

Reproductive incompatibility and fitness components in *Neoleucinodes elegantalis* races (Lepidoptera, Crambidae) from three Solanaceae hosts

Incompatibilidad reproductiva y componentes de la eficacia biológica en razas de *Neoleucinodes elegantalis* (Lepidoptera, Crambidae) provenientes de tres hospederos de Solanaceae

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ABSTRACT

Neoleucinodes elegantalis (Lepidoptera, Crambidae) is a Neotropical Solanaceae pest that has evolved into four host races due to host plant association. In this study, prezygotic and postzygotic isolation were evaluated in parental and F1 generations collected from *Solanum lycopersicum* (S. l), *S. quitoense* (S. q) and *S. betaceum* (S. b), representing medium size (S. l, S. q) and large size genitalia races (S. b). 617 adults were obtained in the laboratory and 106 copulated. One spermatophore per female was found in the *bursa copulatrix*, suggesting monoandry. Crosses between adults mostly occurred assortatively. A third of the female's eggs laid occurred, but females from ♀S. b x ♂S. l, ♀S. l x ♂S. b and S. l x S. l did not. In the cross, ♀S. l x ♂S. q and its reciprocal F₁ progeny developed into larvae. Reproductive success only occurred in three crosses: S. q x S. q, ♀S. q x ♂S. b and its reciprocal progeny. All the progeny from these crosses reached adulthood. The parental population showed differences in pupae and adult measurements according to host. The results on reproductive isolation obtained here suggest speciation in *N. elegantalis* due to host race differentiation which is relevant for the improvement of the management of this species, especially when the species has evolved reproductive isolation among races. We suggest that the use of their host plants in sympatry might reduce *N. elegantalis* densities in the field since hybrids between its races have low survival rates and to evaluate the possibility of pheromone composition differentiation among races given the results on assortative mating obtained here.

Key words. Assortative mating, copula, host plant association, reproductive isolation, speciation, tomato fruit borer.

RESUMEN

Neoleucinodes elegantalis (Lepidoptera, Crambidae) es una plaga Neotropical de las solanáceas que ha evolucionado en cuatro razas por asociación a hospederos de esta familia. En este estudio, se evaluó el aislamiento precigótico y postcigótico en generaciones parentales y F₁ colectadas en *Solanum lycopersicum* (S. l), *S. quitoense* (S. q) y *S. betaceum* (S. b) representando razas con tamaños de genitales medio (S. l, S. q) y grande (S. b). Se obtuvieron

617 adultos en el laboratorio y 106 de ellos copularon. Se encontró un espermátforo en la *bursa copulatrix* de cada hembra sugiriendo monoandria. Los cruces entre los adultos fueron en su mayoría asociativos. Un tercio de las hembras ovipositaron, pero las hembras de los cruces ♀S. b x ♂ S. l, ♀S. l x ♂S. b y S. l x S. l no lo hicieron. En el cruce ♀S. l x ♂ S. q y su recíproco, la progenie F1 se desarrolló hasta larva. El éxito reproductivo sólo ocurrió en los cruces: S. q x S. q, ♀ S. q x ♂ S. b y su recíproco, toda su progenie alcanzó la adultez. La población parental mostró diferencias en las mediciones de pupa y adulto de acuerdo con su hospedero. Los resultados del aislamiento reproductivo sugieren que la diferenciación en diferentes razas es relevante para la mejora del manejo de la especie, especialmente cuando se presenta aislamiento reproductivo entre estas. Se plantea el uso de las plantas hospederas en simpatria como una alternativa para el manejo del insecto, puesto que los híbridos tienen baja supervivencia y evaluar la posibilidad de diferenciación de componentes de feromonas por los resultados obtenidos en el apareamiento asociativo en la especie.

Palabras clave. Cruce asociativo, cópula, asociación hospedero planta, aislamiento reproductivo, especiación, perforador o pasador del fruto del tomate.

INTRODUCTION

One way to describe a speciation process is the evolution of genetic differences between populations that are adapted to different conditions as a result of divergent selection (Rundle and Nosil 2005). The ecological species concept is founded on the importance of disruptive selection on the formation of host races while, on the other hand, the biological species concept emphasizes the evolution of pre and postzygotic isolation for species divergence (Dres and Mallet 2002). In the latter case, isolation is an indispensable factor in reducing encounters between populations, thus allowing the maintenance of races and species, a process that has been widely reported for insects (Coyne and Orr 2004, Drès and Mallet 2002). Both species concepts are applicable to pest species when reproductive barriers prevent gene flow. Examples of host race evolution include the well-known fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) on corn (*Zea mays* L.) and rice (*Oryza sativa* L.) in all of the Western Hemisphere (Prowell *et al.* 2004). Studies on prezygotic and postzygotic isolation carried out in Colombia have shown partial assortative mating since corn strain females do not mate with rice strain males

(Saldamando-Benjumea *et al.* 2014); and F₁, F₂ generations have reduced viability, fertility, and fitness, particularly in females, suggesting the Haldane Rule (Velásquez-Vélez *et al.* 2011). Another case is the moth species *Ostrinia nubilalis* (Hbn., 1796) (Lepidoptera, Crambidae), also known as the European corn borer. In France, Bourguet *et al.* (2000) found that populations of this insect in maize (*Zea mays*) are genetically different from sympatric populations found on sagebrush (*Artemisia* sp.). Genetic differences between these two races are due to differences in pheromone composition and attraction (Malausa *et al.* 2005).

