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Host Plant Association and Genetic Differentiation of Corn and Rice Strains of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Colombia

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ABSTRACT - *Spodoptera frugiperda* (Smith) is a polifagous insect of major economic impact in the western hemisphere and exhibits two strains (i.e., corn and rice) that are morphologically identical but differ in ecology, genetics and physiology. In this work we identified these strains and their respective hybrids by using a PCR-RFLP of the COI gene and PCR of the tandem region FR. Moreover, we performed a population structure analysis by using 253 larvae from Tolima, a region where *S. frugiperda* is a pest on corn, rice, sorghum and cotton. Corn strain was found on 42% in corn, 34% in cotton, 19% in sorghum and 0.04 % in rice and rice strain on 35% in corn, 0.06% in cotton, 0.06% in sorghum and 53% in rice, demonstrating that corn strain specificity is superior to rice strain. Hybrids between these strains were more abundant in corn. The distributions on their host plants reflect a population genetic differentiation in *S. frugiperda* with values of PhiPT (COI) = 0.31, $P < 0.0001$, PhiPT (FR) = 0.17, $P < 0.0001$ for all crops and PhiPT (COI) = 0.42, $P < 0.01$, PhiPT (FR) = 0.13, $P < 0.01$ for the sixteen sampled farms. The dendrograms showed two clusters representing both strains. The results obtained in this study suggest that the management of this insect must differ on each host plant, given the specialization that both strains present, particularly in corn and rice.

KEY WORDS: Strain, population genetics, pest

Among insects, speciation mainly involves the evolution of distinct populations due to local adaptation to certain host plants (Feder 1998). Examples for speciation through host plant adaptation are the apple maggot *Ragolethis pomonella* (Walsh) on hawthorn and domestic apple (Bush 1969, Bush & Smith 1998, Feder 1998), the larch budmoth *Zeiraphera diniana* (Guénée) on European larch and cembran pine (Emelianov *et al* 2001), or the european corn borer *Ostrinia nubilalis* (Hübner) on corn and mugwort (Martel *et al* 2003).

The fall armyworm (FAW) *Spodoptera frugiperda* (Smith) is a key pest of several crops such as corn, cotton, sorghum and rice (Nagoshi & Meagher 2003b, 2004, Busato *et al* 2004, Prowell *et al* 2004, Vélez-Arango *et al* 2008). The fall armyworm equally exhibits two strains associated to its main host plants: corn and rice (Prowell 1986, Prowell *et al* 2004). However, the corn strain has also been collected from cotton and sorghum and the rice strain has been found in pasture grasses (Nagoshi & Meagher 2003a). Speciation in *S. frugiperda* merits special attention for several reasons: 1) *S. frugiperda* strains exhibit varying resistance to chemical and biological control agents (Adamczyk *et al* 1997); 2) FAW strains are particularly interesting to explore (sympatric) speciation process through host plant adaptation (Dres & Mallet 2002); 3) both strains hybridize under laboratory and field conditions (Nagoshi & Meagher 2003b, Prowell *et al* 2004); 4) FAW presents evidence for prezygotic isolation (Prowell & Martin 1987, Prowell *et al* 1992, Lu *et al* 1994); and 5) reproductive isolation may be associated to X linked traits or maternally inherited traits (Prowell 1998).

The presence of *S. frugiperda* strains has been reported in various countries in the western hemisphere (Busato *et al* 2004, Prowell *et al* 2004), including Colombia (Vélez-Arango *et al* 2008). Strains are morphologically identical, but show vast genetic differences in several markers, such as allozymes, esterases, AFLP´s, and ND4 gene (Prowell 1986, McMichael & Prowell 1999, Prowell *et al* 2004). A PCR-RFLP method based on the restriction enzyme *MspI* identifies *S. frugiperda* strains by amplifying products of 569 bp of the COI gene and producing two cleavage sites of 497 bp and 72 bp on the corn strain only (Levy *et al* 2002, Nagoshi & Meagher 2003a,b). Another method used to differentiate the strains is the FR (For Rice) tandem repeated fragment, that produces amplifications (mass smears) above 500 bp in the rice strain and from 0 to 3 faint bands on corn strain (Lu *et*

al 1994, Nagoshi & Meagher 2003a).

