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Field and Forage Crops

Demonstration Using Field Collections that Argentina Fall Armyworm Populations Exhibit Strain-specific Host Plant Preferences

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ABSTRACT *Spodoptera frugiperda*, the fall armyworm, is a major economic pest throughout the Western Hemisphere of corn (maize), cotton, sorghum, and a variety of agricultural grasses and vegetable crops. Studies in the United States, the Caribbean, and Brazil demonstrated the existence of two subpopulations (previously designated "host strains") that differ in their choice of plant host. Specifically, the corn strain is preferentially found in corn and sorghum, while the rice strain is dominant in rice, turf grass, and alfalfa. However, inconsistent results were reported in surveys of fall armyworm in Argentina, with some indicating that the host plant preferences of the two strains might be compromised or even nonexistent. If correct, this would complicate efforts to control this pest by considerably expanding the range of habitats that would have to be considered as potential sources for fall armyworm infestations in specific crops. A reexamination of Argentine fall armyworm, this time with field collections rather than the laboratory colonies used in previous studies, confirmed the existence of the two strains and their host preferences. Specifically, the corn strain was consistently the majority population infesting corn and was usually so in sorghum, while the rice strain was predominant in pasture/turf grasses and alfalfa. The one outlier was a collection from rice, which had a corn strain majority. Overall, the data were generally consistent with strain behaviors observed in other areas of the Western Hemisphere.

KEY WORDS agricultural entomology, crop protection, ecology & population biology, pest management, population genetics

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), the fall armyworm, is one of the major agricultural pests in the Western Hemisphere, infesting corn (*Zea mays* L.), sorghum (*Sorghum* spp), turf grasses, and a number of other crops (Luginbill 1928, Casmuz et al. 2010). Fall armyworm is the most important pest of corn in northern Argentina, causing yield losses that fluctuate from 17 to 72% (Perdiguero et al. 1967; Willink et al. 1993a, b), and thereby promoting the use of genetically modified corn lines expressing *Bacillus thuringiensis* (*Bt*) insecticidal proteins as a control agent (Blanco et al. 2010, Huang et al. 2014). The fall armyworm is reported to be capable of colonizing as many as 190 plant species (Cazmuz et al. 2010, Bohnenblust and Tooker 2012), suggesting that a number of host plants could support pest populations during periods when corn is not available. Identifying the host species most capable of supporting significant numbers of fall armyworm is necessary to identify potential sources of infesting populations and to design effective "refuge" strategies to suppress the generation of resistance to Bt toxins (Tabashnik et al. 2008).

These studies are complicated by the existence of two fall armyworm subpopulations that display specific host plant preferences. The initial characterization was based on genetic distinctions found between morphologically indistinguishable populations feeding on corn ("corn strain") and rice ("rice strain") (Pashley 1986, 1988). The most accurate indicators of strain identity are molecular markers that include allozyme, nuclear genome, and mitochondrial DNA polymorphisms (Pashley 1989, Lu et al. 1994, Lu and Adang 1996, McMichael and Prowell 1999, Levy et al. 2002, Nagoshi and Meagher 2003). Haplotype analysis based on the mitochondrial *cytochrome oxidase subunit I* gene (*COI*) was shown to be at least as accurate as allozymes or whole-genome approaches (Prowell et al. 2004). One portion of the *COI* gene has been developed as a DNA barcoding region capable of distinguishing fall armyworm from other *Spodoptera* species as well as differentiating the two strains (Nagoshi et al.

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2011). A genomic marker of strain identity is the *Tpi* gene, which lies on the Z-chromosome and encodes for the enzyme triose phosphate isomerase (Nagoshi 2010). In previous studies, we showed that the strain designations based on *Tpi* gave results generally consistent with *COI*, making it a useful method to confirm strain identifications (Nagoshi 2010, 2012).

The possibility that only a subpopulation of fall armyworm is a consistent pest of corn could simplify the identification of potential infestation sources if, for example, the corn strain is more limited in its host plant usage. Studies of populations from the United States and Brazil demonstrated a significantly higher proportion of corn strain larvae on sorghum and cotton (*Gossypium hirsutum* L.) and a large rice strain majority on alfalfa (*Medicago sativa* L.) and forage grasses (*Cynodon* spp.) (Nagoshi and Meagher 2004a, Prowell et al. 2004, Nagoshi et al. 2007a, Juárez et al. 2012). This association of the strains with specific plants is not absolute, with genetic markers of the opposing strain frequently observed as a minority component of collections from corn strain- or rice strain-preferred hosts (Prowell et al. 2004, Juárez et al. 2012, Nagoshi et al. 2012). Nevertheless, surveys performed over multiple years and locations in the United States and Brazil have consistently shown strong biases in the distribution of strain-specific genetic markers in specimens collected from corn versus turf/pasture grasses (Nagoshi and Meagher 2004b; Nagoshi et al. 2007b; Machado et al. 2008; Nagoshi 2010, 2012).

We first surveyed Argentine fall armyworm populations by examining laboratory colonies derived from corn, sorghum, or rice plant hosts that were two generations removed from the original field collections (Nagoshi et al. 2012). The results indicated that the expected host plant preferences of the two strains were generally observed in the Argentina populations, indicating that the corn strain was the primary fall armyworm subpopulation infesting corn. However, another study that was also dependent on colonies showed more variable results (Juárez et al. 2012). In this case, out of 18 colonies tested derived from corn, sorghum, or rice, only 8 showed the expected *COI* marker association between strain and host plant. Given these inconsistencies, it is uncertain whether the genetically defined strains in Argentina are showing the expected host preference behavior.

