DOI: 10.1111/j.1570-7458.2010.01033.x

Searching for pheromone strains in the pecan nut casebearer

Emilie A. Hartfield, Marvin K. Harris & Raul F. Medina*
Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843, USA

Accepted: 3 June 2010

Key words: AFLP, *Acrobasis nuxvorella*, pecan, sex pheromone, Lepidoptera, Pyralidae, Juglandaceae, *Carya illinoinensis*, monitoring program, genetic isolation, assortative mating, pherotypes

Abstract

The pecan nut casebearer, *Acrobasis nuxvorella* Nuenzig (Lepidoptera: Pyralidae), is the most damaging insect pest of pecan, *Carya illinoinensis* (Wang) K. Koch (Juglandaceae), in the USA and Mexico. A pheromone monitoring program for *A. nuxvorella* has been developed to assist pecan growers in the timing of insecticide applications. The discovery that there are two pheromone types produced by *A. nuxvorella* has led to complications in the implementation of pheromone monitoring programs. One pheromone (referred to as standard) is attractive to moths in the southern USA, but not in Mexico. The other pheromone (referred to as Mexican) is attractive to moths in the southern USA and in Mexico. Because most male lepidopterans respond only to a specific pheromone, it was suspected that there were two pheromone strains of *A. nuxvorella*, one exclusively present in the northern distribution of *A. nuxvorella* (USA strain) and the other widely distributed from Sonora, Chihuahua, and Durango in northern Mexico to Texas, Georgia, and Oklahoma in the USA (Mexican strain). The goal of this research was to determine whether differences in pheromone response are sufficient to genetically isolate *A. nuxvorella* into pheromone strains. To confirm the existence of the two alleged pheromone strains, amplified fragment length polymorphism (AFLP) markers were obtained and analyzed. Four primer combinations were used to obtain a total of 483 polymorphic AFLP markers. Our results indicated that the standard and Mexican pheromones did not group *A. nuxvorella* into pheromone strains. However, three genetically distinct populations of *A. nuxvorella* were identified. Two of those three populations are sympatric throughout the southern USA and one is allopatric relative to the other two and occurs exclusively in pecan growing regions of Mexico.

Introduction

The pecan nut casebearer, *Acrobasis nuxvorella* Nuenzig (Lepidoptera: Pyralidae), is the most damaging pest of pecan, *Carya illinoinensis* (Wang) K. Koch (Juglandaceae), in the southern USA and Mexico (Harris et al., 1998). Damage to pecans is a result of *A. nuxvorella* larvae burrowing into and feeding on pecan nutlets. *Acrobasis nuxvorella* is mutivoltine, having typically 3–4 generations per year, depending on climate and geographic location (Neunzig, 1972). The first-generation larvae are more destructive to pecan crops due to the small size of the nutlets on which they are feeding. These first-generation larvae are capable of consuming 3–4 nutlets in a cluster (Ring & Harris, 1984). The larvae of subsequent generations require fewer nutlets to complete development and thus are less damaging to the crop. *Acrobasis nuxvorella* is monophagous on pecan.

Pecan is indigenous to the alluvial plains of the south-central USA and mountain valleys of Mexico (Harris, 1983). Native pecan in Mexico is geographically isolated from plantings in the USA (Harris et al., 2008). *Acrobasis nuxvorella* is distributed throughout the pecan growing regions east of the Rocky Mountains in North America (Figure 1). The range of this pest reaches from Florida west to New Mexico in the USA and as far west as Chihuahua in Mexico (Harris, 1983). *Acrobasis nuxvorella* has recently expanded to pecan growing regions in Sonora, Mexico (Fu Castillo et al., 2005; Harris et al., 2008).