Hybridization between both FAW strains has been evidenced in different US states (Prowell 1986, Nagoshi & Meagher 2003a), French Guyana, Ecuador, Guadalupe, and certain Caribbean islands (Prowell *et al* 2004). These results indicate that interbreeding readily occurs in nature (Prowell *et al* 2004).

To prevent hybridization, certain prezygotic isolation barriers have been evidenced between the two strains, such as temporal isolation (Prowell *et al* 1992), partial assortative mating strategies (Prowell & Martin 1987, Lu *et al* 1994), ecological isolation (Prowell 1986 1998, Prowell *et al* 2004), and differences in pheromone composition, suggesting chemical isolation (Groot *et al* 2008). However, opposite results related to assortative mating have been obtained under laboratory conditions (see Withford *et al* 1988, Quinsenberry 1991, Nagoshi & Meagher 2003a), possibly due to geographic origin of the strains or age of *S. frugiperda* populations used to test fitness components of the species (Prowell 1988). Genetic analysis of FAW populations has been performed in only few countries of South America (Busato *et al* 2004, Martinelli *et al* 2007, 2007, Clark *et al* 2007). However, none of these studies has covered Colombia, and only a limited number of studies in the US have investigated host plant associations (Nagoshi & Meagher 2004, Prowell *et al* 2004).

In Colombia, *S. frugiperd*a is a key pest in corn and a secondary pest on cotton, sorghum and rice, particularly at the department of Tolima, a region where most of the studies are focused on its control (Alvarez y Sanchez 1983) by using transgenic cotton crops, with no effects on the mortality of this species (Zenner de Polania unpublished). To prevent life cycle synchrony between FAW and other key pests, all four crops included in this study are rotated. Although rotation does not reduce FAW densities in the field, knowledge of the strain identification of this insect is critical for the integrated pest management of FAW. Moreover, an analysis of population differentiation between these two strains is also important to determine whether they represent genetically differentiated units with restricted gene flow. For these reasons, the purposes of this work were: a) to identify *S. frugiperda* strains and b) to analyse the population differentiation in this department of Colombia by using a PCR-RFLP of the mitochondrial cytochrome oxidase subunit I (COI) gene and a PCR for the tandem repeated unit FR (FAW rice strain) already standardised by Nagoshi & Meagher (2003a) and Lu *et al* (1994).

Material and Methods

Insect collection. In northern Tolima, FAW larvae were collected from corn, cotton, and rice fields that were positioned at close distance (< 5 km apart). In south and central Tolima, larval collections were done in closely positioned cotton, sorghum and corn fields (Table 1). Collections were made during late 2006 and early 2007. Upon collection, larvae were stored in 2.5 ml plastic tubes with 70% ethanol, labelled according to the collecting site, and sent to the laboratory Biotecnología Vegetal UNALMED-CIB (Corporación para

Table 1 Number of larvae of *Spodoptera frugiperda* genotyped on sixteen farms of Tolima department (central Colombia).

