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IDENTIFYING HOST STRAINS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) IN FLORIDA USING MITOCHONDRIAL MARKERS

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ABSTRACT

Two molecular techniques were used to identify host strains of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) from male moths captured in pheromone-baited traps in north-central and central Florida. Moths collected in 1998 were analyzed using direct detection of mitochondrial DNA (mtDNA) RFLPs generated from restriction endonuclease digestion of total DNA, while moths collected in 2000 and 2001 were analyzed using a mitochondrial cytochrome oxidase subunit I (COI) gene PCR-RFLP marker. Both techniques could distinguish between rice and corn strain moths, however, the COI PCR-RFLP marker was more robust as indicated by a time interval experiment that showed that moths held for up to 15 days in a "bucket trap" could still be used for strain diagnosis. In a field study, our strategy gave results consistent with expectations. Rice strain moths were common in habitats with large areas of small grasses, corn strain moths were common in large areas planted to corn, and habitats with mixed large- and small-grass plantings contained both strains. Our methodology of combining pheromone traps with PCR-RFLP analysis will provide a valuable sampling system to determine the population ecology habits and strain isolating mechanisms of fall armyworm populations in numerous habitats, including overwintering areas of southern Florida.

Key Words: *Spodoptera frugiperda*, host strain identification, PCR-RFLP

RESUMEN

Dos ténicas moleculares fueron utilizadas para identificar razas hospederas del cogollero, *Spodoptera frugiperda* (J. E. Smith) a partir de polillas machos capturadas en trampas cebadas con feromonas en la región centro-norte y central de Florida. Las polillas colectadas en 1998 fueron analizadaas utilizando la detección directa de los PLFR (Polimorfismo en la Longitud de los Fragmentos de Restricción [RFLP en ingles]) del ADN mitocondrial (mtADN) generados a partir la digertión por la endonucleasa de restricción del ADN total, mientras que las polillas colectadas en el 2000 y 2001 fueron analizadas utilizando un marcador PCR-RFLP de la subunidad I del gen citocromo oxisasa (COI) miticondrial. Ambas técnicas pudieron distinguir entre las razas de polillas del arroz y las del maíz, sin embargo, el marcador COI PCR-RFLP fue masrobusto tal como lo indico un experimento de intervalo de tiempo en el cual las polillas que se mantuvieron en una "trampa de balde" hasta por 15 días, todavía podían ser utilizadas para diagnosticar su raza. En un estudio de campo realizado, nuestra estrategia produjo resultados consistentes con las expectativas. Las razas de polillas de del arroz fueron communes en habitats con amplias áreas de pastos bajos, las razas de polillas del maíz fueron mas comunes en amplias áreas sembradas con maíz, y en áreas sembradas con una mezcla de pastos altos y bajos se consiguieron ambas razas. Nuestra metodología de combinar las trampas de feromonas con el análisis de PLFR-PCR proveerá un importante sistema de muestreo para determinar hábitos ecológicos de las poblaciones y los mecanismos de aislamiento de ls poblaciones de cogollero en numerosos habitats, incluyendo las áreas de hibernación en el sur de Florida

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a migratory polyphagous pest that attacks several important crops such as maize, sorghum, forage grasses, rice, cotton and peanuts (Luginbill 1928; Sparks 1979; Knipling 1980). Two morphologically indistinguishable host strains have been identified that are possibly in the initial stages of speciation (Pashley 1986; Prowell 1998). One strain was identified from populations feeding on corn and sorghum (corn strain), and the other strain was identified from populations feeding on rice and bermudagrass (rice strain). Strains exhibit polymorphisms at five allozyme loci (Pashley 1986), in their mitochondrial DNA (mtDNA) (Pashley 1989; Lu & Adang 1996) and in their nuclear DNA (Lu et al.