*Correspondence: E-mail: rfmedina@tamu.edu

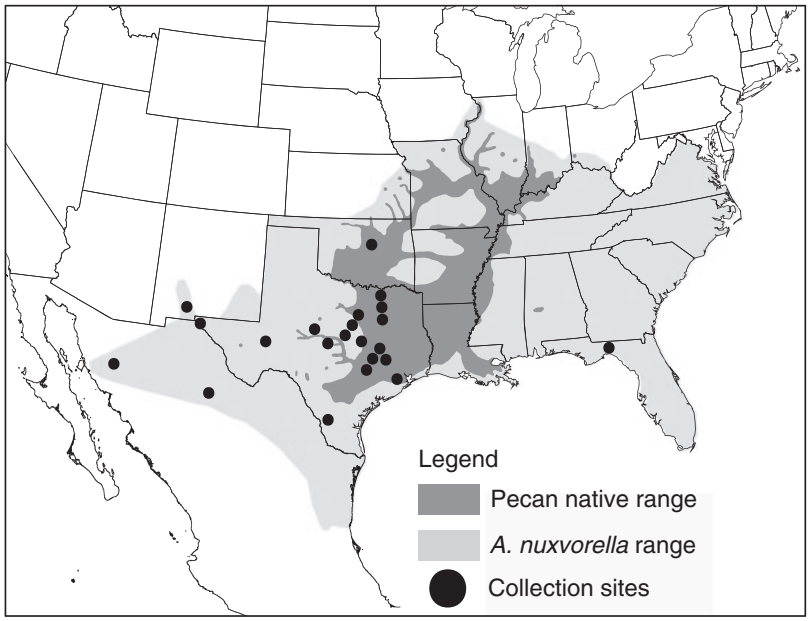

Figure 1 Native pecan distribution, range of *Acrobasis nuxvorella*, and collection sites. Pecan is native to the alluvial plains of the south-central USA and mountain valleys of Mexico, but has expanded due to cultivation. *Acrobasis nuxvorella* is present in pecan growing regions in the USA east of the Rocky Mountains and in pecan growing regions in northern Mexico.

The current IPM strategy for controlling *A. nuxvorella* consists of monitoring for this pest with sex pheromone baited sticky traps and using a degree-day prediction model (Stevenson et al., 2003) combined with a sequential sampling plan (Ring et al., 1989) to ensure only needed insecticide treatments are applied at the correct time. These strategies have been helpful in timing pesticide applications, allowing for more efficient pesticide use. Chemical control against *A. nuxvorella*, when needed, targets hatching first instars to prevent nut entry and to kill them before they gain access to a refuge inside the pecan nut. The current IPM strategic plan for pecan has significantly reduced the amount of pesticides used to control *A. nuxvorella* compared to when calendar sprays were used to control this pest (Stevenson et al., 2003). This reduced pesticide use was achieved without a reduction of crop quality or yield and has conserved natural enemies to more effectively manage foliar pecan pests, such as aphids, mites, and leaf miners, that occur later in the pecan growing season (Harris et al., 1998).

Two synthetic sex pheromones have been developed and are currently used to detect the flight of *A. nuxvorella* adults (Millar et al., 1996; Harris et al., 2008). The first pheromone developed (henceforth standard pheromone) was identified to be a single aldehyde (9*E*,11*Z*)-hexadecadienal (9*E*,11*Z*-16:Ald) (Millar et al., 1996). This pheromone has been used to attract male *A. nuxvorella* in the USA for over 10 years, but interestingly, the standard pheromone does not attract *A. nuxvorella* male moths in Mexico, even though *A. nuxvorella* is known to be present in Mexico (Fu Castillo et al., 2005; Harris et al., 2008). Due to the failure of the standard pheromone to attract *A. nuxvorella* in Mexico, the second *A. nuxvorella* pheromone (henceforth Mexican pheromone) was developed (Harris et al., 2008). The Mexican pheromone was identified as 1:1 (9*E*,11*Z*)-hexadecadien-1-yl acetate (9*E*,11*Z*-16:Ac):(9*E*,11*Z*-16:Ald). This pheromone attracts moths in the USA and in Mexico. The discovery that there are two sex pheromones able to attract *A. nuxvorella* (Millar et al., 1996; Harris et al., 2008) has improved the pheromone monitoring program currently in place for this pest.

Sex pheromones are used by many insects to facilitate mate location and recognition (Löfstedt, 1993). In the majority of moth species, it is the female that produces the chemical signal and the male that responds. Sex pheromones are the primary source of communication among mating moths (Tamaki, 1985). The responder is capable of distinguishing among pheromones and is attracted to a very specific chemical signal produced by a conspecific female (Svensson, 1996). There are several examples of lepidopterans that have polymorphic sex pheromones within the species. A thoroughly studied example is *Ostrinia nubilalis* Hübner: females of one pheromone race produce and males respond to a 3:97 E/Z-11-tetradecenyl acetate pheromone blend, whereas in a second pheromone race, females produce and males respond to a 99:1 E/Z