Another moth recently studied in Colombia that has evolved into host races is the tomato fruit borer *Neoleucinodes elegantalis* (Guennée, 1854) (Lepidoptera: Crambidae). This insect is a quarantine pest that causes relevant economic damage to Solanaceous fruits, including the *Solanum* sp. and *Capsicum* sp. genera (USDA 2005, Díaz and Solis 2007, Díaz *et al.* 2011) and to vegetables across the Western Hemisphere. Actually, it is the main entomological limitation for Solanaceae fruit exportation in Colombia. *N. elegantalis* has adapted to different ecological zones and elevations (0-2600 m a.s.l.) including six Holdridge life zones

(Díaz *et al.* 2011). Laboratory studies have shown that its life cycle is from 25.6 to 124.1 days, and the number of eggs laid depends on both temperature and host fruit (such as tomato, eggplant, or lulo) used for larvae development (Marcano 1991a, b, Serrano *et al.* 1992). Oviposition and pupation habits studied in laboratory and nature also show host plant variation (Marcano 1991a, b, Serrano *et al.* 1992). Natural enemies are diverse and depend on the host (Serrano *et al.* 1992, Viafara *et al.* 1999, Díaz-M and Brochero 2012).

This species meets the criteria described in Dres and Mallet (2002) for host race speciation through host plant association, partial sympatric coexistence of the species range of distribution and genetic differentiation (Díaz and Solis 2007, Díaz *et al.* 2011, Díaz-Montilla *et al.* 2013). Díaz-Montilla *et al.* (2015) described the evolution of four races in a study based on collections of larvae attacking cultivable and wild species of the Solanaceae family throughout Colombia. These races were divided into four groups according to variation in six genitalia sizes as follows: a) *S. acerifolium* (small genitalia), b) *S. atropurpureum* (small-medium genitalia), c) *Capsicum annum*, *S. hirtum*, *S. lycopersicum*, and *S. quitoense*, (medium-sized genitalia), d) *S. betaceum*, *S. crinitum*, and *S. melongena* (large genitalia). In addition, Díaz-Montilla *et al.* (2013) sequenced the mitochondrial gene COI (cytochrome oxidase I) of 103 individuals from Colombia, finding four haplotypes that were genetically structured ($F_{st} = 0.57$, $P < 0.0001$) due to both host plant association and the Andean mountains acting as a barrier to gene flow. Finally, Obando (2011) applied a morphometric wing analysis to approximately 1500 males and females sampled on a variety of hosts in Holdridge life zones, demonstrating that wing size and wing shape varied significantly according to host. These three separate studies support the

possibility of genetic divergence and host race formation facilitating speciation.

The purpose of this study was to determine the reproductive compatibility of *N. elegantalis* in Colombia using intrapopulation (homotypic) and interpopulation (heterotypic) crosses between adults collected on *S. lycopersicum*, *S. quitoense* and *S. betaceum*. We raised insects under laboratory conditions, characterized pupae and adults from Solanaceae hosts, measured postzygotic isolation parameters in parental and F1 generations and proposed that investigating the evolutionary biology of *N. elegantalis* is also important in determining the potential of host-specific associations for use as an additional control tactic.

MATERIALS AND METHODS

Two trials were carried out at two locations and seasons of the year to facilitate larvae collection. In both experiments, populations of *N. elegantalis* were collected from tree tomato (*S. betaceum*), tomato (*S. lycopersicum*) and lulo (*S. quitoense*) in the Departments of Antioquia, Caldas, Risaralda and Valle del Cauca (Colombia, South America) (Table 1). These populations were chosen because they differ in female genitalia size and genetics according to DNA sequencing of mitochondrial gene COI (cytochrome oxidase I). *S. betaceum* females have large genitalia and *S. quitoense* and *S. lycopersicum* females have medium-sized genitalia (Díaz-Montilla *et al.* 2015) and are genetically differentiated based on COI haplotype composition (Díaz-Montilla *et al.* 2017). The other host *N. elegantalis* populations were not included in this study as the most affected crops in Colombia are *S. betaceum*, *S. lycopersicum*, and *S. quitoense*.