One possible explanation for the conflicting results is that even short-term artificial rearing might have unforeseen effects on haplotype frequencies. These include founder effects and unintended selection for certain genotypes (Arias et al. 2005, Liu et al. 2015). To address these concerns, we performed another survey of Argentina fall armyworm populations using field-collected specimens from corn, sorghum, alfalfa, and rice host plants over a large geographical area. The use of field collections avoids the potential influences of artificial rearing. In addition, a preliminary statistical analysis was performed to assess the feasibility of correlating the geographical distribution of the two strains with climatic regions.

Materials and Methods

Insect Collections. Fall armyworm larvae were collected from commercial and subsistence farming fields in 11 Argentine provinces. The crops sampled were corn, rice, sorghum [(*Sorghum bicolor* (L.) Moench ssp. *bicolor*)], alfalfa, and different wild grasses [Bermuda grass, *Cynodon dactylon* (L.), Guinea grass, *Megathyrsus maximum* (Jacq.)] that were collectively labeled as "grasses" (Fig. 1; Table 1). Collections took place in February 2012. At each sampling site, a minimum of 250 larvae (from instars 3-6) were collected and placed individually in glass tubes (12 cm in height and 1.5 cm in diameter) in addition to leaves of the host plant.

Fall Armyworm Sample Preparation. Field-collected larvae were preserved in 70% ethanol until DNA isolation or were placed in growth chambers under controlled conditions [27 ± 2°C, 70–75% RH, 14:10 (L:D) h photoperiod] until adult emergence. The adults were conserved dry until DNA isolation. Late larval instars and adults were examined using taxonomic morphological markers to confirm that all individuals were fall armyworm. Sampled insects from each of these populations were deposited as voucher specimens at the collection of Sección Zoología Agrícola, Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina.

DNA Isolation and PCR Analysis. Genomic/mitochondrial DNA for use in PCR amplifications were isolated from individual specimens using Zymo-Spin III columns (Zymo Research, Orange, CA) as described previously (Nagoshi et al. 2007b). PCR amplification for all reactions used the profile: 94°C (1 min); followed by 32 cycles of 92°C (30 sec), 56°C (30 sec), and 72°C (1 min); and a final segment of 72°C for 3 min. All primers used were synthesized by Integrated DNA Technologies (Coralville, IA). Amplification of the *COI* region used the primer pairs *COI-45F* (5′-TTCGAGCTGAATTAGGGACTC-3′) and *COI-1058R* (5′-ACACCTGTTAATCCTCCTACAG-3′). Amplification of the *Tpi* gene used primers *Tpi*-632F (5′-GGTTGCCCATGCTCTTGAGTCCGGACT-GAAG-3′) and *Tpi*-1195R (5′-AGTCACTGACCCAC-CATACTG-3′). Digestions with restriction enzymes (New England Biolabs, Beverly, MA) used manufacturer-provided buffers. Each reaction used 10-20 units of restriction enzyme and was incubated at 37°C for 3 h to overnight. For gel electrophoresis, 6 µl of 6X gel loading buffer was added to each reaction and the entire sample run on a 2% agarose horizontal gel containing GelRed (Biotium, Hayward, CA) in 0.5X Tris-borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). Fragments were visualized on a long-wave UV light box using the Alpha Imager Mini photodocumentation system (Cell Biosciences Inc., Santa Clara, CA).

Fall Armyworm Strain Analysis. PCR amplification of *COI* sequences using the primers *COI*-45F/*COI*-1058R produce a 958-bp amplified product that contains an *Msp*I site at two locations, one specific to the rice strain and the other to the corn strain