pheromone blend (Klun et al., 1973; Kochansky et al., 1975). Studies have found that *O. nubilalis* pheromone production and response can be attributed to insect genetics (Klun & Maini, 1979; Dopman et al., 2004) and to host plant use (Pelozuelo et al., 2004). Likewise, pheromone races in *Spodoptera frugiperda* JE Smith are linked to host plant preference (Groot et al., 2008). In contrast, an aberrant pheromone blend resulting from a single autosomal gene mutation was discovered in a laboratory population of *Trichoplusia ni* Hübner in which mutant females were unattractive to normal males (Hunt & Haynes, 1990). Two distinct pheromone blends and one intermediate pheromone blend were discovered for *Hemileuca eglanterina* Boisduval (McElfresh & Millar, 2001). It is believed that these two distinct blends are a result of reproductive character displacement and that the intermediate blend is a result of mating between the two pheromone races where they occur in sympatry (McElfresh & Millar, 2001). Other examples of moth species that exhibit polymorphic pheromone production include *Agrotis segetum* Denis & Schiffermüller (Löfstedt et al., 1986; Hannson et al., 1990), *Argyrotaenia velutinana* Walker (Miller & Roelofs, 1980), and *Pectinophora gossypiella* Saunders (Collins & Cardé, 1985). Each of these cases offers a unique angle to learn more about the evolution of chemical signaling in Lepidoptera.

Löfstedt (1990) hypothesized that aberrant pheromone blends in lepidopteran populations are maintained due to the existence of males that are able to respond to a wide range of pheromone blends. Additionally, it is thought that the specificity of sex pheromones can lead to reproductive isolation among lepidopteran populations (Roelofs & Comeau, 1969); however, few studies have tested this hypothesis in the field.

The objective of this study was to determine the presence or absence of reproductive isolation that would result from assortative mating between the postulated pheromone strains of *A. nuxvorella* over many generations. This will allow us to determine whether pheromone strains are present within this species. Amplified fragment length polymorphism (AFLP) markers were used to assess the existence of pheromone strains in this insect (Vos et al., 1995). Amplified fragment length polymorphism markers have been successfully used to confirm the existence of biotypes or strains in other insect species (Cervera et al., 2000; Salvato et al., 2002; Zhu-Salzman et al., 2003; Busato et al., 2004; Zhang et al., 2005). The data presented in this study are the first genetic data available for this pest species. We hypothesized that *A. nuxvorella* pheromone strains (i.e., standard and Mexican) were reproductively isolated, thus genotypic differences were expected between the different pherotypes (i.e., conspecific males that show differential response to pheromone blends) (Zada et al., 2008) at each of our study sites.

Materials and methods

Amplified fragment length polymorphism markers (Vos et al., 1995) were used to detect differences in the genotypes of male *A. nuxvorella* that responded to different sex pheromones. Amplified fragment length polymorphism markers were chosen because they are capable of surveying the whole genome of an organism when no prior genetic information is available. Additionally, they can be obtained fairly inexpensively and they produce many markers in a relatively short period of time (Pejic et al., 1998; Althoff et al., 2007).

Specimen collection

Acrobasis nuxvorella used for this study were collected by peer and producer cooperators from pheromone-baited sticky traps (Trece, Adair, OK, USA) from 21 locations in the southern USA and Mexico (Figure 1). Each trap was baited with a rubber septum impregnated with 100 μg of synthetic pheromone. At each of the locations in the USA and Mexico, 3–6 Trece Pherocon III™ sticky traps baited with synthetic standard pheromone lures and 3–6 sticky traps baited with synthetic Mexican pheromone lures were used to capture male moths. Traps were placed in pecan trees 2 m above the ground and at least 50 m from other traps. Trapped male moths were transferred to microcentrifuge tubes with 70% alcohol at the collection locations. Each cooperator collected 10 random specimens from each of the two pheromone trap types. The specimens were then sent to Texas A&M University in College Station (TX, USA) and stored at −80 °C until used for genetic analysis. Samples were collected for two consecutive years (between the months of April and September of 2007 and 2008). A total of 181 specimens were selected at random for AFLP analysis.