1992). Additionally, a tandemly repeated (189 bp) DNA sequence has been shown to be unique to the rice strain (Lu et al. 1994). Two recent techniques have been used to improve strain discrimination, including one that uses amplified fragment-length polymorphisms (AFLPs) (McMichael & Prowell 1999), and another that employs amplification of a region of the mitochondrial cytochrome oxidase C subunit I gene (COI) followed by restriction enzyme digestion (PCR-RFLP) (Levy et al. 2002).

Strain identification is important because research has shown biological, behavioral, toxicological, and host genotypic differences between strains. Both strains attained similar larval and pupal weights when fed bermudagrass or rice, but when reared on maize, corn strain larvae attained larger weights (Pashley et al. 1995; Veenstra et al. 1995). Behavioral reproductive incompatibilities, such as the lack of successful mating between corn strain females and rice strain males, have been tentatively identified (Pashley & Martin 1987), although successful matings were achieved with moths held in culture for over three years (Whitford et al. 1988). Temporal partitioning of calling/mating times has been presented as a strain isolation mechanism (Pashley et al. 1992). Rice strain larvae were shown to be more susceptible to various insecticides such as carbaryl, diazinon, cypermethrin, methyl parathion, and methomyl, while corn strain larvae were more susceptible to carbofuran (Pashley et al. 1987b; Adamczyk et al. 1997). Rice strain larvae were also more susceptible to transgenic *Bacillus thuringiensis* Berliner (Bt) cotton than corn strain larvae (Adamczyk et al. 1997). Laboratory and field studies have shown distinct differences in feeding of bermudagrass genotypes, with rice strain larvae generally able to gain more weight and consume more plant material than corn strain larvae (Pashley et al. 1987a; Quisenberry & Whitford 1988).

Although fall armyworm overwinters in southern Florida counties and can build up large populations in central and north-central Florida (Pashley et al. 1985; Mitchell et al. 1991), strain identification of Florida populations is limited. Late instar larvae collected from corn in southern Florida (Hendry Co.) were identified as corn strain in 1983 and 1984 (Pashley et al. 1985). Both corn and rice strain populations were identified from southern Florida, although no information regarding location or collection habitat were provided (Pashley 1988). Corn strain larvae were collected in early 1989 from southern Florida, but again collection sites and habitats were not disclosed (Pashley et al. 1992). Strain identification of populations from non-overwintering areas in north or central Florida has not been attempted. The objective of this research was to identify the host strain of fall armyworm moths collected from sex pheromone traps for periods up to 15 days in north-central and central Florida using suitable molecular methods.

Materials and Methods

Moth Collection

Standard plastic Unitraps (bucket traps) baited with commercial fall armyworm sex pheromone [(*Z*)-9-tetradecen-1-ol acetate, (*Z*)-11-hexadecen-1-ol acetate and (*Z*)-7-dodecen-1-ol acetate; either Scentry® (Ecogen, Inc., Langhorne, PA) or Scenturion® (Scenturion, Inc., Clinton, WA) lures] were placed in field locations in 1998, 2000, and 2001. All traps contained insecticide strips (Hercon® Vaportape II containing 10% 2,2-dichlorovinyl dimethyl phosphate, Hercon Environmental Co., Emigsville, PA) to kill the moths. In 1998, locations in Alachua Co., FL were used to collect moths. One was near the Dairy and Agronomy Forage Research Unit of the University of Florida, and the second location was a commercial corn field near the town of Alachua. The research unit was located in the northern half of the county and contained plantings of field corn and pasture grasses.

In 2000, three traps were placed along State Route 121 in Levy Co., FL. Several hundred hectares of forage grasses bordered the route. In 2001, three traps were placed at the University of Florida Range Cattle Research and Education Center in Ona, FL. This center has over 1150 h of natural and improved forage grasses. Two traps were also placed beside sugarcane plantings in the Everglades Agricultural Area near the University of Florida Everglades Research and Education Center, Belle Glade, FL, and the USDA, ARS, Sugarcane Field Station, Canal Point, FL. Fall armyworm larvae were also collected from Ona and resulting adults were analyzed to determine their host strain.

