# BIOLOGICAL DIFFERENCES BETWEEN FIVE POPULATIONS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) COLLECTED FROM CORN IN MEXICO

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ABSTRACT

Biological characterizations of five fall armyworm populations, *Spodoptera frugiperda* (J. E. Smith) (FAW) collected from corn, Zea mays L., in Mexico, were reared and evaluated under laboratory conditions. The period from larvae to pupal stage, pupal weights, and survival rates were determined. The reproductive compatibility of adults, and the neonatal susceptibility to Endosulfan, Carbofuran and *Bacillus thur-*

*ingiensis* (Bt) were also evaluated. Populations from Aguascalientes, Colima, Nuevo León, Sinaloa, and Yucatán were reared on corn at 25°C. The Colima population reared on corn leaves required the least number of days to reach the pupal stage (13.04 D). Significant differences between the pupal weights of the different populations were found, ranging from 0.215 to 0.156 g. Survival rates varied from 80 to 45%, the Colima and Sinaloa populations had the highest survival. The Aguascalientes, Nuevo León and Yucatán populations were reproductively compatible as they produced progeny when paired. However, no progeny were obtained when the Colima and Sinaloa populations were paired with any other populations. The Aguascalientes, Nuevo León and Sinaloa populations tested for susceptibility to *B. thuringiensis* resulted in $LC_{50}$ values, from 0.001 to 0.045 mg/ml). The Aguascalientes and Yucatán populations showed similar susceptibility to Carbofuran and Endosulfan insecticide with an $LC_{50}$ ranging from 0.033 to 0.188 mg/ml, and 0.023 to 0.054 mg/ml, respectively. The Nuevo León population was the least susceptible. Results suggest that two corn FAW strains may have developed reproductive isolation due to geographic isolation. One strain formed by the Yucatán, Aguascalientes and Nuevo León populations, which are distributed along the Coastal Gulf and the geographic center of Mexico, and the other corn strain is formed by the Colima and Sinaloa populations found along the Mexican Pacific Coast, as the two strains produce no progeny when paired.

Key Words: *Spodoptera frugiperda*, reproductive compatibility, control, Mexican populations

RESUMEN

Cinco poblaciones de gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) se evaluaron en condiciones de laboratorio. Se determinaron el número de días a pupa, el peso de las pupas y su sobrevivencia. También se evaluaron la compatibilidad reproductiva de los adultos y la susceptibilidad a controles con insecticidas químicos y *Bacillus thuringiensis* (Bt). Las poblaciones de Aguascalientes, Colima, Nuevo León, Sinaloa y Yucatán se alimentaron con follaje de maíz y se mantuvieron a 25°C. La población de Colima fue la más precoz a la pupación (13.04 d). En cuanto al peso de pupa se encontraron diferencias significativas en un rango de 156 a 215 mg. El rango de sobrevivencia osciló del 45 al 80%, las poblaciones de Colima y Sinaloa presentaron la mayor sobrevivencia. Entre las poblaciones de Aguascalientes, Nuevo León y Yucatán aparentemente existió compatibilidad reproductiva; sin embargo, no se obtuvo descendencia cuando estas poblaciones se aparearon con Colima y Sinaloa, aparentemente no existió compatibilidad reproductiva entre Colima y Sinaloa, ya que al aparearlas tampoco produjeron progenie. Se determinó la susceptibilidad al Bt en las poblaciones de Aguascalientes, Nuevo León y Sinaloa, las CL50 obtenidas oscilaron entre 0.001 y 0.045 mg/ml. Las poblaciones de Aguascalientes y Yucatán presentaron resultados semejantes en cuanto a la susceptibilidad al Carbofuran y Endosulfan, con rangos en las EL50 de 0.033 a 0.188 y 0.023 a 0.054 mg/ml, respectivamente. La población de Nuevo León presentó los valores más bajos. Los resultados sugieren que existen 2 cepas o biotipos entre las poblaciones de gusano cogollero del maíz. Estas podrían haber desarrollado su aislamiento reproductivo tal vez, debido al aislamiento geográfico. Una cepa es formada por las poblaciones de Yucatán, Nuevo León y Aguascalientes localizadas en la Costa del Golfo de México y la segunda por Colima y Sinaloa, localizadas en la Costa del Océano Pacífico, ya que no produjeron progenie entre ellas.