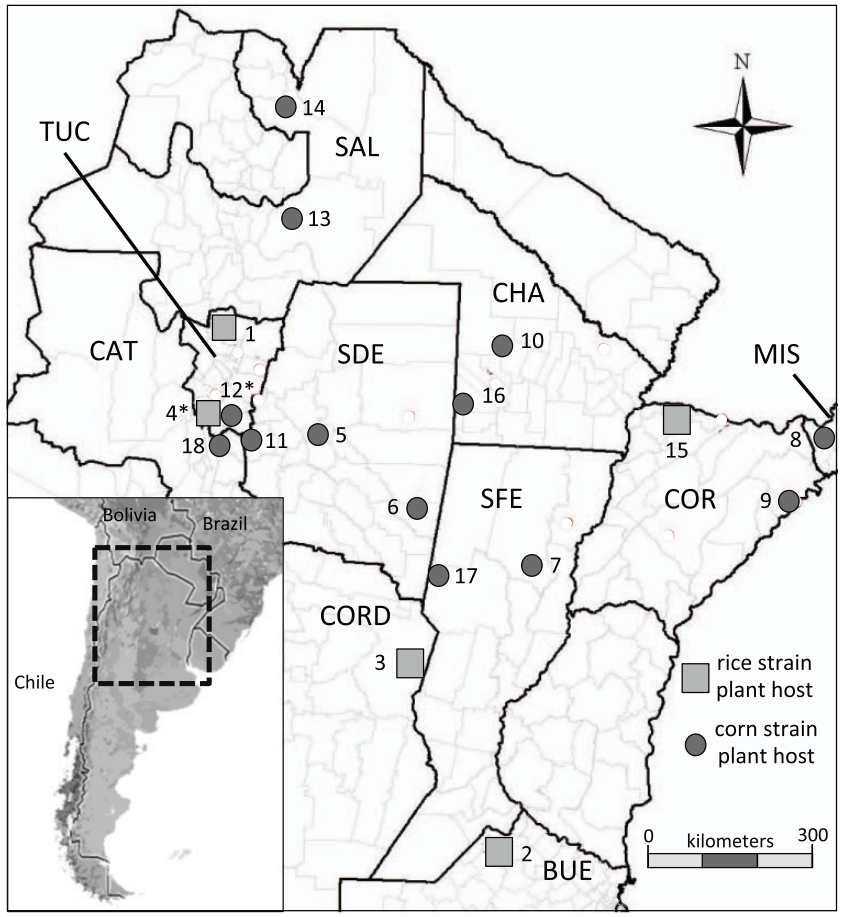

Fig. 1. Map of locations in Argentina where fall armyworm larvae were collected. Abbreviations of provinces, BUE: Buenos Aires, CAT: Catamarca, CHA: Chaco, CORD: Córdoba, COR: Corrientes, MI: Misiones, SAL: Salta, SFE: Santa Fé, SDE: Santiago del Estero, TUC: Tucumán. Numbers identify site locations as described in Table 1. Rice-strain plant hosts (square) indicate larvae collected from rice, pasture grasses, or alfalfa. Corn-strain plant hosts (circle) indicate collections from corn or sorghum. Asterisks (*) indicate collections from different plant hosts in the same location.

Table 1. Location information for the sampling sites

Site	Province	Nearest City	Longitude	Latitude	Host plant
1	Tucumán (TUC)	Vipos	−65.3000	−26.4667	Alfalfa
2	Buenos Aires (BUE)	Pergamino	−60.6641	−33.7026	Grasses
3	Córdoba (CORD)	San Francisco	−62.2298	−31.4264	Grasses
4	Tucumán (TUC)	La Cocha	−65.5001	−27.7484	Grasses
5	Santiago del Estero (SDE)	Fernández	−63.7942	−27.9671	Sorghum
6	Santiago del Estero (SDE)	Bandera	−62.4833	−28.7222	Sorghum
7	Santa Fé (SFE)	Margaritas	−60.2502	−29.6807	Sorghum
8	Misiones (MI)	Cerro Azul	−55.4386	−27.6574	Corn
9	Corrientes (COR)	Santo Tomé	−56.1078	−28.4497	Sorghum
10	Chaco (CHA)	Concepción del Bermejo	−60.8855	−26.6299	Sorghum
11	Santiago del Estero (SDE)	San Pedro	−65.1619	−27.9801	Sorghum
12	Tucumán (TUC)	La Cocha	−65.5001	−27.7484	Corn
13	Salta (SAL)	Las Lajitas	−64.1871	−24.8806	Corn
14	Salta (SAL)	Coronel Cornejo	−64.7981	−23.8257	Corn
15	Corrientes (COR)	Scorza Cue	−57.9755	−27.3412	Rice
16	Chaco (CHA)	Gancedo	−61.6641	−27.4865	Corn
17	Santa Fé (SFE)	Ceres	−62.0936	−29.7222	Sorghum
18	Catamarca (CAT)	Los Altos	−65.3996	−28.1412	Corn

Sites are numbered in order of ascending sample score as determined by correspondence analysis (CA). Pasture and turf grass host plants are of mixed species and are generically labeled "grasses".

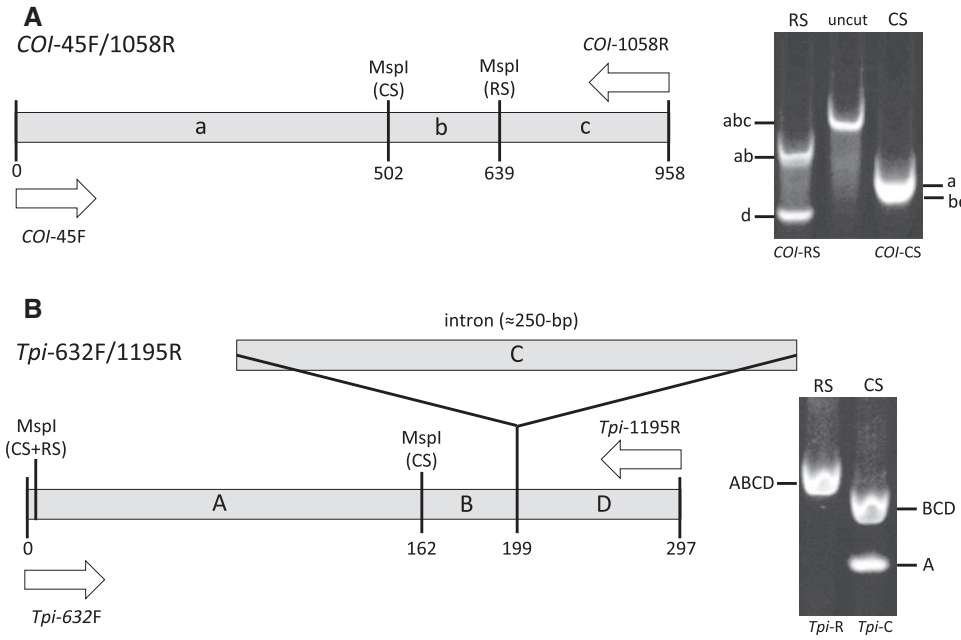

Fig. 2. Diagrams of the regions from the *COI* and *Tpi* genes used for the strain analysis. A: Region of the *COI* gene used for strain identification with location of the two strain-specific *Msp*I sites indicated. B: Portion of the fall armyworm *Tpi* gene including exon-4 (A+B), intron-4 (C), and a part of exon-5 (D). Number of base pairs from the diagram origin (0) for selected sites is shown. Block arrows designate locations and directions of primers used for PCR amplification. Gel pictures show strain-specifying bands produced by digestion of the PCR product with the restriction enzyme *Msp*I. Letters indicate interpretation of fragment based on diagram. CS: corn-strain-specific. RS: rice-strain-specific.