Insect collection and rearing/raising

Trial 1 collections of *N. elegantalis* larvae took place between May and November 2009 from *S. betaceum*, *S. lycopersicum*

and *S. quitoense* infested fruits in the departments indicated in Table 1. All samples were taken to ICA's (Instituto Colombiano Agropecuario) Tulio Ospina Entomology Laboratory in Bello, Antioquia: at 1459 m, 6°19'12.8" North, 75°33'13.4" West. The average temperature at this location is 23.95 °C and RH 76 %. Trial 2 collections were made from January to June of 2014 in *S. lycopersicum*, *S. quitoense* and *S. betaceum* fields in the municipality of Anserma in the Department of Caldas (5°45'58.2" North,

75°45'07.8" West) located at 1846 m. This second trial was made five years later due to difficulties in sampling conditions. All fruits were transported to the Agrosavia La Selva Research Center in Rio Negro, Antioquia (6°7'57.9" North, 75°25'8.7" West) at 2120 m. There they were kept at room conditions described as follows: Photoperiod 12:12 day/night, average temperature 14.25 ± 1.26 °C (CV 8.8 %), with a maximum average temperature 18.02 ± 4.76 °C (CV 26%) during the day and a minimum average temperature

Table 1. Collection sites and hosts studied for the analysis of reproductive compatibility and fitness differences in *N. elegantalis* host races in Colombia.

Collection Date	Department	Municipality	Location/ Farm/	Elevation (m)	Geographic coordinates	Crops
5/5/2009	Antioquia	Santo Domingo	Santa Rita/El Fierro uno	1972	6°28'37.1" N 75°08'14.2" W	<i>S. betaceum</i> <i>S. quitoense</i>
5/15/2009		Jardín	El Tapado/El Llano	1653	5°36'04.1" N 75°50'17.3" W	<i>S. quitoense</i>
07 - 12/08/ 2009		Jardín	El Tapado/El Llano	1653	5°36'04.1" N 75°50'17.3" W	<i>S. quitoense</i>
08 - 13/08/ 2009		El Peñol	Santa Inés/ Santa Inés	2174	6°15.404" N 75°16.288" W	<i>S. betaceum</i>
05 - 23/09/ 2009		El Peñol	Santa Inés/ Santa Inés	2174	6°15.404" N 75°16.288" W	<i>S. betaceum</i>
10/27/2009	Valle del Cauca	Palmira	Corpoica	1003	3°31' N 76°18' W	<i>S. lycopersicum</i>
01/10/2009	Caldas	Chinchiná	San Andrés/ Los Alpes	1522	4°56'0.8" N 75°36'48.8" W	<i>S. quitoense</i>
01/10/2009		Manizales	Bajo Tablazo Hoyo Frío/ Los Eucaliptos	1796	5°01'15.2" N 75°31'59.4" W	<i>S. betaceum</i>
01/10/2009	Risaralda	Santa Rosa de Cabal	El Embol/ La Laguna	1679	4°55'11.8" N 75°37'58.6 W	<i>S. lycopersicum</i>
11/20/2009	Valle	Palmira	Corpoica	1003	3°31' N 76°18' W	<i>S. lycopersicum</i>

16.82 ± 4.24 °C (CV 25 %) at night, RH 82.11 ± 19.2 % (CV 23 %), sun radiation 202.68 W/m² (CV 136 %). For both trials, sampled fruits were separated according to host and were kept in different cages with paper towels for pupation. To obtain virgin adults, all pupae were sexed (Serrano *et al.* 1992) and deposited separately in plastic containers (11.5 cm x 7.5 cm); they were left there until emerging as adults.

Trial 1: Reproductive compatibility

Paired homotypic and heterotypic crosses consisted of introducing one male and one female in a cage (42 cm x 42 cm x 64 cm) with a *S. lycopersicum* Torrano hybrids plant with fruit from 1.5 to 4 cm long. Males and females were between 0-2 days old to ensure having virgin adults for mating behavior experiments. After mating, the number of copulated females was counted by examining the spermatophore transfer produced at the “bursa copulatrix” (Burns 1968). The “bursa copulatrix” was stained following Prophet *et al.* (1995). Dissections were kept in 2.5 ml tubes with glycerin.

Postzygotic isolation

Once mating occurred, adults were removed from each cage to evaluate female fertility, and the number of eggs produced per female per fruit on each tomato plant was counted in parental generations. To test for differences in oviposition behavior, orifices generated by females were identified with a pen mark, and then classified as basal, medial, and superior, according to position/location on the fruit. Paper towels were placed on the bottom of each cage fifteen days after hatching to facilitate pupation. Pupae were removed from each cage daily and placed in plastic containers (5 cm x 7.5 cm). All pupae were sexed and kept separated until adulthood. The following life history traits were recorded from the parental and F₁ generations from the different sampling sites

to test for postzygotic isolation: 1) number of pupae, 2) pupae longevity (days), 3) pupae weight (gr), 4) adult longevity (days) and 5) proportion of sexes in all hosts.

Trial 2. Reproductive compatibility

Virgin individuals were obtained from previously sexed pupae, as mentioned above. The number of homotypic and heterotypic crosses depended on the availability of both adults and plants. In this case, paired crosses consisted of placing five males and five females in an entomological cage (42 cm x 42 cm x 64 cm) with either a *S. lycopersicum*, a *S. quitoense* or a *S. betaceum* plant. Plants were grown in greenhouses to facilitate female oviposition and larvae development because this species does not feed on artificial laboratory diets and does not easily mate in captivity. On this trial, females were enclosed with their native host species for oviposition. The number of copulations that occurred per female was recorded by counting the number of spermatophores transferred to the “bursa copulatrix” (Burns 1968). Dissected genitalia were kept in glycerin in 2.5 ml tubes.