Region	Farm	Crop	Total number	Category
Center	Algodonera Andina	Cotton	28	2 Rice; 21 Corn; 5 Hybrids
	CI Nataima	Corn	14	9 Corn; 5 Hybrids
	Semilas Valle	Sorghum	13	10 Corn; 3 Hybrids
	Triángulo saldaña	Corn	11	1 Rice; 5 Corn; 5 Hybrids
North	Armero-Guayabal	Corn	14	9 Corn; 5 Hybrids
	Hacienda pajonales	Rice	33	23 Rice; 10 Hybrids
	Hacienda Potossi	Corn	5	1 Rice; 2 Corn; 11 Hybrids
	Pajonales	Cotton	33	1 Rice; 23 Corn; 9 Hybrids
South	La Colmena	Rice	4	1 Rice; 3 Corn
	Natagaima 1	Rice	10	2 Rice; 4 Corn; 4 Hybrids
	Natagaima 2	Corn	20	7 Rice; 8 Corn; 5 Hybrids
	Natagaima 3	Sorghum	6	1 Rice; 5 Corn
	Oticuno	Cotton	9	9 Corn
	Pazos	Cotton	10	6 Corn; 4 Hybrids
	Predio llano grande	Corn	5	2 Rice; 3 Corn
	Predio llano grande	Sorghum	15	1 Rice; 11 Corn; 3 Hybrids
	Vereda baloca	Corn	23	6 Rice; 16 Corn; 1 Hybrid

Investigaciones Biológicas), where they were subsequently kept at -70ºC until processing.

Insect genotyping. Genotyping was performed on 253 individuals by using a PCR-RFLP of the COI gene at the mitochondrial DNA and a PCR for the nuclear region FR (Nagoshi & Meagher 2003a). DNA extraction was performed following modified protocols of Sambrook -& Russell (2001). Details on PCR-RFLP´s on COI gene and PCR of the tandem FR region are described elsewhere (Vélez-Arango *et al* 2008).

Data analysis. A binary data matrix was created for each marker, representing presence (1) or absence (0) of a certain band. Both *MspI* and FR markers were considered neutral and dominant, since the first marker is haploid and is part of the mitochondrial cytochrome oxidase subunit I (COI) gene and is maternally inherited (Levy *et al* 2002), while the second marker produces either a smear pattern higher than 500 bp or 0-3 faint bands with molecular weight below 500 bp. This marker is thought to be linked to the Y and X chromosomes in *S. frugiperda* (Nagoshi & Meahger 2003a) and hence considered diploid. Since both markers are dominant, Hardy Weinberg equilibrium was not assumed (Excoffier *et al* 1992, Hedrick 2004), and an AMOVA (Analysis of Molecular Variance) was used to determine genetic differentiation of *S. frugiperda* strains. Population structure analyses were conducted separately between crops and between individual farms for each molecular marker using the software GenAlEx 6 (Peakall & Smouse 2006), following methods of Excoffier *et al* (1992), and Michalakis & Excoffier (1996).

On the other hand, Popgene 1.31 (Yeh *et al* 1997) was used to calculate Nei´s genetic distances and produce two dendrograms with UPGMA algorithm for each marker (Sneath & Sokal 1973). Dendrograms were constructed on the four crops and on the sixteen farms sampled with Mega 4.0 (Tamura *et al* 2007). Nei genetic distance was chosen on this study because it does not assume Hardy Weinberg equilibrium (Hedrick 2004). In addition, since *S. frugiperda* strain data are categorical, three contingency tables (Sokal & Rohlf 1995) were performed in Genstat 5.0 (2003) to asses host plant association of *S. frugiperda* strains to their respective host plants. Also, a logistic regression was used to test three effects on *S. frugiperda* strains distributions in Tolima: a) an effect of the region were collections were made, b) an effect of the host plant where larvae were collected and c) an effect of surrounding crops of the sampling location. Logistic regression was carried out in Minitab 15 (2007).

Results and Discussion

From all 253 collected FAW larvae, a total of 143 individuals were genotyped as corn strain, and 49 individuals were genotyped as rice strain (Table 1). Electrophoretic patterns for each marker of corn and rice strains were similar to those defined by Nagoshi & Meagher (2003a).