DNA extraction

Microcentrifuge tubes containing *A. nuxvorella* male individuals selected for molecular analyses were taken out of −80 °C storage and placed in liquid nitrogen. Entire individuals were frozen in liquid nitrogen and crushed to start DNA extraction procedures. Only a sub-sample of specimens collected per each pheromone at each of the study sites were used for molecular marker studies and the remaining specimens were kept as vouchers. DNA was extracted using Qiagen DNEasy Tissue Kit (Qiagen, Valencia, CA, USA) following Qiagen recommended protocol (Qiagen, 2002). The quantity (ng of nucleic acid per μl of

solution) and quality (ratio of sample absorbance at 260 and 280 nm) of DNA was measured using a NanoDrop® spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

AFLP development

Amplified fragment length polymorphism markers were used to determine genetic differentiation among *A. nuxvorella* from different presumed pheromone strains in our 21 locations. Digestion of genomic DNA by the restriction enzymes *Eco*RI and *Mse*I and ligation of oligonucleotide adaptors compatible with these endonucleases (Applied Biosystems, Foster City, CA, USA) were accomplished in a single reaction mixture. Each reaction aliquot contained approximately 605 ng of template DNA. Pre-selective polymerase chain reaction (PCR) amplification was performed using the Applied Biosystems AFLP Preselective Mix (Applied Biosystems). The PCR program for preselective amplification was: 95 °C for 1 min followed by 20 cycles of 95 °C for 10 s, 56 °C for 30 s, and 72 °C for 90 s, with a final hold at 75 °C for 5 min. The amplified product was then diluted 20-fold using 15 nM Tris–HCl buffer (pH 8.0) containing 0.1 mM EDTA. For the selective amplification of restriction fragments, already prepared custom primers for recognition of *Eco*RI and *Mse*I adaptors were used. The four primer pairs selected were M-CAT and E-ACT, M-CTC and E-AAC, M-CAC and E-ACG, and M-CAA and E-ACT. Fragments were visualized by attaching a fluorescent dye to the 5′ end of each *Eco*RI selective amplification primer with no modification made to the *Mse*I primer. The PCR program for the selective amplification process consisted of an initial warm-up at 95 °C for 30 s, 12 cycles of 95 °C for 10 s, 65 °C for 40 s with a lowering of 0.7 °C per cycle, 72 °C for 90 s, followed by 35 cycles of 95 °C for 11 s, 56 °C for 40 s, 72 °C for 90 s, and finally a hold of 75 °C for 5 min before storing the samples at 4 °C.

Data analysis

Amplified fragment length polymorphism markers were separated by capillary electrophoresis in a 3100 Genetic Analyzer from Applied Biosystems and analyzed using GeneMapper software (Applied Biosystems, 2005). Markers with a dye signal larger than 100 luminescent units were considered as present. Each AFLP marker was considered a locus and was assumed to have two possible alleles (represented in binary form, i.e., 0 for no allele present and 1 for a present allele). The number of specimens and primer combinations necessary to detect genetic differences in this study was assessed using SESim values (Medina et al., 2006). The four primer pairs mentioned above were considered sufficient based on a SESim <0.05.

The possibility of two pheromone strains of *A. nuxvorella* was investigated using STRUCTURE (Pritchard et al., 2000; Falush et al., 2007). STRUCTURE is a Bayesian inference method that uses a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into populations and measures the probability that an individual is a member of different populations based on genotypic data (Pritchard et al., 2000; Falush et al., 2007). The STRUCTURE burn-in period was fixed at 10 000 with a run length of 10 000 under the admixture model with correlated and uncorrelated allele frequencies. To determine the number of populations present in the data (K), 20 replications were completed for each K value between 1 and 5. The number of populations (K) indicated by STRUCTURE was verified using the ΔK method of Evanno et al. (2005).

Results

A total of 181 individuals from 21 locations were used for AFLP analyses. The average concentration of DNA for the 181 specimens was 110 ng of nucleic acid per µl of solution. The average quality of DNA samples as measured by the ratio of light absorbance at 260 and 280 nm was 2.00. The four primer combinations used yielded 483 polymorphic AFLP markers. This number of individuals and AFLP markers resulted in a SESim value of 0.0262. A SESim value of <0.05 indicates that the sample size of individuals and genetic markers is sufficient to accurately capture the genetic variability of the two postulated strains of *A. nuxvorella* at the geographic scale of this study (Medina et al., 2006).