Interval Testing

This test was designed to determine if the host strain of moths held for up to 15 d in a pheromone trap could still be accurately identified. Fall armyworms used in this test were reared in the laboratory on a pinto bean-based artificial diet according to the procedures of Guy et al. (1985). Pupae were sexed and placed in 163-ml (5.5 oz.) paper cups (Sweetheart, Chicago, IL) that were placed in 24 × 24-cm screen cages for eclosion. Pupae were maintained under reversed photoperiod (14:10, light:dark) in an environmental chamber held at 26°C and 70% RH. Adult males had access to cotton balls saturated with distilled water and a honey-sugar solution. Live male moths aged 2-5 d were placed in bucket traps with insecticide strips and removed for testing at 1, 2, 4, 7, 10 and 15 d. Moths usually died within an hour after ex-

posure to the insecticide strips. Three to 10 moths were sampled for each time point.

Total DNA Extraction and Strain Identification

Two techniques were used to identify strains. For both techniques, total DNA was extracted from one fall armyworm adult that was ground in liquid nitrogen and then homogenized in 1 ml of extraction buffer (100 mM Tris-HCl, pH 7.5, 20 mM EDTA, pH 8, 500 mM NaCl, 2% (w/v) SDS). The remainder of the extraction procedure was performed according to Lu et al. (1992) except that final resuspension of the DNA was in 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.1). The technique of Lu & Adang (1996) was employed to identify strains of moths collected in 1998. Approximately 50 µg of total DNA was double-digested with the endonucleases *Hae*III and *Msp*I, and 8 µg of the digested DNA was electrophoresed on a 1% agarose gel in TBE buffer. Gels were then stained with ethidium bromide and visualized under UV illumination. The corn strain produces four mitochondrial bands of 5.5, 4.3, 3.8 and 1.3 kb (cannot see this smaller band due to masking by nuclear DNA), while the rice strain produces only two bands of 10.4 and 4.4 kb.

Strain identification of moths collected in 2000 and 2001 was accomplished using a mitochondrial PCR-RFLP marker (Levy et al. 2002). Two PCR primers (5'GAGCTGAATTAGGGACTCCAGG3'-forward and 5'ATCACCTCCACCTGCAGGATC3'-reverse) flanking a diagnostic *Msp*I restriction site (CCGG) within the mitochondrial COI gene sequence were used to amplify a 569 bp product. The PCR mixture contained 25 ng of total DNA, 0.25 µM primers, 200 µM dNTPs, 0.5 U *Taq* polymerase, and 1× PCR buffer (Perkin Elmer GeneAmp kit) in a total volume of 25 µl. Amplification conditions were as follows: denaturation, 94°C for 30 sec; annealing, 58°C for 1 min; extension, 72°C for 1 min, with a final cycle extension of 72°C for 10 min. Reactions were run for 40 cycles in a thermal cycler (MJ Research). Following amplification, 5 µl of the PCR mixture was used for *Msp*I digestion and the resulting products were surveyed using 2% agarose gel electrophoresis. The corn strain PCR product contains the *Msp*I restriction site while this site is missing in the rice strain product. Consequently, *Msp*I digestion of the corn strain 569 bp PCR product results in two bands of 497 bp and 72 bp, whereas this PCR product from the rice strain moths is unrestricted and remains intact.

Results and Discussion

Both corn and rice strain moths were collected from north-central and central Florida (Table 1). Digestion of total DNA with *Hae*III fragments genomic DNA made observation of mtDNA fragments easier. Digestion of mtDNA with *Msp*I produced three visible bands of 5.4, 4.3, and 3.8 kb representing corn strain moths, and two bands of 10.4 and 4.3 kb representing rice strain moths as previously established by Lu & Adang (1996) (Fig. 1). The size of the mtDNA genome of fall armyworm was estimated as approximately 14.8 kb (Lu & Adang 1996). The smallest band (1.3 kb) from corn strain moths was not visible because of masking by nuclear DNA. This method therefore resulted in a "3-band pattern" and "2-band pattern" of corn and rice strain moths, respectively (Lu & Adang 1996). The PCR-RFLP marker correctly identified corn strain moths by way of *Msp*I digestion of the 569 bp COI amplified fragment into two bands of 497 bp and 72 bp (Fig. 2). The rice strain PCR product was not digested by *Msp*I (Levy et al. 2002).