Two types of hybrids were found: a) 37 individuals that presented both digestions of 497 bp and 72 bp with the enzyme *MspI* and smear amplifications higher than 500 bp with FR primers (subsequently termed hybrids + /+) and b) 24 individuals that did not present neither a digestion of 497 bp and 72 bp with the enzyme *MspI* or smear amplification products >500 pb with FR primers (named hybrids -/-). The hybrids could be the product of bidirectional crosses between the strains, producing F1 generations, or backcrosses of F1 individuals to parentals, suggesting that interstrain mating occurs easily in Tolima. However, opposite observations have been made in FAW populations of Florida where Nagoshi & Meagher (2003b, 2004) found that hybrids were the product of unidirectional interstrain matings between rice females and corn males. Similarly, Lu *et al* (1994) found restricted interstrain mating in nature on FAW populations in Georgia, USA arguing that bi-directional crosses are rare or absent in nature. Contrary to above findings and in correspondence with our work, Prowell *et al* (2004) found evidence of bi-directional crosses in nature in a multitude of FAW populations, with 54% possible offspring of rice strain females mated with corn strain and 46% reciprocal cross. In our study, 41% of hybrids were collected from corn, 26% on cotton, 9% on sorghum and 23% on rice, supporting Prowell *et al* (2004) who reported that the majority (i.e., 62%) of presumptive hybrids in the corn habitat.

AMOVA results show genetically differentiated FAW populations between the four crops (corn, cotton, sorghum and rice) with significant PhiPT values for both markers: PhiPT = 0.309 (df = 3, 249; $P < 0.0001$) for the COI region and PhiPT = 0,168 (df = 3, 249; $P < 0.0001$) for the FR marker. Indeed, pair wise comparisons between crops showed restricted gene flow between corn and rice, cotton and rice and sorghum and rice (Table 2). Nevertheless, this gene flow is existent since hybrids between both strains were found in Tolima, perhaps due to the coexistence of corn and rice

Table 2 PhiPT values for the pair wise comparisons of *Spodoptera frugiperda* populations collected from four crops, 9999 permutations were used for the COI region and FR marker.

Marker	Crop 1	Crop 2	PhiPT	Nm	P
COI	Corn	Cotton	0.002	128.898	0.322
	Corn	Rice	0.460	0.294	< 0.001
	Cotton	Rice	0.554	0.202	< 0.001
	Corn	Sorghum	0.000	∞	0.337
	Cotton	Sorghum	0.000	∞	0.255
	Rice	Sorghum	0.521	0.230	< 0.001
FR	Corn	Cotton	0.044	5.389	0.014
	Corn	Rice	0.313	0.549	< 0.001
	Cotton	Rice	0.473	0.278	< 0.001
	Corn	Sorghum	0.043	5.593	0.036
	Cotton	Sorghum	0.000	∞	0.378
	Rice	Sorghum	0.457	0.298	< 0.001

Nm: number of migrants. Bonferroni correction $\alpha = 0.05/12 = 0.004$.

Table 3 PhiPT values for the pair wise comparisons of *Spodoptera frugiperda* populations collected from sixteen farms, 99 permutations were used for the COI region.