When the Evanno ΔK method (Evanno et al., 2005) was used to predict the number of populations generated by STRUCTURE, the results indicated that there were three distinct populations under the admixture and correlated allele frequencies model. The first cluster (MEX) consists of only the individuals collected from pheromone traps in Mexico (Figure 2). Due to the inability of the standard pheromone to attract *A. nuxvorella* males in Mexico, all of the individuals in the first cluster (i.e., MEX) were captured using the Mexican pheromone only. The second (US 1) and third (US 2) clusters contained all of the individuals collected in pheromone traps in the USA. The second cluster (US 1) contains individuals captured in pheromone traps baited with the Mexican pheromone and individuals captured in pheromone traps baited with the standard pheromone from each of the collection sites in the USA. Similarly, the third cluster (US 2) contains individuals captured in pheromone traps baited with the Mexican pheromone and individuals captured in pheromone traps baited with the standard pheromone from each of the USA col-

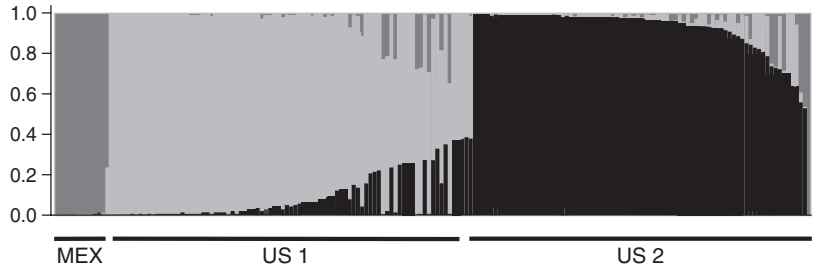

Figure 2 Results of STRUCTURE (Pritchard et al., 2000). Each vertical line on the x-axis represents one individual. The y-axis corresponds to the probability that an individual is a member of a particular population: dark gray (Mexican population), light gray (US population 1), and black (US population 2). The most likely number of clusters was 3 according to the ΔK method of Evanno et al. (2005).

lection sites. Thirteen markers were only present in individuals belonging to the Mexican cluster and absent in all individuals in the two USA clusters.

Discussion

The results of this study indicate that there is no evidence supporting pheromone strain formation among populations of *A. nuxvorella*. There was, however, a distinct difference between *A. nuxvorella* captured in Mexico and the USA. The westward expansion of the pecan industry in the USA and Mexico has recently been followed by the expansion of *A. nuxvorella* into areas where pecan is not indigenous, but has been cultivated since the late 1800s (Brison, 1974). This pest was found infesting pecan plantings in the El Paso Valley of Far West Texas for the first time in 1988 (Harris et al., 1988). *Acrobasis nuxvorella* is autochthonous in some regions of Mexico and expanded to pecan plantings in the Mexican state of Sonora as recently as 2001 (Fu Castillo et al., 2005). Most likely this westward expansion of *A. nuxvorella* is a result of the importation of infested plant materials from Chihuahua into these areas. Genetic differences between the USA and Mexican moths are most likely due to the continued geographic isolation of pecan in Mexico from those in the USA.

The absence of the standard pheromone in Mexico may be attributed to the fact that the sugarcane borer, *Diatraea saccharalis* Fabricius, also produces the same sex pheromone (9*E*,11Z-16:Ald) (Santangelo et al., 2002). It is possible that *A. nuxvorella* in Mexico are unresponsive to this pheromone as a mechanism to avoid cross attraction between the two species. If male *A. nuxvorella* were attracted to female *D. saccharalis*, the result would be wasted reproductive effort for those individuals attracted to incompatible females.

Diatraea saccharalis is one of the most damaging pests of corn in Mexico (Maredia & Mihm, 1991). Corn is grown in every state in Mexico, thus there is considerable spatial overlap between pecan growing regions and corn growing regions in Mexico leading to *A. nuxvorella* and *D. saccharalis* occurring in sympatry. In Mexico, there is also a great deal of temporal overlap between *A. nuxvorella* and *D. saccharalis*. The adults of the first generation of *A. nuxvorella* begin emerging in late April and the flight of the final generation ends in early September (Fu Castillo et al., 2005). In northern Mexico, *D. saccharalis* adults begin emerging in March and subsequent generations continue their flights into September (Rodriguez Del Bosque et al., 1995).