Postzygotic isolation

The following parameters were measured daily in parental and F₁ generations: 1) number of laid eggs per female per cross on the basal, medium or apical parts of the fruit as well as on the sepals or peduncles; 2) number of larvae produced per female by counting the number of orifices on the fruit epidermis; 3) pupae longevity from oviposition time to pupae formation, this measurement is performed when larvae left the fruit to produce perforations for pupae formation; 4) the number of pupae per fruit; 5) pupae development time (days); 6) pupae weight (gr); 7) wingspan (cm); 8) adult longevity (days) and 9) proportion of sexes. In cases where fruit damage was recorded without identifying pupae, transversal cuts on the exocarpace or epicarpace were made

to locate larvae and evaluate survival rate (Chiarini and Barboza 2007).

Data analysis

To test whether differences in the number of copulas among *N. elegantalis* paired crosses (*i.e.* homotypic vs heterotypic) were significant, a Chi-square test was employed in both trials. In Trial 1, a Yates correction was carried out due to the low number of copulas observed in this experiment (Sokal and Rohlf 1995). In addition, differences in oviposition behavior of *N. elegantalis* females (from intrapopulation and interpopulation crosses) were tested using another Chi-square analysis. To analyze fitness components in both trials, comparisons tests (ANOVA, Welch-F, Kruskal Wallis or Mann Whitney tests) were carried out on *N. elegantalis* populations obtained from *S. lycopersicum*, *S. quitoense* and *S. betaceum*. To test the assumptions of the variance analysis, Shapiro Wilk tests for normality and Levene's tests for variance homogeneity were conducted (Sokal and Rohlf 1995). Depending on the results of these tests, a parametric variance analysis test (ANOVA), followed by a Tukey test, a Welch F statistic test (followed by a Tukey test), or the non - parametric Kruskal Wallis or Mann Whitney tests were subsequently employed (Sokal and Rohlf 1995). All analyses were carried out using Past 1.34 (Hammer *et al.* 2001) software.

RESULTS

In Trial 1, 138 crosses were made between adults obtained from *S. quitoense* and *S. betaceum*. From these crosses, 119 matings occurred and a total of 257 females were obtained to analyze spermatophore transfer (Table 2a). In Trial 2, 72 crosses were made (29 intrapopulation and 43 interpopulation crosses) and 360 females were obtained from them and analyzed (Table 2b). In total, 617 females were tested in both trials and only 106

copulated. Overall, *N. elegantalis* females mated once with only one spermatophore transfer per bursae copulatrix, thus the moth is a monoandric species.

Mating compatibility in *N. elegantalis* (Prezygotic isolation)

These populations exhibit a higher number of homotypic than heterotypic crosses (Table 3). On the first trial, we found differences between the homotypic and heterotypic crosses between individuals from *S. lycopersicum* and *S. betaceum* ($P < 0.05$) (Table 3a). In fact, reproductive isolation was found between individuals from *S. lycopersicum* and *S. betaceum* where no crosses were obtained regarding sex. On the second trial, more crosses were obtained between individuals with similar genitalia size ($n = 48$), including the S. q x S. q, S. l x S. l and S. b x S. b homotypic crosses and the two heterotypic crosses, ♀ *S. lycopersicum* x ♂ *S. quitoense*, and their reciprocal progeny. Lesser successful crosses were obtained between individuals with medium size genitalia and individuals with large sized genitalia ($n = 24$), including ♀ *S. betaceum* x ♂ *S. quitoense* and it's/their reciprocal progeny, and ♀ *S. lycopersicum* x ♂ *S. betaceum* and it's/their reciprocal progeny (Table 3b). These differences were also statistically significant, thereby supporting the results obtained in the first trial (Table 3a).

Postzygotic isolation in *N. elegantalis*

On Trial 1, according to the number of females that copulated (Table 2), *N. elegantalis* produced more successful crosses between homotypic populations than the heterotypic populations. However, only the homotypic cross from *S. quitoense* and the heterotypic cross (♀ S. b x ♂ S. q) produced eggs that later reached pupae and adulthood. On Trial 2, the most successful crosses were between

Table 2. Reproductive incompatibility obtained for *N. elegantalis* evaluated in populations collected on *S. lycopersicum* (S. l), *S. quitoense* (S. q) and *S. betaceum* (S. b). **a** = Trial 1, **b** = Trial 2.

Crosses	Prezygotic				Postzygotic			Duration (days)	
	No. crosses	Copulated females/ total obtained	No. crosses with oviposition	No. prepupae	No. pupae F ₁	No. adults F ₁	Prepupae	Pupae	Adults
<i>S. q</i> x <i>S. q</i>	38	5/38	4	33	32	29	2-4	12-13	1-6
<i>S. b</i> x <i>S. b</i>	51	7/51	2	0					
♀ <i>S. b</i> x <i>S. q</i> ♂	23	1/23	1	3	3	3	3-3	12-12	
♀ <i>S. q</i> x <i>S. b</i> ♂	26	5/26	1	0					
<i>S. l</i> x <i>S. l</i>	53	7/53	2	0					
<i>S. b</i> x <i>S. b</i>	51	7/51	2	0					
♀ <i>S. b</i> x <i>S. l</i> ♂	6	0/6							
♀ <i>S. l</i> x <i>S. b</i> ♂	9	0/9							