	Algodonera Andina	Triangulo Saldaña	Vereda Baloca	Oticuno	CI Nataima	La Colmena	Armero Guayabal	Hacienda Pajonales	Hacienda Potossi	Nataga-ima1	Nataga-ima2	Nataga-ima3	Pajona-les	Pazos	Predio Llano Grande	Semillas Valle
Algodonera Andina	**X**	ns	Ns	ns	ns	ns	ns	***0.010***	ns	***0.010***	***0.010***	Ns	ns	ns	ns	ns
Triangulo Saldaña	0.125	**X**	0.260	***0.030***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vereda Baloca	0.004	0.000	**X**	ns	ns	ns	ns	***0.020***	ns	ns	ns	ns	ns	ns	ns	ns
Oticuno	0.059	0.367	0.172	**X**	ns	***0.050***	ns	***0.010***	ns	***0.010***	***0.020***	ns	ns	ns	ns	ns
CI Nataima	0.000	0.000	0.000	0.173	**X**	ns	ns	***0.040***	ns	ns	ns	ns	ns	ns	ns	ns
La Colmena	0.138	0.000	0.000	0.544	0.000	**X**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Armero Guayabal	0.032	0.000	0.000	0.246	0.000	0.000	**X**	***0.050***	ns	ns	ns	ns	ns	ns	ns	ns
Hacienda Pajonales	0.406	0.062	0.239	0.570	0.252	0.000	0.170	**X**	0.320	0.240	0.360	0.060	***0.010***	***0.030***	***0.010***	***0.010***
Hacienda Potossi	0.025	0.000	0.000	0.392	0.000	0.000	0.000	0.073	**X**	0.310	0.360	0.240	0.540	0.210	0.270	0.310
Natagaima1	0.297	0.000	0.103	0.539	0.108	0.000	0.029	0.000	0.000	**X**	0.240	0.160	0.020	0.350	0.040	0.120
Natagaima2	0.294	0.000	0.122	0.482	0.127	0.000	0.054	0.000	0.000	0.000	**X**	0.160	0.010	0.300	***0.020***	0.110
Natagaima3	0.000	0.044	0.000	0.072	0.000	0.033	0.000	0.362	0.000	0.215	0.226	**X**	0.520	0.220	0.360	0.240
Pajonales	0.000	0.126	0.005	0.060	0.000	0.138	0.033	0.406	0.024	0.298	0.295	0.000	**X**	0.240	0.270	0.430
Pazos	0.000	0.000	0.000	0.206	0.000	0.000	0.000	0.225	0.000	0.074	0.097	0.000	0.000	**X**	0.340	0.280
Predio Llano Grande	0.000	0.081	0.000	0.081	0.000	0.079	0.001	0.367	0.000	0.245	0.248	0.000	0.000	0.000	**X**	0.390
Semillas Valle	0.000	0.025	0.000	0.120	0.000	0.001	0.000	0.316	0.000	0.177	0.190	0.000	0.000	0.000	0.000	0.000

Nm: number of migrants. PhiPT values below diagonal. Probability values based on 99 permutations are shown above diagonal.

Table 4 PhiPT values for the pair wise comparisons of *Spodoptera frugiperda* populations collected from sixteen farms, 99 permutations were used for the FR marker.

	Algodonera Andina	Triangulo Saldaña	Vereda Baloca	Oticuno	CI Nataima	La Colmena	Armero Guayabal	Hacienda Pajonales	Hacienda Potossi	Nataga-ima1	Nataga-ima2	Nataga-ima3	Pajona-les	Pazos	Predio Llano Grande	Semillas Valle
Algodonera Andina	**X**	ns	ns	ns	ns	ns	ns	***0.010***	ns	ns	ns	ns	ns	ns	ns	ns
Triangulo Saldaña	0.000	**X**	ns	ns	ns	ns	ns	***0.010***	ns	ns	ns	ns	ns	ns	ns	ns
Vereda Baloca	0.074	0.000	**X**	***0.040***	ns	ns	***0.020***	***0.010***	ns	ns	ns	ns	ns	ns	ns	ns
Oticuno	0.030	0.075	0.213	**X**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CI Nataima	0.000	0.000	0.138	0.000	**X**	***0.040***	***0.010***	***0.010***	ns	ns	***0.050***	ns	ns	ns	ns	ns
La Colmena	0.000	0.000	0.000	0.217	0.000	**X**	ns	***0.020***	ns	ns	ns	ns	ns	ns	ns	ns
Armero Guayabal	0.061	0.131	0.258	0.000	0.000	0.336	**X**	ns	***0.040***	***0.010***	***0.010***	ns	ns	ns	***0.040***	ns
Hacienda Pajonales	0.863	0.895	0.684	0.000	0.954	0.928	0.000	**X**	***0.010***	***0.010***	***0.010***	***0.010***	***0.010***	***0.010***	***0.010***	ns
Hacienda Potossi	0.094	0.000	0.000	0.392	0.228	0.000	0.499	0.841	**X**	ns	ns	ns	ns	ns	ns	ns
Natagaima1	0.000	0.000	0.000	0.097	0.000	0.000	0.159	0.891	0.000	**X**	ns	ns	ns	ns	ns	ns
Natagaima2	0.075	0.000	0.000	0.221	0.142	0.000	0.270	0.699	0.000	0.000	**X**	ns	ns	ns	ns	***0.040***
Natagaima3	0.000	0.000	0.000	0.072	0.000	0.000	0.153	0.939	0.000	0.000	0.000	**X**	ns	ns	ns	ns
Pajonales	0.000	0.000	0.068	0.036	0.000	0.000	0.066	0.844	0.082	0.000	0.069	0.000	**X**	ns	ns	ns
Pazos	0.000	0.000	0.077	0.000	0.000	0.000	0.035	0.948	0.114	0.000	0.079	0.000	0.000	**X**	ns	ns
Predio Llano Grande	0.075	0.000	0.000	0.221	0.142	0.000	0.270	0.699	0.000	0.000	0.000	0.000	0.069	0.079	**X**	***0.030***
Semillas Valle	0.056	0.122	0.250	0.000	0.000	0.316	0.000	0.000	0.481	0.148	0.261	0.139	0.061	0.027	0.261	**X**