Diatraea saccharalis is also a major pest of corn, rice, and sugarcane in the USA, but it is only a pest in the warmer parts of the Gulf Coast States (i.e., Florida, Louisiana, and Texas) (Capinera, 2007). *Diatraea saccharalis* adults begin emerging in April or May in the Gulf Coast States and subsequent generations continue flight into the autumn months (Capinera, 2007). In the USA, *A. nuxvorella* adults begin flying in late April and subsequent generations continue into October (Bilsing, 1926; Stevenson et al., 2003; MK Harris, unpubl.). Thus, although there is a temporal overlap between *D. saccaralis* and *A. nuxvorella* in the USA, *A. nuxvorella* in the USA are somehow able to maintain the integrity of both chemical signals, probably because the spatial overlap of these two pest species is limited to the Gulf Coast Region.

The two sympatric USA populations of *A. nuxvorella* (Figure 2) are both comprised of individuals captured at each location with each of the sex pheromones. This is an indication that male *A. nuxvorella* in the USA are able to recognize and respond to both of the pheromones produced by *A. nuxvorella* in the USA. This widened response by moths in the USA can be explained by Phelan's asymmetric tracking hypothesis which states that male moths will be most sensitive to the most common pheromone present within a population, but that male response should be wide enough to recognize potential mates of the same species, even if the calling female belongs to a different

pheromone race (Phelan, 1992). If this is the case for *A. nuxvorella*, it is likely that there is a pheromone polymorphism in females, but there is not a response polymorphism in males.

Liu & Haynes (1994) showed that *T. ni* males from what they refer to as mutant sex pheromone producing colonies preferred females that produced normal sex pheromones. After 49 generations the males from mutant colonies displayed a widening response that included both pheromones and would respond to normal and mutant pheromones with equal frequency. Another example of a wide response to sex pheromones is exhibited between *O. nubilalis* and its congener *Ostrinia furnacalis* Guenée. These two species have similar pheromone blends and showed cross attraction between species when tested in a wind tunnel (Linn et al., 2003, 2006).

Alternatively, it is possible to have two pherotypes within the same species that are not reproductively isolated, and there are examples of this in the Lepidoptera (Liu & Haynes, 1994; McElfresh & Millar, 2001). The two pherotypes of *A. nuxvorella* could be two different phenotypes (much the same as color or size) that although explained by differences in one or few genes, do not lead to reproductively isolate the two *A. nuxvorella* pherotypes into distinct populations. Thus, it is possible that in Mexico, *A. nuxvorella* respond to the Mexican pheromone because genes associated with recognition of the Mexican pheromone were selected for while genes responsible for the response to the standard pheromone were selected against, possibly because of signal competition from *D. saccharalis*. In the USA, moths are attracted to both the standard pheromone and the Mexican pheromone perhaps because they experience less selective interference from *D. saccharalis*.

Not much is currently known about the mating behavior of *A. nuxvorella*, and the question still remains as to why male *A. nuxvorella* in the USA are responsive to the Mexican and standard pheromones while in Mexico the standard pheromone seems to be absent. It is possible that the Mexican pheromone evolved long ago to reduce interspecific competition between *A. nuxvorella* and *D. saccharalis*. Further research is needed in order to explain the existence of the two characterized *A. nuxvorella* pherotypes.

Acknowledgements

We would like to thank all of the pecan producers and peer cooperators that collected samples for genetic analyses. We also thank Allen Dean for assisting with this project. We are grateful to the two anonymous reviewers for their insight that helped to improve this manuscript. This research was funded By USDA-CSREES award 2007-34103-18109.

References

Althoff DM, Gitzendanner MA & Segraves KA (2007) The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. Systematic Biology 56: 477–484.

Applied Biosystems (2005) GeneMapper Computer Program, Version 4.0. Applied Biosystems, Foster City, CA, USA.

Bilsing SW (1926) The life history and control of the Pecan nut casebearer (*Acrobasis caryae*). Texas Agricultural Experiment Station Bulletin 328. The Texas A&M University System, College Station, TX, USA.

Brison FR (1974) Pecan Culture. Capital Printing, Austin, TX, USA.

Busato GR, Grutzmacher AD, De Oliveira AC, Vieira EA, Zimmer PD et al. (2004) Analysis of the molecular structure and diversity of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) populations associated to the corn and rice crops in Rio Grande do Sul State, Brazil. Neotropical Entomology 33: 709–716.

Capinera JL (2007) Sugarcane borer, *Diatraea saccharalis* (Fabricius) (Insecta: Lepidoptera: Pyralidae). Florida Cooperative Extension Service Bulletin EENY-217. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, USA.