(Fig. 2A). Digestion by *Msp*I produces diagnostic banding patterns of 319 bp and 639 bp associated with the rice strain (*COI*-RS) or 456 bp and 502 bp for the corn-strain (*COI*-CS) haplotypes (Meagher and Nagoshi 2010). This method has the advantage of requiring *Msp*I activity to occur to diagnose the strain. Therefore, the failure of the enzyme to digest an existing site, perhaps the most likely potential technical artifact, would result in a single 958-bp band that is distinguishable from the strain diagnostic patterns. This was only rarely observed, and in each case was resolved by repeating the PCR and restriction digest. Analysis of the *Msp*I restriction digest pattern was shown to be as accurate as DNA sequencing for determining strain identity in specimens from the United States, Puerto Rico, and Brazil (Nagoshi 2012). It has the additional advantage of being much faster and less expensive than DNA sequencing, allowing examination of larger sample populations.

PCR amplification of *Tpi* sequences using the primers *Tpi*-632F/*Tpi*-1195R amplify an approximately 600-bp fragment that extends from the beginning of *Tpi* exon-4 (segments A+B) to the middle of exon-5 (segment D) and includes a variable length intron (segment C) that is typically about 250 bp in length (Fig. 2B)(Nagoshi 2012). The PCR fragment carries an *Msp*I site present in both strains within the *Tpi*-632F primer region and a second *Msp*I site that is present only in the *Tpi*-C haplotype (Fig. 2B). In a *Tpi*-R sample found in the rice strain, an *Msp*I restriction digest of the PCR amplified fragment produces a band of greater than 500 bp (fragment ABCD, Fig. 2B). The presence of the *Msp*I site in the *Tpi*-C haplotype results in two bands, a diagnostic 162-bp fragment (fragment A) and a larger one that contains the variable length intron and parts of exon-4 and exon-5 (fragment BCD). This *Msp*I restriction method gave strain identity results comparable to DNA sequence analysis (Nagoshi 2012). Statistical analyses of the haplotype and strain frequencies include two-tailed *t*-tests and one-tailed ANOVA with appropriate post hoc analyses. These were performed using GraphPad Prism version 6.0d for MacOS X, GraphPad Software, La Jolla, California, USA, www.graphpad.com.

Correspondence Analysis. The collected individuals of fall armyworm were divided into four classes or genotype configurations according to the different marker combinations: 1) *COI*-RS and *Tpi*-C (discordant class RC); 2) *COI*-CS and *Tpi*-R (discordant class CR); 3) *COI*-RS and *Tpi*-R (concordant class RR); 4) *COI*-CS and *Tpi*-C (concordant class CC) (first letter always referring to the *COI* haplotype). The genotypic profile of each site was based on the relative frequency of the haplotypes detected. In the initial analysis, the 444 individuals characterized were cross-tabulated according to locality of occurrence and genotype configuration. Then, a correspondence analysis (CA) was performed using a contingency table such that both genetic markers and underlying crops could be projected in a postulated environmental space (or ordination space). CA assumes that the genetic markers have unimodal response curves. A genotype is located in the

location of space where it is most frequent. The solution obtained by CA through an eigenanalysis has important mathematical properties. The first axis consists of the ordering of genetic markers and sites that produces the maximum possible correlation between markers and sites scores. Second and higher axes also have maximal site-species correlation subject to the constraint that axes are orthogonal (Pielou 1984). The first axis usually turns out to be related to important environmental gradients. Importantly, first axis scores of both genotypes and sites are simultaneously ordinated along the same first axis. CA was applied on our data through R package (Nenadic and Greenacre 2007).

Once the sequential arrangement of sites was established, we bisected the respective gradient (dictated by the genotype prevalence) into two subsets of sites base on whether the RR was in the majority (>50%) or not. The bipartition of studied localities that best agree with the two pools of sites previously polarized was identified using a simple bisector tracked on the geographical map. To track the bisector, we have carried out an original algorithm consisting of the following computational steps: 1) polygonal tessellation of the geographically projected point localities (Thiesen's polygons) and the drawing of the dual Delaunay triangulation; 2) segregation of localities into two pools of contiguous points using simple bisectors (equidistant line between dot clouds without backtracking and self-intersection) running across the Delaunay triangulation; 3) calculating the degree of agreement between the spatial and genotype bipartitions of the same data points (using a confusion matrix for that purpose) for the spatial scission induced by each bisector; and 4) selecting the bisector that best fit the classification. The algorithm itself has been coded with the R language.

Results

Association of *COI* and *Tpi* Haplotypes. The *COI* and *Tpi* strain diagnostic markers were determined for 444 specimens, allowing a direct assessment of their association across the samples (Table 2). The concordant configurations of *COI*-C/*Tpi*-C (CC) and *COI*-R/*Tpi*-R (RR) were far more frequent than the discordant groups, representing 84% of specimens. Two-tailed paired *t*-test analysis of the 18 colonies showed a significant positive correlation between the *COI* and *Tpi* markers ($r = 0.84$; $P < 0.001$). The *COI*-R/*Tpi*-C (RC) or *COI*-C/*Tpi*-R (CR) discordant configurations were found in all collections except at Site 1 (Table 2). The overall percentage of discordant individuals was 16% (69/444), with the frequency of the RC discordants (14%) much higher than the reciprocal CR configuration (2%). This bias was observed in collections from all plant hosts tested except for the single sampling from alfalfa, where no discordants were found (Fig. 3). Discordant fall armyworms were predominantly RC regardless of host plant or the concordant genotype frequency of the population.

One simple explanation for the discordant class is that they resulted from matings between strains that occurred at some point in their ancestry (Nagoshi 2010). Support for this came from a comparison of discordant frequencies in collections with different concordant proportions. A significantly higher frequency of discordants was observed in collections where RR and CC specimens were both present (25%), which should provide more opportunities for interstrain mating, compared to those with only RR (16%, $P = 0.047$) or CC (11%, $P = 0.018$), as determined by two-tailed, unpaired *t*-test analysis.