Crosses	Prezygotic				Postzygotic			Duration (days)	
	No. crosses	Copulated females/ total obtained	No. Ovipositions	No. prepupae	No. Adults F ₁	Pupae weight mean (gr)	Egg- prepupae	Pupae	
<i>S. q</i> x <i>S. q</i>	20	26/100	191	0 - 87	3	0.027	79.33	21.00	
<i>S. b</i> x <i>S. b</i>	2	3/10	71	0 - 27	0				
<i>S. l</i> x <i>S. l</i>	7	3/35	0						
♀ <i>S. q</i> x <i>S. b</i> ♂	13	22/65	109	0 - 38	12	0.042	124.5	17.55	
♀ <i>S. q</i> x <i>S. l</i> ♂	11	8/55	49	0 - 9	0				
♀ <i>S. b</i> x <i>S. q</i> ♂	1	2/5	6	7	0				
♀ <i>S. b</i> x <i>S. l</i> ♂	1	2/5	22	12	0				
♀ <i>S. l</i> x <i>S. q</i> ♂	8	3/40	2	0					
♀ <i>S. l</i> x <i>S. b</i> ♂	9	5/45	0						

S. q x S. q followed by ♀ S. q x ♂ S. b, and ♀ S. q x ♂ S. l as more copulated females were found on them. Ovipositions occurred on all crosses except for S. l x S. l and S. l x S. b, and pupae in all crosses except for S. l x S. q. However, only the *S. quitoense* homotypic cross produced eggs that reached adulthood and, of the heterotypic crosses, only the reciprocal progeny from Trial 1 (♀ S. q x ♂ S. b) (Tables 2b, 4). On fruits without observable pupae, the larvae apparently died in/during mesocarpus since no more damage was found. We did not find sex bias in this species; sex ratios were 1:1 in all crosses. Longevity

or duration of pupae and adult longevity were shorter in Trial 1 than in Trial 2.

Fitness components in parental generations

In both trials, *N. elegantalis* pupae longevity or duration were correlated with environmental conditions. The higher the temperature and the stronger the solar radiation, the lower the pupae duration (Table 4). This result was observed in this work as two different locations were considered for rearing *N. elegantalis* and thus they were crucial to determine the importance of

Table 3. Pre and postzygotic isolation tests of *N. elegantalis* among populations from *S. quitoense* (S. q), *S. betaceum* (S. b) and *S. lycopersicum* (S. l). a= Trial 1, b= Trial 2. Numbers in parenthesis correspond to expected values

a			
Crosses	Number of copulas	Contingency table (Yates correction)	P
<i>S. q x S. q</i>	5 (4.5)	4.44	<i>P</i> > 0.05
<i>S. b x S. b</i>	7 (4.5)		
♀ <i>S. b x S. q</i> ♂	1 (4.5)		
♀ <i>S. q x S. b</i> ♂	5 (4.5)		
<i>S. l x S. l</i>	7 (3.5)	14.28	<i>P</i> < 0.05
<i>S. b x S. b</i>	7 (3.5)		
♀ <i>S. b x S. l</i> ♂	0 (3.5)		
♀ <i>S. l x S. b</i> ♂	0 (3.5)		
b			
Crosses	Number of copulas	Contingency table	P
<i>S. q x S. q</i>	26 (10.66)	14.6	<i>P</i> << 0.01
<i>S. b x S. b</i>	3 (10.66)		
<i>S. l x S. l</i>	3 (10.66)		
♀ <i>S. q x S. b</i> ♂	22 (7)	15.31	<i>P</i> < 0.001
♀ <i>S. q x S. l</i> ♂	8 (7)		
♀ <i>S. b x S. q</i> ♂	2 (7)		
♀ <i>S. b x S. l</i> ♂	2 (7)		
♀ <i>S. l x S. q</i> ♂	3 (7)		
♀ <i>S. l x S. b</i> ♂	5 (7)		

Table 4. Fitness components evaluated for *N. elegantalis* parental generations. **a** = Trial 1, **b** = Trial 2.