Nm: number of migrants. PhiPT Values below diagonal. Probability values based on 99 permutations are shown above diagonal.

strains in corn, one of the crops where hybrids were more abundantly collected. Moreover, AMOVA tests support the result mentioned above when FAW populations amongst sixteen farms were considered: PhiPT = 0.414 (df = 15, 237; P < 0.01) for the COI region and PhiPT = 0,129 (df = 15, 237; P < 0.01) for the FR marker.

The population differentiation was mainly produced between the farms Hacienda Pajonales (rice farm, n = 33), Algodonera Andina (cotton farm, n = 28) and CI Nataima (corn farm, n = 14) with the other thirteen farms (Table 3, 4). This can be explained due to FAW population composition, with the rice strain and hybrids predominant on the first farm, and corn strain and hybrids principally found on the second and third farm. Furthermore, pairwise comparisons amongst farms for both markers were mostly not significant, particularly between corn, sorghum and cotton farms, since corn strain was predominant on them. All these results are in line with findings of Busato *et al* (2004), Clark *et al* (2007) and Martinelli *et al* (2007), because in all these separated studies there were differentiated populations of *S. frugiperda* in Argentina, Brasil, and US. However, only in the present work this analysis was focused on strain identification previously to a population structure analysis. It is important to mention that the molecular markers used here are more useful for strain identification than for population analysis, since they do not provide much information about variation within the strain. However they were adequate to provide the required information for the proposed analysis of the strain and hybrid composition within farms and crops.

Contingency tables indicated a differential distribution of both FAW strains on the four host plants (Table 5), with corn strain mainly present in corn, sorghum and cotton, and rice strain predominantly in rice and in lower proportions on the other crops (Fig 1). Similar results were reported by Prowell *et al* (2004). We also obtained clustering for dendrograms obtained from each marker based on Nei genetic distances for the four studied crops, with a first cluster composed of *S. frugiperda* larvae from corn, sorghum and cotton and a second cluster from rice crops (Fig 2a). Similar findings were made by Prowell (1988). In addition, we recorded differences in host distribution, with (+/+) hybrids being more abundant on corn and cotton, while (-/-) hybrids on rice and cotton. Clustering of hybrids on dendrograms was not possible since Nei genetic distances were separately calculated for both markers.

Table 5 Contingency table for the molecular markers COI, FR analysed separately and together used to test whether *Spodoptera frugiperda* strains are differentially distributed amongst corn, cotton, Sorghum and rice crops in Tolima department.