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The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (FAW), is a tropical insect species that is endemic to the Western Hemisphere, where it ranges from Brazil

northward, throughout Central America and North America (Mitchell 1979a). It is considered a major pest of corn, rice, and forage grasses (Pashley 1989) and is found in almost all of Mexico's agricultural areas, causing its greatest damage in the southern and eastern tropical States (Andrews 1980). Crop losses caused by the FAW range from $300 to $500 million annually in the United States of America U.S.A. (Mitchell, 1979b).

The genetic structure of FAW populations and patterns of genetic similarity among samples from different regions can provide a better understanding of migratory pathways and assist researchers in developing improved pest management strategies (Pashley, 1986). Interestingly, the study of the movement of insects in North America has greatly increased during the last 10 years (Johnson 1987). The movements of noctuids have been the focus of much of this interest, because many species are highly mobile and cause serious damage to major food and forage crops over large areas (Rabb & Kennedy 1979; Johnson & Mason 1986). Long-range movements of FAW moths from the state Mississippi to Canada on a low-level jet stream were documented on one occasion using weather maps (Johnson 1987). Many FAW moths have been detected in the Gulf of Mexico as far as 250 km from land, indicating the possibility of seasonal trans-Gulf migration between the USA and the tropics (Johnson 1987). Alternating wet and dry seasons on the east and west coasts of Central America and elsewhere in the tropics makes only one habitat available at a time. These weather fluctuations have led to the establishment, maintenance and evolution of colonizing patterns of the FAW (Johnson 1987).

Pashley et al. (1995) reported physiological differences between FAW strains. She also detected significant differences in larval development, but potential fitness consequences of adults were not examined. Pashley (1988) reported that the strains may represent one of three types of taxa: they may be biotypes in which genetic differences are due to a selectively-mediated polymorphism within a single randomly-mating species; they may be host races in the initial stages of speciation in which interbreeding is reduced due to differences in host preference, or they may be sibling species that are either capable of hybridizing to a limited degree or completely reproductively isolated.

In Mexico the differences among corn FAW populations have not been determined. Furthermore, any possible relationship between Gulf Coast populations and populations inhabiting other geographic areas of Mexico has not been established. The purpose of our research is to present information about five different FAW populations found on corn within Mexico. Two of these populations (from the states of Yucatán and Nuevo León) are found along the Gulf Coast; one population (the state of Aguascalientes) is found in the geographical Center of the Mexican Republic and two populations (the states of Colima and Sinaloa) are located along the Pacific Coast. These populations were compared by determining the biological characteristics of larvae, their susceptibility to chemical and biological insecticides, and the potential for adult interbreeding.

## Materials and Methods

### Insect Origin

FAW larvae for this study were collected from corn plants from different Mexican geographic areas: the Gulf Coast (Yucatán and Nuevo León) (October, 1995); the Central Highlands (Aguascalientes) (August, 1995); and the Pacific Coast (Colima and Sinaloa) (March, 1995). Four random samples from 25 consecutive plants per row were taken per geographic area (Pair et al. 1986).

Insect Rearing

The larvae were taken from the field to the laboratory and placed into individual plastic containers (100 ml), containing freshly cut corn leaves. Larvae were fed on foliage of the "Ancho" cultivar of corn until pupation. Containers were placed in the dark for 24 h to minimize phototactic responses at 25 ± 1°C and > 50% relative humidity (Quisenberry 1991; Pashley et al. 1995; Lezama-Gutiérrez et al. 1996). Corn was planted at different dates to provide fresh foliage for the larvae during the experiment. Pupae were then sexed and each group was placed in 500 ml containers with coconut fiber moistened with sterile distilled water until the emergence of adults. Adults were then placed in 30 × 30 cm cages with paper towels as oviposition substrate (Burton & Perkins 1989) and fed with 10% honey-water solution (Poitout & Bues 1974). Egg masses were collected every 24 h and placed into containers which were lined with filter paper discs and moistened with sterile distilled water until larval emergence (Whitford et al. 1992). The second generation of neonatal larvae from each population was then used to determine the number of days required to reach the pupal stage. Pupal weight, susceptibility to Endosulfan, Carboufan and *Bacillus thuringiensis* Berliner, and survival rates were recorded for each FAW population.