a			
Trait	Comparison test		Mean ± SD
Pupa longevity	F Welch	Tukey	
Females	F = 22.52	S. q vs S. l	♀S.q: 10.98 ± 0.18 n = 79
	df = 141	P < 0.0001	♀S.l: 10.17 ± 0.07 n = 79
	P < 0.0001	S. l vs S. b	♀S.b: 10.91 ± 0.10 n = 79
		P = 0.0001	
Males	F Welch	Tukey	♂S.q: 11.40 ± 0.17 n = 79
	F = 41.79	S. q vs S. l	♂S.l: 10.04 ± 0.075 n = 79
	df = 146.1	P < 0.0001	♂S.b: 10.87 ± 0.01 n = 79
	P < 0.0001	S. q vs S. b	
		P = 0.004	
		S. l vs S. b	
		P < 0.0001	
Pupae weight	ANOVA	Tukey	
Females	F = 8.597	S. q vs S. l	♀S.q: 0.034 ± 0.002 n = 22
	df = 65	P = 0.04	♀S.l: 0.026 ± 0.002 n = 22
	P < 0.0001	S. l vs S. b	♀S.b: 0.040 ± 0.003 n = 22
		P < 0.0001	
Males	F Welch	Tukey	♂S.q: 0.031 ± 0.007 n = 22
	F = 14.36	S. q vs S. l	♂S.l: 0.024 ± 0.001 n = 22
	df = 39.99	P = 0.01	♂S.b: 0.037 ± 0.002 n = 22
	P < 0.0001	S. l vs S. b	
		P = 0.0001	
Adult longevity			
Females	t(equal var) = 1.11		♀S.l: 4.15 ± 0.25 n = 26
	P = 0.26		♀S.b: 4.55 ± 0.24 n = 26
Males	t(unequ var) = 2.53	S. l vs S. b	♂S.l: 4.55 ± 0.17 n = 40
	P = 0.01		♂S.b: 5.67 ± 0.40 n = 40
b			
Trait	Comparison test		Mean values +/- SD
Pupae longevity (duration)	F Welch = 0.0697	KW = 0.6654	Sq _{Median=IQR} : 17 ± 2 n = 16
	P = 0.93	P = 0.70	Sb _{Median=IQR} : 17 ± 0 n = 12
	df = 2.626		Sl _{Median=IQR} : 18 ± 7.3 n = 2

(Continued)

Table 4. Fitness components evaluated for *N. elegantalis* parental generations. **a** = Trial 1, **b** = Trial 2. (continuation)

b

Trait	Comparison test		Mean values +/- SD
Pupae weight vs time	Anova	Tukey	
1 day	F = 15.88 $P << 0.01$ df = 2.130	Sq vs Sl $P = 0.001$ Sq vs Sb $P = 0.04$ Sl vs Sb $P << 0.01$	Sq: 0.049 ± 0.001 n = 114 Sl: 0.033 ± 0.003 n = 13 Sb: 0.060 ± 0.004 n = 6
6 day	Anova F = 16.34 $P << 0.01$ df = 2.120	Tukey Sq vs Sl $P = 0.001$ Sq vs Sb $P = 0.15$ Sl vs Sb $P = 0.001$	Sq: 0.048 ± 0.001 n = 105 Sl: 0.031 ± 0.003 n = 13 Sb: 0.057 ± 0.0051 n = 5
12 day	Anova F = 16.27 $P << 0.01$ df = 2.118	Tukey Sq vs Sl $P = 0.008$ Sq vs Sb $P = 0.007$ Sl vs Sb $P = 0.0002$	Sq: 0.045 ± 0.001 n = 105 Sl: 0.029 ± 0.003 n = 12 Sb: 0.062 ± 0.0017 n = 4
Wingspan (cm)	t = 4.5162 $P << 0.01$		Sq: 2.58 ± 0.032 n = 57 Sb: 2.20 ± 0.083 n = 11
Adult longevity			
S. q x S. q	Man Whitney Z = -1.026 $P = 0.30$		♀S.q: 5.5 ± 1.6 ♂S.q: 5.2 ± 1.5
S. b x S. b	t = 2.508 $P = 0.02$		♀S.b: 5.5 ± 1.4 ♂S.b: 4.1 ± 1.1
S. l x S. l	Man Whitney Z = -2.199 $P = 0.02$		♀S.l: 5.5 ± 1.7 ♂S.l: 4.7 ± 0.28
S. q x S. b	t = -0.0335 $P = 0.97$		♀S.q: 5.5 ± 1.7 ♂S.b: 5.5 ± 1.6
S. q x S. l	t = -0.4009 $P = 0.69$		♀S.q: 5.5 ± 2.13 ♂S.l: 5.7 ± 2.05
S. b x S. q	Man Whitney Z = -0.3869 $P = 0.70$		♀S.b: 4.2 ± 0.49 ♂S.q: 4.0 ± 0.26

(Continued)

Table 4. Fitness components evaluated for *N. elegantalis* parental generations. **a** = Trial 1, **b** = Trial 2. (*continuation*)

b		
Trait	Comparison test	Mean values +/- SD
Adult longevity		
S. b x S. l	t = 1.768	♀S.b: 5.6 ± 1.5
	P = 0.11	♂S.l: 4.6 ± 0.55
	Man Whitney	♀S.l: 5.6 ± 2.7
S. l x S. q	Z = -0.0680	♂S.q: 5.4 ± 2.1
	P = 0.94	
S. l x S. b	t = 0.3673	♀S.l: 5.9 ± 1.7
	P = 0.71	♂S.b: 5.7 ± 1.9

temperature and radiation in pupae longevity in this pest. In Trial 1, we found that pupae with the longest average duration came from *S. quitoense* fruit, followed by *S. betaceum* and *S. lycopersicum* ($P < 0.05$, Table 4). On both trials, the heaviest pupae were found in *S. betaceum*, followed by *S. quitoense*, and *S. lycopersicum* (Table 4). On Trial 1, differences were found between pupae from *S. lycopersicum* and the other hosts. On Trial 2, pupae weight differed significantly among hosts (Table 4). We found differences in the longevity of males by the host on Trial 1 (Table 4). On Trial 2, we only observed longevity differences between *S. betaceum* and *S. lycopersicum* males and females.