Table 2. Number and frequency of genotype configurations and agreement with host plant expectation for the different sampling sites

Site	Host	Numbers				Frequency		Agree?[a]
		RR	CR	RC	CC	RR	CC	
1	Alfalfa	22	0	0	0	1.0	0.0	Yes
2	Grasses	21	1	1	0	0.9	0.0	Yes
3	Grasses	22	1	3	0	0.8	0.0	Yes
4	Grasses	26	0	5	0	0.8	0.0	Yes
15	Rice	0	0	4	29	0.0	0.9	No
Avg ± SD			0.7 ± 0.4a			0.2 ± 0.4b		
5	Sorghum	10	0	2	4	0.6	0.3	No
6	Sorghum	9	1	7	8	0.4	0.3	No
7	Sorghum	6	0	5	8	0.3	0.4	No
8	Corn	1	0	3	9	0.1	0.7	Yes
9	Sorghum	0	2	5	32	0.0	0.8	Yes
10	Sorghum	0	1	8	38	0.0	0.8	Yes
11	Sorghum	0	0	4	18	0.0	0.8	Yes
12	Corn	0	0	5	23	0.0	0.8	Yes
13	Corn	0	0	4	21	0.0	0.8	Yes
14	Corn	0	0	2	12	0.0	0.9	Yes
16	Corn	0	1	1	21	0.0	0.9	Yes
17	Corn	0	0	1	9	0.0	0.9	Yes
18	Corn	0	0	2	26	0.0	0.9	Yes
Avg ± SD			0.1 ± 0.2b			0.7 ± 0.2a		

One-way ANOVA was conducted to compare mean RR and CC frequencies in host plants associated with the two fall armyworm strains. Averages associated with different letters (a, b) are significantly different based on the Tukey's multiple comparisons post hoc test.

[a]Do RR and CC distributions agree with host plant?

Association of Strain Markers with Plant Hosts. Based on past studies, the fall armyworm rice strain is expected to be the majority strain on rice, alfalfa, turf, and pasture grasses, while the corn strain should predominate on corn and sorghum. Of the 18 collections, 14 showed the majority *COI-Tpi* configuration predicted by the host plant (Fig. 4A). Over 80% of the specimens in each the alfalfa and grass collections were RR, while CC was present in over 65% in all six collections from corn and four of seven collections from sorghum. However, an ambiguous distribution was found in two sorghum populations (Sites 6 and 7) where neither RR nor CC reached 50%. Unexpected results were observed in the sorghum collection from Site 5, where 63% of the specimens were RR, and in the one collection from rice (Site 16), which was 88% CC. Despite these exceptions, an analysis of variance by one-way ANOVA showed that the effect of RR and CC frequency was significant ($F = 13.85$, df = 3, 32, $P < 0.001$; Table 2). Post hoc analyses using the Tukey's multiple comparison test indicated that the mean frequency of RR was significantly higher than that of CC in collections from expected rice-strain hosts (rice,

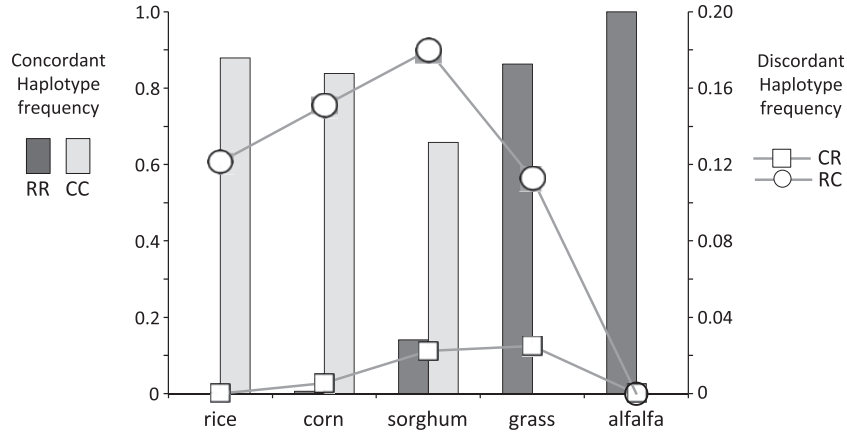

Fig. 3. Comparison of concordant (RR and CC) and discordant (RC and CR) haplotype frequencies as observed in different host plant collections. The concordant haplotype frequencies are diagrammed as a bar graph (left y-axis). The discordant haplotype frequencies are depicted by line graphs (right y-axis).

grasses, alfalfa) and of RR found in the corn-strain hosts (sorghum, corn) (means and standard deviations in Table 2). Similarly, the mean frequency of CC in corn-strain hosts was significantly higher than mean frequencies of RR in corn-strain hosts or CC in rice-strain hosts. Overall, the results were consistent with expectations of strain-specific biases in plant host usage.

Generally similar results were obtained by using either the *COI* or *Tpi* markers individually, with both identifying the same 15 collections as being consistent with expectations as well as the two collections that were divergent (Fig. 4B, C). Some differences were observed, particularly with respect to the ambiguous populations. Over 50% of the specimens from Sites 6 and 7 were *COI*-R, while the *Tpi* analysis of the same collections showed a *Tpi*-C majority. This opposing strain bias of the two markers was consistently observed in the other collections tested. The average *COI*-R frequency for the 18 populations tested was 42%, compared to 29% for the average *Tpi*-R frequency.