Rice strain moths predominated in large areas of small grasses such as Levy Co. Rt. 121 and Ona (Table 1), while corn strain moths were found more frequently in the only large corn site tested in Alachua Co. (Table 1). Mixed areas of corn and small grasses, and the peanut habitat contained moths of both strains. The agroecosystem near

Table 1. Corn and rice strains of fall armyworm adult males collected in pheromone traps at different field sites in Florida, 1998, 2000, 2001. Moths collected in 1998 were analyzed using the method of Lu & Adang (1992); moths collected in 2000 and 2001 were analyzed using the method of Levy et al. (2002).

Location	Date	Habitat	Strains (No.)	
			Corn	Rice
Dairy/Forage, Alachua Co.	4/22, 5/29, 6/12/98	grass, corn	3	1
Corn fields, Alachua Co.	5/25/98	corn	7	1
Levy Co., Rt. 121	8/10, 8/14, 8/16/00	grass	1	13
Levy Co.	8/20, 9/3/01	peanuts	17	10
Everglades Agric. Area	8/17/01, 11/14/01	sugarcane	1	6
Ona, RCREC	6/7, 7/12, 8/30, 11/14/01	grass	0	39
Ona, RCREC	11/14/01	colony	0	5

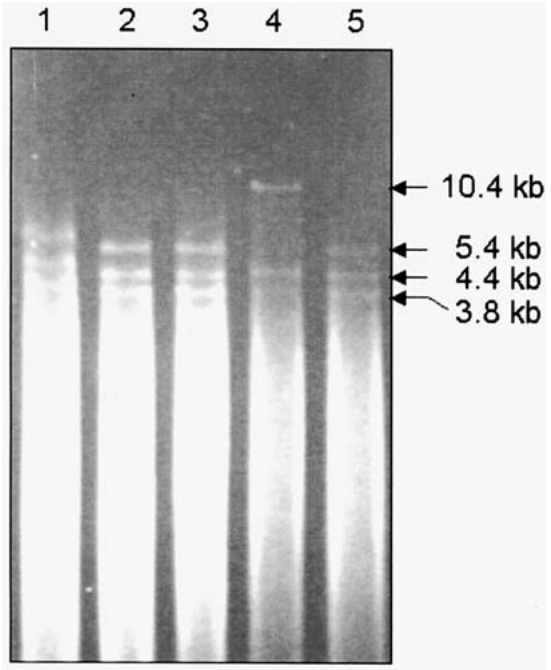

Fig. 1. Fall armyworm total DNA digested with *Hae*III and *Msp*I. Lanes 1-3 and 5 are corn strain, lane 4 is a rice strain.

the peanut site also contained large tracts of pasture, easily accessible by moths. Therefore, the presence of both strains was expected, although it is not known which strain is physiologically better adapted to peanut as a host plant. Previous studies of peanut host plant resistance used laboratory colonies that were probably corn strain (Leuck & Skinner 1971; Garner & Lynch 1981; Lynch et al. 1981), although strain analysis was not performed on these colonies. Further studies are underway to determine whether one strain is better adapted to peanuts than the other. The Everglades Agricultural Area also contained moths of both strains. The habitat in this area is dominated by large grasses such as sugarcane and corn and small grasses such as rice and "wild" grass species. Larger sample sizes from these habitats are needed to determine which strain is more common in this important and fragile agroecosystem.