Microorganism Culture

The *B. thuringiensis* Kenyae serovar 07 inoculum, obtained from the "Centre de Rèférence de l'Organisation Internationale de Lutte Biologique" (Pasteur Institute), consisted of a spore powder and was kept at 4°C. The inoculum was seeded into a liquid culture medium as described by Kalfon & De Barjac (1985). Dry powder obtained had $2.4 \times 10^8$ colony forming units per g.

Development Rate of FAW Populations

Groups of 50 neonatal larvae of each FAW population were individually placed into plastic containers (100 ml) containing two pieces freshly cut corn leaves. The leaves were cut into 2-cm-long segments and fed to the larvae every 24 h. The development period of each larval instar was recorded every 24 h until reaching the pupal stage. The pupae (obtained 24 h after pupation) were weighed by an analytical balance (Ohaus Model GA22) with a sensitivity of 0.001 g. Also, the survival rate of each population was determined from neonate larvae to adult emergence. The differences in the development period of the larval stage, the pupal weight, and the survival rate were statistically analyzed (ANOVA) and the means were separated using the Student-Newman-Keuls multiple range test.

Reproductive Compatibility Between FAW populations

Fifty pupae of each FAW population were sexed and each sex group was placed in plastic containers (100 ml) with coconut fiber moistened with sterile distilled water until the emergence of adults. Plastic containers with pupae were placed into other plastic pots (1000 ml). One male and one female from different FAW populations were paired in the plastic pots (as above) with paper towels as oviposition substrate (Burton & Perkins 1989) and fed with 10% honey-water solution (Poitout & Bues 1974). Ten adult females or 10 adult males of each population were mated as single-pairs in each test. Eggs were collected daily and placed into plastic containers which were lined with filter paper discs and moistened with sterile distilled water, until larva emergence (Whitford et al. 1992). The presence of progeny from each single-pair was recorded.

*B. thuringiensis* Bioassays

Susceptibility of FAW larvae from Aguascalientes, Colima, Nuevo León, and Sinaloa to *B. thuringiensis* Kenyae serotype 07 was determined by $LC_{50}$ values (Dulmage et al. 1976). *B. thuringiensis* dilution series were performed (0.003, 0.001, 0.0003 and 0.0001 mg/ml). Ten ml of sterile distilled water with Tween 0.1% of each dilution was taken. Fresh corn leaves were cut into segments (2 cm long) and inoculated by immersion for 30 s. After excess water evaporated, one piece of foliage was placed into a plastic petri dish (50 × 15 mm) lined with filter paper Whatman No. 1. The filter paper was moistened with sterile distilled water and 25 neonatal larvae per dose were used, in 2 replicates (Kalfon & De Barjac 1985; Hernández 1988). Petri dishes were placed at 25°C and kept dark for 24 h to minimize phototactic responses. Controls consisted of the same number of larvae from each FAW population fed foliage without *B. thuringiensis*. Mortality was recorded after 72 h and data were subjected to Probit analysis (Finney 1971). Maximum likelihood estimates of median lethal concentrations of *B. thuringiensis* for each FAW population were calculated. $LC_{50}$ values were expressed as mg/ml.

Insecticide Bioassay

Based on $LC_{50}$ values the susceptibility of the FAW neonatal larval populations from Aguascalientes, Nuevo León, and Yucatán to Carbofuran, and the FAW population from Aguascalientes and Yucatán to Endosulfan were determined (Leibee & Savage 1992). Five dilution series of each insecticide were prepared from 0.0000588 to 58.8 mg/ml of Carbofuran, and 0.0125 to 0.125 mg/ml of Endosulfan. Ten ml of sterile distilled water with Tween 0.1% of each dilution was taken. Fresh corn leaves were cut into segments (2 cm long) and inoculated by immersion for 30 s. After the excess water evaporated, one piece of foliage was placed into each plastic container (30 ml) and 5 neonatal larvae/container; 25 neonate per dose were used, in 2 replicates. Controls consisted of the same number of neonatal larvae from each FAW population fed foliage without insecticide. Mortality was recorded after 48 h and data were subjected to Probit analysis (Finney, 1971).