Geographical Distribution of Genotype Configurations. Correspondence analysis ordering (CA) was performed on the collections and a first axis rank based on genotype configuration frequency was calculated (Table 1). A CA biplot showed a clear segregation into two pools of sites (Fig 5). Sites 1-7 (CA rank) were located at the negative side of the first CA axis and characterized by the low prevalence (<50%) of unambiguous corn strain (CC), while Sites 8-19 were clumped in a narrow range of positive values of the first CA axis and characterized by the high prevalence (>50%) of the CC genotype. This bipartition of sites corresponded to a crisp bisection of the underlying gradient. Geographically, our algorithm detected a bisector running from Northwest (NW) to Southeast (SE) between two sets of localities that were broadly consistent with the bipartition of sites established on the grounds of first CA axis (Fig. 6A). All sites located at the northern zone (Sites 8-11, 14-17) had a predominantly CC genotype configuration, including the two sorghum sites (9, 11) and the single rice site (16). In comparison, the rice strain was more prevalent in the southern sites both on the expected host plants (alfalfa, pasture grasses) as well as in three of the five sorghum sites (5, 6, 7).

Discussion

The strain-specific bias in the use of host plants was consistently observed in our multiple surveys of United States and Brazilian fall armyworm field populations (Nagoshi 2012, see also Prowell et al. 2004). The rice strain typically predominates in pasture and turf grass habitats, making up 80% or more of the larvae collected from these locations, as identified with either the *COI* or *Tpi* markers. In comparison, the corn strain is most frequently found associated with corn and sorghum hosts, but the proportions are more variable, with an average of about 60% of the larvae from these host plants showing the corn strain diagnostic COI-C marker and almost 80% with the Tpi-C marker (Nagoshi 2012). That the host plant specificities of the two strains are not absolute could be due to several reasons, including plasticity in host choice for one or both strains or compromises in the linkage between the genetic markers and strain identity because of inter-strain mating.

The results of the Argentine field collections were generally consistent with our earlier study based on Argentine laboratory colonies (Nagoshi et al. 2012). In both cases, the majority of collections gave *COI* and *Tpi* haplotypes consistent with the relevant host plant. When using both markers for identification, 14 of the 18 collections had the expected strain predominating based on host plant, with two collections from La Cocha, Tucumán being particularly compelling. Field collections from pasture grass (Site 4) and corn (Site 13) showed diametrically different strain proportions despite their geographical proximity, demonstrating the strong host preferences of the two strains. Differences in strain identification with the two markers occurred

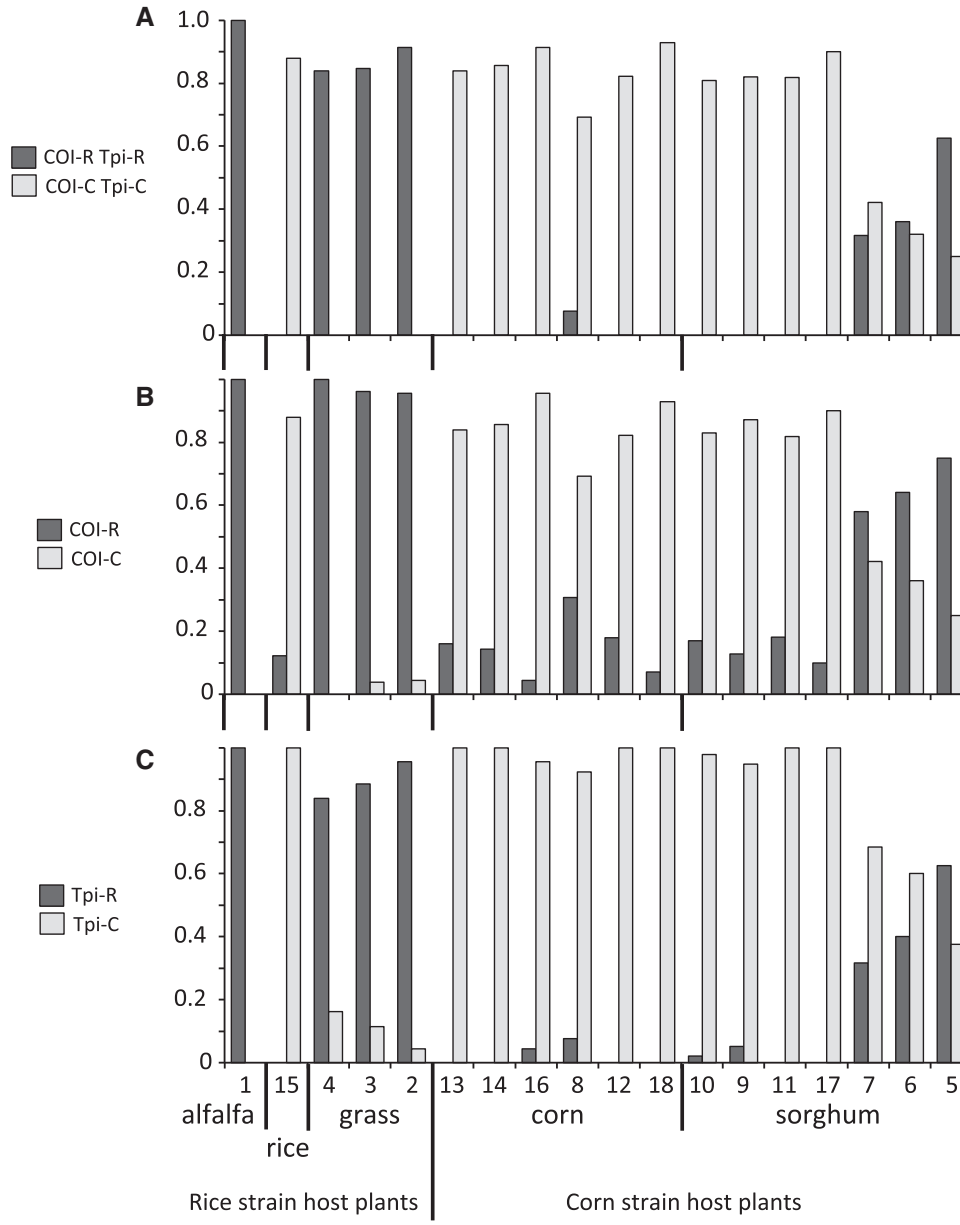

Fig. 4. Comparisons of the concordant *COI* and *Tpi* proportions with those of the individual markers as observed in different host plant collections. A: Proportions of the concordant double marker combinations. B: Proportions of the *COI* strain haplotypes. C: Proportions of the *Tpi* strain haplotypes.