Cervera MT, Cabezas JA, Simon B, Martinez-Zapater JM, Beitia F & Cenis JL (2000) Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. Bulletin of Entomological Research 90: 391–396.

Collins JT & Cardé RT (1985) Variation in and heritability of aspects of pheromone production in the pink bollworm moth, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Annals of the Entomological Society of America 78: 229–234.

Dopman EB, Bogdanowicz SM & Harrison RG (2004) Genetic mapping of sexual isolation between E and Z pheromone strains of the European corn borer (*Ostrinia nubilalis*). Genetics 167: 301–309.

Evanno G, Regnaut S & Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Falush D, Stephens M & Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7: 574–578.

Fu Castillo A, Harris M, Grageda J, Nava Camberos U & Villa B (2005) Evaluación demétodos de pronó stico para control del ''Gusano barrenador de la nuez'' *Acrobasis nuxvorella* en la Costa de Hermosillo, Sonora presented to Congress international in Mexicali, Baja California Norte. Memoria Tecnica 19: 52–60.

Groot AT, Marr M, Schöfl G, Lorenz S, Svatos A & Heckel DG (2008) Host strain specific pheromone variation in *Spodoptera frugiperda*. Frontiers in Zoology 5: 20.

Hansson BS, Tóth M, Löfstedt C, Szöcs G, Subchev M & Löfqvist J (1990) Pheromone variation among eastern European and a western Asian population of the turnip moth *Agrotis segetum*. Journal of Chemical Ecology 16: 1611–1622.

Harris MK (1983) Integrated pest management of pecans. Annual Review of Entomology 28: 291–318.

Harris MK, Jackman JA & Ring DR (1988) Sound management vital to minimize impact of pecan nut casebearer. Pecan South Including Pecan Quarterly 22: 4–6.

Harris MK, Ree B, Cooper J, Jackman JA, Young J et al. (1998) Economic impact of pecan integrated pest management implementation in Texas. Journal of Economic Entomology 91: 1011–1020.

Harris MK, Fu AAA, Nunez H, Aranda-Herrera E, Moreira JA et al. (2008) A new pheromone race of *Acrobasis nuxvorella* (Lepidoptera: Pyralidae). Journal of Economic Entomology 101: 769–776.

Hunt RE & Haynes KF (1990) Periodicity in the quantity and blend ratios of pheromone components in glands and volatile emissions of mutant and normal cabbage looper moths, *Trichoplusia ni*. Journal of Insect Physiology 36: 769–774.

Klun JA & Maini S (1979) Genetic basis of an insect chemical communication system: The European corn borer. Environmental Entomology 8: 423–426.

Klun JA, Chapman OL, Mattes KC, Wojtkowski PW, Beroza M & Sonnet PE (1973) Insect sex pheromone: minor amount of opposite geometrical isomer critical to attraction. Science 181: 661–663.

Kochansky J, Cardé RT, Liebherr J & Roelofs WL (1975) Sex pheromone of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae) in New York. Journal of Chemical Ecology 1: 225–231.

Linn CE Jr, O'Connor M & Roelofs WL (2003) Silent genes and rare males: a fresh look at pheromone blend response specificity in the European corn borer moth, *Ostrinia nubilalis*. Journal of Insect Science 3: 15.

Linn CE Jr, Musto CJ & Roelofs WL (2006) More rare males in *Ostrinia*: response of Asian corn borer moths to the sex pheromone of the European corn borer. Journal of Chemical Ecology 33: 199–212.

Liu YB & Haynes KF (1994) Evolution of behavioral responses to sex pheromone in mutant laboratory colonies of *Trichoplusia ni*. Journal of Chemical Ecology 20: 231–238.

Löfstedt C (1990) Population variation and genetic control of pheromone communication systems in moths. Entomologia Experimentalis et Applicata 54: 199–218.

Löfstedt C (1993) Moth pheromone genetics and evolution. Philosophical Transactions of the Royal Society of London, Series B: Biological Science 340: 167–177.

Löfstedt C, Löfquist J, Lanne BS, Van Der Peers JNC & Hansson BS (1986) Pheromone dialects in European turnip moths *Agrotis segetum*. Oikos 46: 250–257.

Maredia KM & Mihm JA (1991) Sugarcane borer (Lepidoptera: Pyralidae) damage to maize at four plant growth stages. Environmental Entomology 20: 1019–1023.