## RESULTS

Development Rate of FAW Populations

The development rate of FAW populations from neonate to pupal stage varied between 13.04 to 16.17 d. The Colima population developed significantly faster than the other 4 populations. The pupal weights among populations varied from 0.156 to 0.215 g. Aguascalientes pupae were significantly heavier than the other four populations; with the Yucatán and Colima pupae being the lightest. The survival rate of populations from neonate until adult emergence was the highest for the Colima and Sinaloa populations (80%) while the other populations ranged between 45 to 54% (Table 1).

Reproductive Compatibility Between FAW Populations

The reproductive capacity among the different populations showed that the Aguascalientes, Nuevo León and Yucatán populations were able to produce progeny when they were paired among themselves (Table 2). On the other hand, the Colima and Sinaloa populations would not cross with any of the other populations.

TABLE 1. DEVELOPMENT RATE, PUPAL WEIGHT AND SURVIVAL RATE OF FAW POPULATIONS GROWN ON EXCISED CORN LEAVES.

| FAW Populations | Number of Days to Pupation[1] | Pupal Weights (g)[1] | Survival Rate (%) |
|---|---|---|---|
| Aguascalientes | 14.20 a | 0.215 a | 54.00 |
| Colima | 13.04 b | 0.157 c | 80.00 |
| Nuevo León | 15.37 a | 0.168 bc | 54.00 |
| Sinaloa | 15.60 a | 0.178 b | 80.00 |
| Yucatán | 16.17 a | 0. 156 c | 45.00 |

[1]Means within a column followed by the same letter are not significantly different (P < 0.05, LSD).

Larval Susceptibility to *B. thuringiensis*

Larvae from the Nuevo León and Aguascalientes populations were the most susceptible to *B. thuringiensis* with a $LC_{50}$ range of between 0.001 to 0.005 mg/ml. The Sinaloa population had a significantly higher $LC_{50}$ than the Aguascalientes and Nuevo León populations (Table 3).

Larval Susceptibility to Insecticides

There were no significant differences among the three FAW populations from Aguascalientes, Nuevo León and Yucatán to Carbufuran. $LC_{50}$ values ranged from 0.033 to 1.36 (Table 4). Likewise, the Aguascalientes and Nuevo León populations resulted in a similar susceptibility to Endosulfan (Table 5).

## DISCUSSION

Pashley (1986) reported that the FAW is composed of two genetically different strains, each of which exhibits different host specificity; one feeds on corn (genotype ABAA) and another on rice and Bermuda grass (genotype BABB). Also, no significant genotypic differences exist within the corn strain. Our results showed that, based on the development rate, the period from neonate to pupae was different between some

TABLE 2. REPRODUCTIVE COMPATIBILITY AMONG FAW POPULATIONS.

| FAW Populations | Aguascalientes | Colima | Nuevo León | Sinaloa | Yucatán |
|---|---|---|---|---|---|
| Aguascalientes | + | - | + | - | + |
| Colima | - | + | - | - | - |
| Nuevo León | + | - | + | - | + |
| Sinaloa | - | - | - | + | - |
| Yucatán | + | - | + | - | + |

+ = Progeny obtained; - = No progeny obtained.

TABLE 3. $LC_{50}$ S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAL POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF *B. THURINGIENSIS* SEROVAR *KENYAE* 07.

| FAW Population | $LC_{50}$ | 95% FL | Intercept | Slope |
|---|---|---|---|---|
| Aguascalientes | 0.005 | 0.043 to 0.003 | 5.27 | 0.90 |
| Nuevo León | 0.001 | 0.005 to 0.0001 | 4.56 | 0.41 |
| Sinaloa | 0.045 | 0.056 to 0.037 | 3.38 | 2.49 |

of the corn FAW populations. The Colima population required the fewest number of days to pupation (13.4 days). Results showed that the corn foliage used to feed the larvae produced the same growth rate for all of the larval populations except for Colima, which is apparently adapted to consume this corn genotype. Isenhour et al. (1985) reported that the number of days to pupation for their FAW larvae population at 25°C ranged from 21.2 to 28.4 days when fed on corn foliage.