infrequently, as indicated by the low (16%) discordance rate in our 18 field collections. In only two cases would the determination of the majority strain in a collection have been affected by the choice of strain markers. Two sorghum collections (Sites 6 and 7) showed a majority rice strain using the *COI* marker, a majority corn strain with the *Tpi* marker, and no majority when diagnosed with both markers (Table 2).

Of the four exceptions to the expected plant host bias, Site 15 (rice) and Site 5 (sorghum) had a majority of the opposing strain, and two others from sorghum (Sites 6 and 7) showed no RR or CC majority (Table 2). Relatively high proportions of larvae with the discordant (CR and RC) marker configurations were observed at Sites 6 (8/25) and 7 (5/19), suggesting that interstrain hybridization might contribute to the discrepancies at these locations. Significant departures from the expected strain specificity have now been documented in field collections from corn, sorghum, and rice hosts in Florida and South America (Nagoshi and Meagher 2004a, Prowell et al. 2004, Nagoshi et al. 2007b), with indications in southern Florida that such exceptions in corn may be related to seasonal effects (Nagoshi and Meagher 2004a, Nagoshi et al. 2006).

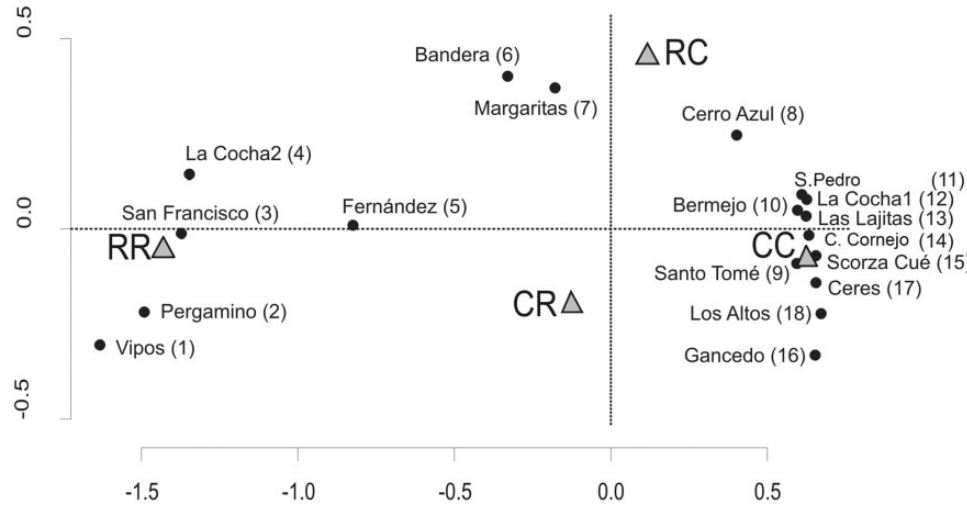

Fig. 5. Biplot of correspondence analysis between sampling sites and genotype configurations of fall armyworm according to the different marker combinations Numbers correspond to the ranks of sites calculated from their scores in the first axis.