	Corn	Cotton	Sorghum	Rice	Total	χ^2	gl
COI region							
Corn	80	57	31	11	179		
Rice	22	10	6	36	74	63.5	3
FR region							
Corn	64	55	30	16	166		
Rice	38	12	7	31	87	32.9	3
COI + FRa							
Corn	60	48	28	7	143		
Rice	17	3	3	26	49		
Hybrid ++	21	9	3	4	37		
Hybrid --	4	7	3	10	24	76.64	9

Nei genetic distances and dendrograms obtained for each marker and all sixteen farms analyzed (Fig 2b) failed to separate corn, sorghum and cotton farms from rice farms, the explanation for this result being that more molecular markers are needed for the construction of dendrograms between these farms or simply that the association between corn and rice strains to corn and rice as hosts is not so strong, so both strains coexist in both crops, but their frequency is different, given that corn strain is more abundant in corn and rice strain in rice.

Finally, logistic regression (ML = -273,775; G = 22,047; df = 3, P < 0.0001) indicates that *S. frugiperda* strain distribution was not affected by sampling region or by surrounding crops, but by the host plant (i.e., crop) on which larvae were collected.

In general, our study shows that host plant association is the main cause of the genetic differentiation of FAW populations in Tolima, Colombia. This finding is comparable to the association of *O. nubilalis* with corn and mugwort in France (Martel *et al* 2003, Malausa *et al* 2007). Both lepidopterans may be in a similar stage of speciation as host plant associated populations' origin relative to other insects, such as *R. pomonella* (Feder 1998). Identification of *S. frugiperda* strains is crucial for regional FAW integrated pest management, as the department of Tolima is considered a major producer of all main FAW hosts plants. In addition, since corn strain is apparently more resistant than rice strain to several insecticides and to the endotoxin *Cry1Ac* on transgenic cotton crops (Adamczyk *et al* 1997), corn, cotton and sorghum crops may have to be managed in a different way that rice crops are.

Finally, current crop rotation may not be the most effective management strategy for FAW in the region, as corn strains will likely shift from corn or sorghum to cotton, while the rice strain will shift from rice to pasture grasses from the first to the second semester of the year. We suggest corn, sorghum and cotton crops to be sawed in allopatric sites if they are produced at the same semester of the year in order to prevent migration of corn strain amongst them, and production of rice crops at separated distances from pasture grasses, to reduce movement of rice strain between them. In rice, *S. frugiperda* is not a major pest in Tolima as the other three crops, since it is produced in wetlands where FAW larvae are incapable to survive.

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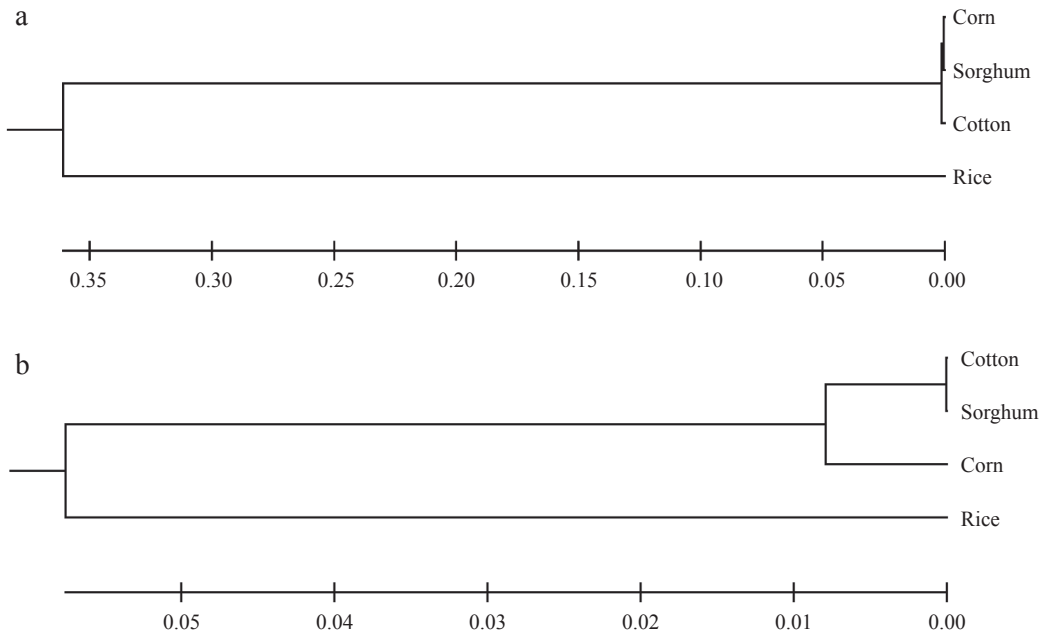