Previous physiological studies suggested that rice strain larvae were more specialized and affected by their host plant than were corn strain larvae (Pashley et al. 1995; Veenstra et al. 1995). However, larval collections in the field disclosed that rice strain larvae occur in both large and small grass habitats, whereas corn strain larvae rarely occupied small grass habitats (Pashley 1988; Pashley et al. 1995; McMichael & Prowell 1999). Our study detected few corn strain moths in small grass habitats (Levy Co. Rt. 121, Ona). Although host-plant specialization is likely mediated by adult behavioral attributes rather than larval physiological characteristics (Pashley et al. 1995), studies determining adult attributes such as mating behavior and ovipositional preference have not provided clear results.

Pheromone traps provide a convenient means of collecting wild males in the field and represent one of the few methods of directly trapping adult fall armyworm. However, such field-collected specimens may be in traps for up to two weeks before they can be analyzed, with significant degra-

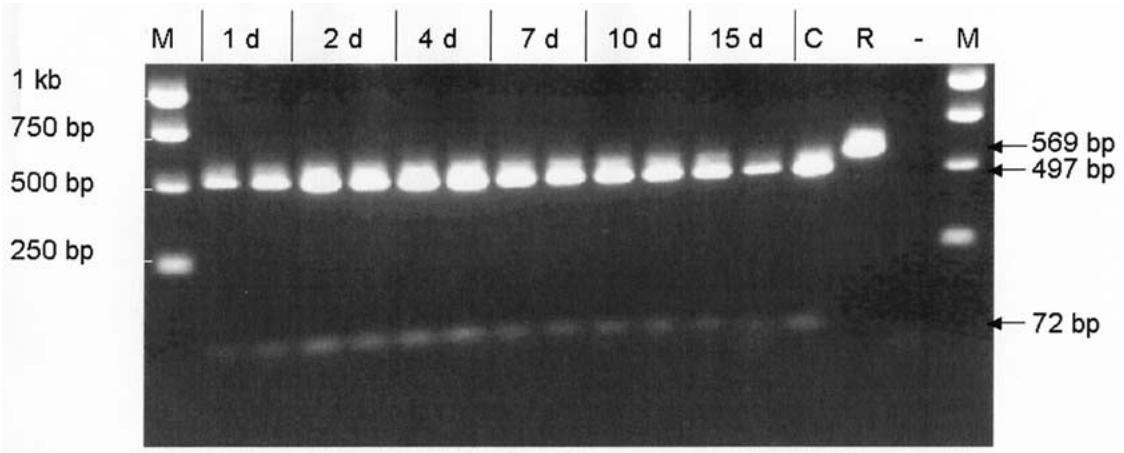

Fig. 2. Time interval experiment showing that adult fall armyworm held for up to 15 days could still be used for strain diagnosis by using the COI PCR-RFLP marker. All laboratory-reared moths from a corn strain colony were killed in the traps and exposed to outdoor climate for 1, 2, 4, 7, 10 or 15 d before collection for strain diagnosis. Positive controls included freshly collected corn [C] and rice [R] strains and the negative control was a PCR reaction containing no DNA template (-). M = 1 kb ladder.

dation of DNA likely. Therefore, it is necessary that the diagnostic molecular techniques employed for strain identification be robust enough to distinguish between the strains under these field conditions. The time interval experiment showed that moths held for at least 15 d could still be used for strain diagnosis when using the COI PCR-RFLP marker (Fig. 2). In comparison, results from the non-PCR based mt DNA RFLP method of Lu & Adang (1996) were highly variable and this method could not be used to identify strains held in traps longer than four days (data not shown). Therefore, the PCR-based method now makes it possible to obtain consistent and accurate strain identification of moths collected by standard pheromone trapping methods.

The combination of the pheromone trapping method with PCR-RFLP provide a valuable sampling system. Biological attributes such as strain isolating mechanisms, intra- and inter-strain mating behavior, and within-field populations in monocot and dicot crops are potential future studies. Additionally, strain analysis of overwintering fall armyworm populations in southern Florida is a important component to understanding population flow of this neotropical migrant.

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