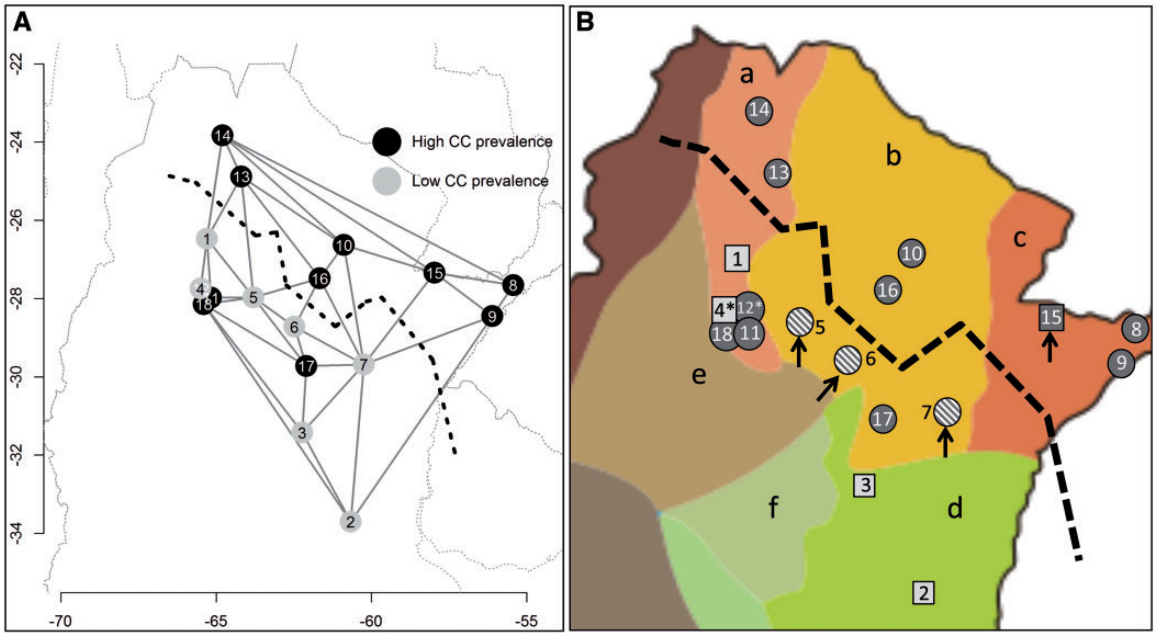

Fig. 6. Mapping of sampled localities with respect to genotypic configurations and climate zones. A: Delaunay's triangulation analysis applied on the sampled localities. Black dots: Collections dominated by genotypic configuration CC. Gray dots: Collections not dominated by CC. The bisector running from NW to SE segregates maximally both categories of populations. B: Bisector and sampled localities mapped relative to climate zones. Localities identified by rice-strain (square) or corn-strain (circle) plant host and whether have more than 70% RR (dark), more than 70% CC (light), or both RR and CC less than 70% (stripes). Climate zones are identified by color and number, and include subtropical mountain (1), subtropical with dry season (2), subtropical without dry season (3), temperate and humid pampas (4), arid hills and valley (5), and temperate mountain (6). Asterisks indicate collections from different plant hosts in the same location. Arrows indicate deviations from the expected strain frequency for the given host plant.

However, these incidents were relatively rare events, with the majority of collections displaying the strain-specific genetic markers consistent with the host plant. Similarly, the large majority of our collections in two years of surveys from Argentina, as well as in Brazil (Nagoshi et al. 2007b, Machado et al. 2008) confirm that the strain-specific host plant preferences previously described for U.S. populations are observed throughout the Western Hemisphere.

These conclusions differ from those of two other studies of Argentina fall armyworm populations that indicated a much weaker association between the strain

markers and host plant using *COI* (Juárez et al. 2012) or both *COI* and *Tpi* (Juárez et al. 2014). Most of the deviations reported involved rice as a host plant, which we note was also an exception in our study (Table 2). While the rice strain was originally detected on rice plants in Puerto Rico (Pashley et al. 1985) and was found to be the majority strain in a small number of rice fields sampled in Brazil (Nagoshi et al. 2007b, Machado et al. 2008), the number of such studies are limited, largely because major fall armyworm infestations in rice are infrequent. Therefore, it is possible that strain specificity with respect to rice may be more variable than originally thought. Furthermore, because Juarez et al. (2012) and Juarez et al (2014) both used laboratory colonies for their analyses, the observed marker frequencies even after only a single generation under artificial conditions may not accurately reflect that of the source population. Given these observations, it is possible that most, if not all, of the disagreement between these studies and our results can be explained by our analysis of field specimens and our greater use of pasture grasses to assess rice strain host preference.

The accurate assessment of fall armyworm host plant preferences has important implications on the appropriate use of Bt-expressing crops. Naturally arising resistance to some Bt-toxins has been reported in fall armyworm with the potential for significant economic impact (Storer et al. 2010, Farias et al. 2014). The primary strategy to delay the incidence of field resistance to Bt crops is the use of refuges where non-Bt host plants are grown capable of supporting an abundant susceptible pest population (Tabashnik et al. 2013). When Bt corn is grown intensively on large, homogeneous farms, the refuge requirement specifies conventional corn. However, refuges with alternative hosts are possible if Bt corn is grown in smaller farms with more diverse cropping systems, (Wu et al. 2002, Ravi et al. 2005). In Argentina, a study that did not take into account host strains found that corn, sorghum, alfalfa, Bermudagrass, and Guinea grass appeared to support a sufficient density of fall armyworm larvae to act as a potential Bt corn refuge (Murúa et al. 2009). However, our study indicates that the corn strain fall armyworm in Argentina is unlikely to consistently use alfalfa and pasture grasses (like Bermudagrass and Guinea grass) as a host, so these will not serve as effective refuges for this pest subpopulation.

A statistical analysis of the geographical distribution of the strain haplotype frequencies identified a bisecting line running northwest to southeast (Fig. 6B), with substantially higher RR proportions in the southern traps (46%) than in the north (1%). This was primarily due to the southern locations of the pasture grass and alfalfa collections sites that were predominantly rice strain (RR > 80%). Comparisons of the corn sites showed no differences between north and south; however, the sorghum collections showed 27% RR from the five southern sites compared to 0% in the two northern sites, suggesting a possibly higher rice-strain population south of the bisector. The geographical bisector broadly parallels the boundary between the mostly tropical and subtropical regions of northern Argentina with the more temperate zones in the south (Fig. 6B). However, 9 of the 11 collection sites lying south of the bisector, including the five sorghum sites, are in the same climate zones as the northern sites and should therefore share similar meteorological conditions. These data indicate that the geographical distribution of the two strains is primarily determined by the availability of their preferred host plants, with no definitive evidence of a more direct effect of climatic factors.

In summary, these results based on field-collected specimens confirm our previous conclusion using laboratory-reared colonies that the fall armyworm strains in Argentina generally show the same host plant preferences as observed in the United States. This represents the largest survey of wild fall armyworm in Argentina to date, and the consistency with two similar studies from Brazil (Nagoshi et al. 2007b, Machado et al. 2008) provides strong support that the host strains are present throughout the Western Hemisphere. The data also confirm that the *COI* and *Tpi* markers are accurate indicators of strain identity, either individually or together. There are small, but detectable, differences between the markers, most notably that *COI* tends to identify more rice strain than *Tpi*. But the differences are usually not large enough to alter the assessment of the majority strain for a given collection. Finally there are preliminary indications that the two fall armyworm strains may differ in their north–south distribution in Argentina, with the rice strain at higher densities in the southern regions. This probably reflects differences in the distribution of the relevant plant hosts. Additional surveys along the north–south axis comparing strain proportions in the same plant host would address this issue.

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