Fig 1 Dendrogram based on Nei's Genetic distance and UPGMA method for a) the COI region and b) FR marker for the four crops sampled in Tolima.

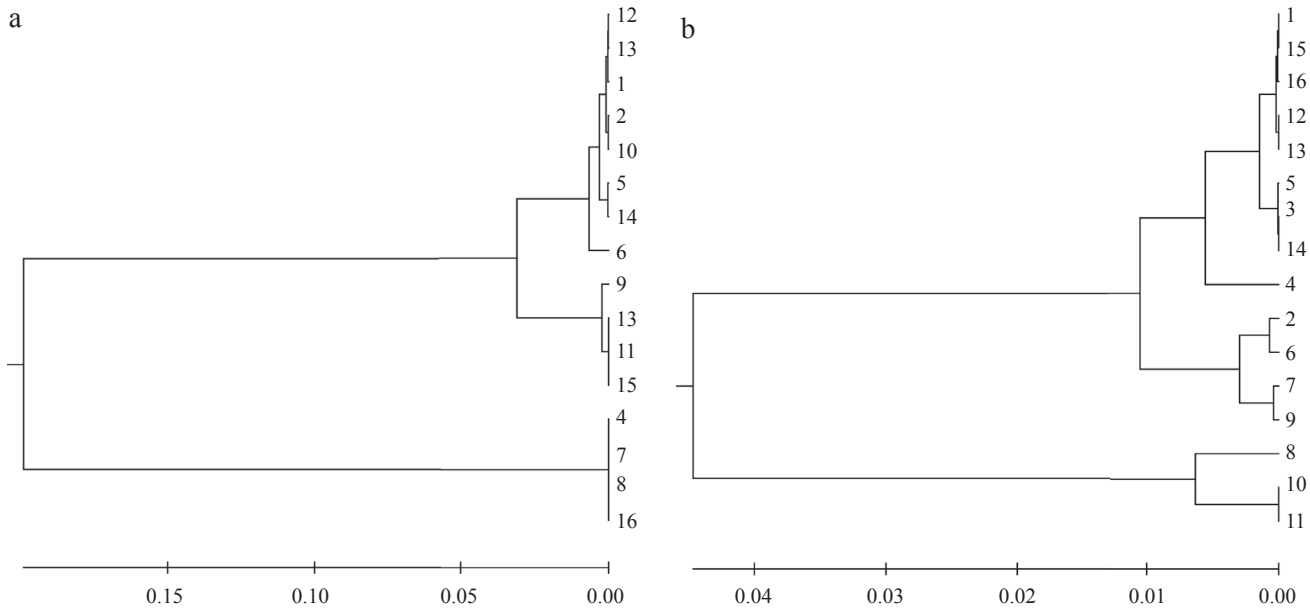

Fig 2 Dendrogram based on Nei's Genetic distance and UPGMA method for the (a) COI region and (b) FR marker for the sixteen farms sampled in Tolima (1 = Algodonera Andina, 2 = Armero Guayabal, 3 = Hacienda Pajonales, 4 = Hacienda Potossi, 5 = Natagaima3, 6 = Pajonales, 7 = Pazos, 8 = Predio Llano Grande, 9 = Semillas Valle, 10 = Triangulo Saldaña, 11 = Vereda Baloca, 12 = Oticuno, 13 = CI Nataima, 14 = La Colmena, 15 = Natagaima1, 16 = Natagaima2).

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