McElfresh JS & Millar JG (2001) Geographic variation in the pheromone system of the saturnid moth *Hemileuca eglanterina*. Ecology 82: 3505–3518.

Medina RF, Barbosa P, Christman M & Battisti A (2006) Number of individuals and molecular markers to use in genetic differentiation studies. Molecular Ecology Notes 6: 1010–1013.

Millar JG, Knudson AE, McElfresh JS, Gries R, Gries G & Davis JH (1996) Sex attractant pheromone of the pecan nut casebearer (Lepidoptera: Pyralidae). Bioorganic and Medicinal Chemistry 4: 331–339.

Miller JR & Roelofs WL (1980) Individual variation in sex pheromone component ratios in two populations of the redbanded leafroller moth *Argyrotaenia velutinana*. Environmental Entomology 9: 359–363.

Neunzig HH (1972) Taxonomy of *Acrobasis* Larvae in Eastern North America (Lepidoptera: Pyralidae). USDA Technical Bulletin no. 1457.

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P et al. (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theoretical and Applied Genetics 97: 1248–1255.

Pelozuelo L, Malosse C, Genestier G, Guenego H & Frerot B (2004) Host-plant specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France. Journal of Chemical Ecology 30: 335–352.

Phelan PL (1992) Evolution of sex pheromones and the role of asymmetric tracking. Insect Chemical Ecology an Evolutionary Approach (ed. by BD Roitberg & MB Isman), pp. 265–314. Chapman and Hall, New York, NY, USA.

Pritchard JK, Stephens M & Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Qiagen (2002) DNEasy® Tissue Handbook. Qiagen, Valencia, CA, USA.

Ring DR & Harris MK (1984) Nut entry by 1st summer generation pecan nut casebearer. Southwest Entomologist 9: 13–21.

Ring DR, Harris MK & Payne JA (1989) Sequential sampling plan for integrated pest management of pecan nut casebearer (Lepidoptera: Pyralidae). Journal of Economic Entomology 82: 906–909.

Rodriguez Del Bosque LA, Smith JW Jr & Martinez AJ (1995) Winter mortality and spring emergence of corn stalkborers (Lepidoptera: Pyralidae) in subtropical Mexico. Journal of Economic Entomology 88: 628–634.

Roelofs WL & Comeau A (1969) Sex pheromone: taxonomic and evolutionary aspects in Lepidoptera. Science 165: 398–399.

Salvato P, Battisti A, Concato S, Masutti L, Patarnello T & Zane L (2002) Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa-wilkinsoni* complex), inferred by AFLP and mitochondrial DNA markers. Molecular Ecology 11: 2435–2444.

Santangelo EM, Coracini M, Witzgall P, Correa AG & Unelius CR (2002) Identification, syntheses, and characterization of the geometric isomers of 9,11-hexadecadienal from female pheromone glands of the sugar cane borer *Diatraea saccharalis*. Journal of Natural Products 65: 909–915.

Stevenson DE, Knutson AE, Ree W, Jackman JA, Dean A et al. (2003) Pecan nut casebearer pheromone monitoring and degree-day model validation across the pecan belt. Southwest Entomologist Supplement 27: 57–73.

Svensson M (1996) Sexual selection in moths: the role of chemical communication. Biological Reviews of the Cambridge Philosophical Society 71: 113–135.

Tamaki Y (1985) Sex pheromones. Comprehensive Insect Physiology, Biochemistry and Pharmacology, Vol. 9 (ed. by GA Kerkut & LI Gilbert), pp. 145–191. Pergamon Press, Oxford, UK.

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T et al. (1995) AFLP – a new technique for DNA-fingerprinting. Nucleic Acids Research 23: 4407–4414.

Zada A, Dunkelblum E, Assael F, Franco JC, Da Silva EB et al. (2008) Attraction of *Planococcus ficus* males to racemic and chiral pheromone baits: flight activity and bait longevity. Journal of Applied Entomology 132: 480–489.

Zhang LP, Zhang YJ, Zhang WL, Wu QJ, Xu BY & Chu D (2005) Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. Journal of Applied Entomology 129: 121–128.

Zhu-Salzman K, Li HW, Klein PE, Gorena RL & Salzman RA (2003) Using high-throughput amplified fragment length polymorphism to distinguish greenbug (Homoptera: Aphididae) biotypes. Agricultural and Forest Entomology 5: 311–315.