Oviposition sites

Contingency tables show that *N. elegantalis* female oviposition sites differ significantly according to host (Table 5). In general, on Trial 1, females of the S. q x S. q cross laid eggs on all parts of the fruit including sepal and peduncle areas, but they mostly oviposited on the apical part of the fruit. Females from the S. b x S. b cross preferred to lay eggs on the apical and basal fruit parts including the stem, and females from the S. l x S. l cross mostly laid eggs at the base

of the sepals. In this last cross, females laid a larger number of eggs compared to other females. In generation Trial 2, females obtained from S. q, mostly laid eggs on the apical fruit parts, females from S. b laid eggs on the basal part of the fruit and on sepals, and females from S. l laid eggs on the sepals.

DISCUSSION

Prezygotic isolation

N. elegantalis host races mated assortatively. This was demonstrated by the number of females from both trials with spermatophore transfer counting both homotypic and heterotypic crosses, suggesting the possibility of chemical and/or mechanical reproductive isolation for this species. In the case of mechanical isolation, [Díaz-Montilla et al. \(2015\)](#) showed that *N. elegantalis* females with large genitalia originated from large fruits and females with small genitalia from small fruits. Therefore, if female genitalia size presents a correlation with fruit size, males of this species might also show this type of correlation demonstrating the possibility of a lock-key hypothesis in the four races of this species. Evolution of male genitalia is one of the most generalized

Table 5. Host oviposition sites used by *N. elegantalis* analyzed from intra and interpopulation crosses. *S. lycopersicum* (S.1), *S. quitoense* (S. q) and *S. betaceum* (S. b). **a** = Trial 1, **b** = Trial 2.

a										
Cross	Pedicel	Sepals	Basal	Medial	Apical	P				
				Fruit		χ^2				
S. q x S. q	2	7	11	3	3	5.73	$P > 0.05$			
S. b x S. b	4	21	21	1		43.12	$P < 0.001$			
S. 1 x S. 1	19	20	20	3	10	82.50	$P < 0.001$			
♀S. q x S. b♂					1	30.35	$P < 0.001$			
♀S. b x S. q♂	5		3			22.35	$P < 0.01$			
b										
Cross	Basal	Medial	Apical	Sepal	Peduncule-	Sepal-flower	Primary	Secondary	c2	P
	Fruit	Fruit		fruit	fruit	(superior)	Stem	stem		
				(superior)						
S. q x S. q	22		118				18		210.21	$P < 0.000^*$
S. b x S. b	22				18	17			18.92	$P < 0.001^*$
S. 1 x S. 1										
♀S. q x S. b♂	7	25	28	24					99.03	$P < 0.000^*$
♀S. q x S. 1♂		9		13		4	6	13	59.48	$P < 0.05$
♀S. b x S. q♂				3					0.63	$P < 0.89$
♀S. b x S. 1♂		8			5				5.15	$P < 0.52$
♀S. 1 x S. q♂				2						
♀S. 1 x S. b♂										

evolutionary trends in animals with internal fertilization, where shapes of male genital traits often provide the only trustworthy characteristic for species identification (Arnqvist 1998). However, the evolutionary processes responsible for this pattern remain unknown. In a study comparing pairs of related clades of insects that differ in mating system, Arnqvist (1998) found that genital evolution in polyandric females is more than twice more divergent than in monandric females. Given that we only found one spermatophore transferred in *N. elegantalis*, our results do not support Arnqvist (1998)'s proposition. Similar observations were made of other Lepidoptera species. For example, Pashley and Martin (1987)'s mating study showed no spermatophore transfer from males to females on *Spodoptera frugiperda* corn and rice strains. Likewise, in Colombia, Saldamando *et al.* (2014) and Velásquez-Velez *et al.* (2011) observed no spermatophore transfer on *S. frugiperda* corn and rice females from a laboratory colony. In both studies, they suggested assortative species mating. In France *Ostrinia nubilalis* (Hbn., 1796), females from maize-Z and mugwort (*Artemisia vulgaris* L.) races mated with homospecific males in 95 % of the cases, indicating a lack of hybridization between them (Malausa *et al.* 2005).

Another possibility of prezygotic isolation in *N. elegantalis* is the evolution of chemical isolation through female pheromone differentiation among host races (Díaz-Montilla *et al.* 2017). Previous studies have shown attraction of *N. elegantalis* males to female pheromones or to synthetic pheromones (Jaffé *et al.* 2007, Díaz-Montilla *et al.* 2017). Jaffé *et al.* (2007) found that *N. elegantalis* males from tomato fruits were more attracted to pheromone blends produced by larger females, and even more attracted to a synthetic sex pheromone blend. Díaz-Montilla *et al.* (2017) tested two sex pheromones synthesized from

females collected on *S. lycopersicum* from Venezuela in crops of *S. lycopersicum*, *S. quitoense*, and *S. betaceum* in Colombia. They obtained higher captures for males on *S. lycopersicum* versus the other crops, suggesting that *N. elegantalis* males are attracted to homotypic females. This attraction pattern could generate a reduction of matings between heterotypic individuals and, thus, a limited spermatophore transfer from one host race to another.