The pupal weight obtained from the different Mexican FAW populations showed statistical differences with ranges from 0.156 to 0.215 g. The Aguascalientes FAW population had the heaviest pupae, while the Colima and Yucatán populations resulted in the smallest pupae. Similar results were reported by Isenhour et al. (1985) for FAW larvae fed corn foliage and by Whitford et al. (1992) to larvae fed artificial diets.

The survival rate obtained from the different FAW populations showed the Colima and Sinaloa populations to be the highest. There was no relationship detected between development rate, the pupal weights, or survival rates for the 5 FAW populations studied.

The reproductive compatibility among the different corn FAW populations can be separated into two, or possibly 3, population groups. The Aguascalientes, Nuevo León and Yucatán populations were able to produce progeny when mated among these 3 populations. However the Colima and Sinaloa populations were unable to produce progeny when mated with the other three populations or with each other. Therefore, there is a good probability of seasonal trans-Gulf migration of these Aguascalientes, Nuevo León and Yucatán populations of FAW between the United States and the tropics (Johnson, 1987). These results support the hypothesis that, two or possibly 3 corn FAW strains have developed because of their geographic isolation relative to each other. Pashley (1989) reports only one corn genotype for the populations in Central America, the Caribbean, and the Southeastern USA. However, our results show that one corn FAW strain is comprised of FAW populations from Yucatán, Aguascalientes and Nuevo León and the other corn FAW strains are found in Colima and Sinaloa, along the Mexican Pacific Coast. Although Aguascalientes is more centrally located

TABLE 4. $LC_{50}$ S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAL POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF CARBOJUAN.

| FAW Population | $LC_{50}$ | 95% FL | Intercept | Slope |
|---|---|---|---|---|
| Aguascalientes | 0.033 | 0.071 to 0.016 | 4.53 | 0.90 |
| Nuevo León | 1.36 | 7.778 to 0.238 | 3.99 | 0.47 |
| Yucatán | 0.188 | 0.300 to 0.096 | 3.4 | 1.26 |

TABLE 5. $LC_{50}$ S (MG/ML) AND PROBIT ANALYSIS STATISTICS OF MORTALITY DATA FOR FAW NEONATE LARVAE POPULATIONS WHEN EXPOSED TO DIFFERENT DOSES OF ENDOSULFAN.

| FAW Population | $LC_{50}$ | 95% CL | Intercept | Slope |
|---|---|---|---|---|
| Aguascalientes | 0.023 | 0.29 to 0.018 | 4.07 | 2.65 |
| Yucatán | 0.054 | 0.061 to 0.048 | 1.58 | 4.66 |

and separated from the Gulf Coast states of Nuevo León and Yucatán by the Sierra Madre Oriental, Aguacalientes is on the eastern side of the Sierra Madre Occidental. The Sinaloa and Colima populations are separated from the other populations by the Sierra Madre Occidental and separated from each other by the Eje Neo Volcanico mountains which reach the Pacific coast in the state of Jalisco.

Our results showed that the Nuevo León and Aguascalientes populations were more susceptible to *B. thuringiensis* than the Sinaloa population. Our results showed that the Nuevo León and Aguascalientes populations have a similar susceptibility to *B. thuringiensis* as the Colima population (Hernandez 1988). These results confirm observations by Hernández (1988) that the *B. thuringiensis* serovar *Kenyae* is highly active against *S. frugiperda* larvae. The FAW larvae from the Sinaloa population, however, were less susceptible to the bacteria. These observations corroborate the findings that the *S. frugiperda* population from Sinaloa may be different from the other Mexican FAW populations.

Based on insecticide susceptibility, the Aguascalientes, Nuevo León and Yucatán populations studied were susceptible to Carbofuran, but the Nuevo León population was the least susceptible. Both Aguascalientes, and Yucatán populations resulted in a similar susceptibility to Endosulfan. More research is needed to determine the differences in susceptibility between the Pacific Coast and Gulf Coast FAW populations.

## ENDNOTE

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