premontane very wet forest (vwf-PM) was compared for both countries. Neolegantol® attracted 17 times more males on *S. quitoense* in Colombia than in Ecuador during the same period of time, and in *S. betaceum* this pheromone attracted 6 times more males in Colombia than in Ecuador. Additionally, 29 males were captured on *S. lycopersicum* in Colombia but no males were recorded in Ecuador. All these results suggest that the ability of this pheromone to attract males is locally dependent in this species. A good example is found in the study on pheromone differentiation made by Wanner et al. (2010) with *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) from Europe. This species has diverged in two races: The Z race is widely distributed across the world and the E race is mainly distributed in Italy. Females of the Z race produce a 97:3 ratio blend of Z11- and E11-tetradecenyl acetate, whereas the E race females produce an opposite 1:99 ratio of the Z and E isomers (Olsson et al., 2010). Differences in pheromone composition have generated different responses in males, since males of this species specifically responds to a blend produced by homotypic females. Similar results might be observed in *N. elegantalis* host races, as differences in the number of male catches has been observed in this work. Jaffe et al. (2007) estimated the variation of the Z:E ratios in *N. elegantalis* since this ratio is particularly high (CV = 184%) for this species compared to other moths, and thus, it is possible that the results obtained here indicate that males of *N. elegantalis* are attracted to pheromones produced by homotypic females according to the four host races evidenced by Díaz-Montilla (2013) and Díaz et al. (2015).

In addition, in Colombia the number of males captured decreases with altitude (Holdridge´s life zones). A separated study made by Díaz, Solis, and Brochero (2011), also found that in Colombia both hosts and life zones had an effect on the densities of this pest. Likewise, Arnal, Ramos, Suárez, and González (2006) demonstrated the effect of altitude in the number of *N. elegantalis* captured males in Venezuela with Neolegantol®. They found that at 1270 m.a.s.l., the mean number of captured males/trap/week was 5.7 adults, at 1670 m.a.s.l. the mean number of captured males was lower with 2.98 males/trap/week, and at an elevation of 1860 m.a.s.l. this number was the lowest with 0.97 males/trap/week on *S. betaceum*. These recordings were made in the Miranda and Aragua states with craft traps (EUGO-TCC-2000 design).

On the other hand, no males were captured with Neolegantol® traps in wild habitats both in Colombia and Ecuador. These results suggest that the sexual pheromone attracted males only when traps were located close to their Solanaceae host plants. Deng, Wei, Huang, and Du (2004) argue that some host plant´s volatile compounds enhance the orientation response of male moths to the sex pheromone source. Apparent positive effects of host odor on sex pheromone responses might be related to the plant´s volatile compounds synergizing attraction of male moths to the sex pheromone (Yang, Bengtsson, & Witzgall, 2004). In the corn earworm moth, *Helicoverpa zea* (Boddie), males are captured in greater numbers when pheromone traps are placed near silking corn plants (Light et al., 1993). Besides, more *H. zea* males are trapped with a combination of a synthetic blend of female sex pheromone and either Z-3-hexenyl acetate or Z-3-hexen-1-ol, both known as host plant odorants, compared to those captured with the sole use of the female sex pheromone. In this scenario, it seems that the semiochemicals of *S. quitoense* might stimulate male catches with Neolegantol® in both countries.

In Colombia, both catches and damage made by *N. elegantalis* were low at lower elevations from

1,200 to 1,600 m.a.s.l. and also in areas located at higher elevations from 1951 to 2200 m.a.s.l. These results might indicate that these areas can be potentially used for fruit production and exportation because they do not suffer infestations generated by this insect.

Finally, in Colombia and Ecuador the sex pheromone Neolegantol® also attracted males from other cryptic species such as *E. conifrons* and *N. dissolvens* that are indistinguishable from *N. elegantalis* and could raise counts in pheromone traps biasing results. This also happens with *Leucania phragmatidicola* Guenée, a species that is superficially identical to *Spodoptera frugiperda* Smith & Abbot (Noctuidae), and that is attracted by the commercial pheromone of S. *frugiperda* on maize (*Zea mays* L.) (Harding & Fleischer, 2000). A possible cause for this result is that the chemical components in the sex pheromone are common to several species of Lepidoptera (Hrudová, 2003) and thus, many species may be attracted to this pheromone in the wild.

Genotyping sampled males from Colombia

Díaz et al. (2013) found that in Colombia, *N. elegantalis* was genetically differentiated in four main haplotypes by sequencing 103 individuals from several host plants. In this study, the authors suggested that this differentiation was due to host plant association. Later on, Díaz et al. (2015) demonstrated that *N. elegantalis* differentiates into four races according to the size of the female genitalia, and this characteristic was correlated to host plant fruit size. They found that the first race (with small genitalia size) included individuals collected from the host species *S. acerifolium* Dunal, the second race (with small-medium sized genitalia) by individuals from *S. quitoense*, *S. lycopersicum*, *S. hirtum*, and *C. annuum*, the third race (with medium sized genitalia) of individuals from *S. atropurpureum*, and the fourth race (with large sized genitalia) of individuals from *S. betaceum*, *S. crinitum*, and *S. melongena*. These results corroborate the ones from Díaz et al. (2015) as the haplotype net estimated in this work produced two clusters: one composed by individuals of *N. elegantalis* collected from host plants of *S. lycopersicum* and *S. quitoense*, and the other cluster by individuals collected from *S. betaceum*. In Colombia, collections made with Neolegantol® traps were bigger for *S. lycopesicum* and *S. quitoense* plants than for *S. betaceum*. Similar results were observed in another Crambidae, *Diatraea saccharalis* Fab., where the components (9Z, 11E)-hexadecadienal and (Z11)-hexadecenal of the sex pheromones produced by this insect pest were identified and quantified in one Colombian and four Brazilian populations (Palacio-Cortés et al., 2010). Three different ratios of the components were observed (9:1, 6:1, and 3:1). These differences in pheromone composition among countries were also found in the genetic differentiation estimated for *D. saccharalis* in 11 haplotypes obtained from mitochondrial DNA sequencing of the gene CO1. This result suggest that the species is diverging in separate strains based on their pheromone differentiation (Palacio-Cortés et al., 2010), just like *N. elegantalis* is diverging into four races that are in the process of differentiation at the morphological, the genetic and the chemical levels.

Conclusions

In conclusion, this study has shown that the attraction of *N. elegantalis* males to sex pheromones is mediated by the evolution of four host races in the species, and that differences in either pheromone components or concentrations may play a role in the attraction, as Neolegantol® was synthetized from females found in the host plant *S. lycopersicum*, attracting therefore more males from *S. lycopersicum* and *S. quitoense* crops than from *S. betaceum*. Also, differences in the number of males caught differ between Colombia and Ecuador. These results suggest that in general, sex pheromones in *N. elegantalis* are differentiated according to host race divergence and this differentiation is also locally dependent. Thus, further studies should concentrate on determining differences in sex pheromones among these races and between countries.

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Disclaimers

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