Postzygotic isolation

Several fitness components tested on the F1 of both trials varied among most of the host races: number of eggs, number of developed larvae, pupae and adults (Tables 2-3). Moreover, on trials, oviposition behavior, pupae weight, pupae longevity, adult weight, adult longevity, and wingspan also varied in parental populations (Tables 4-5). Most data in Trial 2 demonstrated that parental populations had better fitness success compared to other crosses. Despite differences in laboratory conditions, individuals from *S. quitoense* were the most successful in reaching adulthood during both trials. In fact, the only heterotypic crosses that reached pupae and/or adulthood also involved a parental *S. q* male in Trial 1 and a female in Trial 2 (Table 2), suggesting that *S. q* host race populations are better adapted to environmental conditions compared to other *N. elegantalis* races.

In both trials, the ICA-Tulio Ospina Laboratory of Entomology seemed to provide better survival conditions for the *N. elegantalis* colonies tested. This may be attributable to warmer temperatures, lower relative humidity, and lower sun radiation compared to the Agrosavia-La Selva Laboratory. *N. elegantalis* pupae longevity was significantly different in both males and females in the three tested hosts and this longevity was influenced by differences

in environmental conditions between these two trials. In fact, pupae duration was two times longer in individuals maintained at Agrosavia-La Selva compared to ICA-Tulio Ospina laboratories, suggesting that this pest is better adapted to warmer temperatures and lower altitude conditions. In addition, even though pupae weight was significantly different among hosts in both trials; the heaviest pupae originated from *S. betaceum* fruits followed by *S. quitoense*, and *S. lycopersicum*. Pupae from *S. lycopersicum* were the lightest in all trials.

Adult longevity in males was greater in *S. betaceum* than in *S. quitoense* in both trials. In Trial 2, adult longevity was also greater in *S. betaceum* than in *S. quitoense*. Differences in fitness components according to host have been previously found in other Lepidoptera species including *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) (Panizzi *et al.* 2004), *Grapholita* (*Cydia*) *molesta* (Lepidoptera: Tortricidae) (Belluti 2011) and *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Yponomeutidae) (Golizadeh *et al.* 2009), suggesting differences in fitness components due to host plant association.

On Trial 1, *N. elegantalis* females mostly laid eggs on *S. lycopersicum*. This result suggests that semiochemicals produced by this host plant might facilitate *N. elegantalis* oviposition behavior. Also, *N. elegantalis* females used different oviposition sites according to host. Previous studies of *S. lycopersicum* indicated that most *N. elegantalis* egg laying occurs on the peduncle, the sepals and the superior part of the fruit; however, this species never laid eggs on flowers (Marcano 1991b, Salas *et al.* 1991, Blackmer *et al.* 2001, Rodrigues-Fihlo *et al.* 2003). Our results coincide with the field pattern of oviposition explained by Marcano (1991b) and Blackmer *et al.* (2001). Differences in oviposition sites

might be explained as a mechanism used by *N. elegantalis* females to prevent egg parasitoids such as *Trichogramma* sp. that has been widely used to control this pest in tomato (Blackmer *et al.* 2001). Salas *et al.* (1991) demonstrated that *N. elegantalis* females lay eggs in places that are not easily exposed to parasitoids such as hidden places under sepals and fruit receptacles.

The results of postzygotic isolation tests demonstrate the importance of verifying the physiological potential for *N. elegantalis* host race hybrid generation because traits related to this type of isolation are relevant for the improvement of the integrated pest management of this species in nature. One way to reduce population densities of this pest in Colombia could be by planting *S. quitoense* and *S. betaceum* in sympatry because the hybrids obtained between them will have a lower survival rates compared to parents or *S. lycopersicum* x *S. betaceum* and *S. lycopersicum* x *S. quitoense* where, according to our results, no adults were obtained.

In conclusion, *N. elegantalis* mate assortatively as more crosses between homotypic individuals than heterotypic individuals took place in the laboratory. Spermatophore transfer occurred only once, suggesting species monoandry. In addition, postzygotic isolation shows that hybrids between individuals with medium sized genitalia vs large genitalia show a reduction in viability. Our results suggest future research into the potential use for *N. elegantalis* hybrids (between races) in pest management in Colombia. More studies on postzygotic isolation, pheromone differentiation among races, and biological control (using *Trichogramma* sp. to parasite *N. elegantalis* eggs) in this species are necessary. The results obtained here accentuate the importance of studies on the evolutionary biology of the pest in order to improve the management of this species in nature.

AUTHOR'S CONTRIBUTION

AEDM concept design of the project. NBB data collecting for Trial 1 as part of her undergraduate research project at the Universidad del Valle. AEDM, JML, and NBB analysis of Trial 1 and writing the text; AEDM data collecting for Trial 2; AEDM and CISB analysis for Trial 2. AEDM and CISB statistical analysis and writing the text.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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