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MOLECULAR DIAGNOSTICS OF ECONOMICALLY IMPORTANT CLEARWING MOTHS (LEPIDOPTERA: SESIIDAE)

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ABSTRACT

Larvae of many species of Sesiidae, the clearwing moths, are important pests in commercial nurseries, urban landscapes, timber stands, vineyards, and orchards. They cause economic loss by larval boring in stems and roots of herbaceous and woody plants. Researchers and growers often monitor for the presence of economically important sesiid adults with pheromone traps. These traps often attract more than one species of Sesiidae and specimens often degrade making identification difficult or impossible. This can cause problems in monitoring programs where species-specific control programs are used. Polymerase chain reaction (PCR) was used to amplify a 606-bp region of the mitochondrial DNA cytochrome oxidase I (COI), tRNA leucine, and COII gene. This region exhibited 7.7-19.5% genetic variability among 8 species of Sesiidae. Samples were sequenced and restriction sites identified. PCR-restriction fragment length polymorphism (PCR-RFLP) analysis was conducted on 8 species of Sesiidae, *Melittia satyriniformis*, *Paranthrene simulans*, *Pennisetia marginata*, *Synanthedon pictipes*, *S. exitiosa*, *S. scitula*, *S. rileyana*, and *Vitacea polistiformis*, with 2 restriction enzymes, *Dra* I and *Hinf* I. This method is time efficient requiring less than 8 h to perform and cost efficient with each sample about $1. PCR-RFLP provides an accurate method to differentiate 8 species of adult clearwing moths commonly found in traps baited with commercially available pheromone lures.

Key Words: COI, PCR-RFLP, *Melittia*, *Paranthrene*, *Pennisetia*, *Synanthedon*, *Vitacea*

RESUMEN

Las larvas de muchas de las especies de la familia Sesiidae, palomillas de alas claras, son plagas importantes en viveros comerciales, áreas urbanas, árboles maderables, viñas y huertos. Ellas causan perdidas económicas al barrenar los tallos y raices de plantas herbáceas y leñosas. Investigadores y agricultores a menudo realizan un monitoreo para la presencia de los adultos sesiidos de importancia económica usando trampas con feromonas. Estas trampas a menudo atraen mas de una especie de Sesiidae y los especimenes a menudo se degradan y con ello se hace dificil o imposible su identificación. Esto puede causar problemas en los programas de monitoreo donde se usan programas de control para la especie especifica. La reacción en cadena por la polimerasa (RCP) fue usada para amplificar la región de 606-pb de la citocromo-c-oxidasa I (COI) de la ADN mitocondrial, tRNA de leucina y del gene COII. Esta región exhibió una variabilidad genética de 7.7-19.5% entre 8 species de Sesiidae. Las muestras fueron secuenciadas y los centros de restricción identificados. Se realizó un análisis del polimorfismo de la longitud de los fragmentos de restricción (PCR-RFLP) para las 8 especies de Sesiidae, *Melittia satyriniformis*, *Paranthrene simulans*, *Pennisetia marginata*, *Synanthedon pictipes*, *S. exitiosa*, *S. scitula*, *S. rileyana* y *Vitacea polistiformis*, con 2 enzimas de restricción , *Dra* I y *Hinf* I. Este método es eficiente en cuanto a que requiere menos de 8 horas de tiempo para ejecutar y eficiente en cuanto a su costo de $1 por muestra. El PCR-RFLP provee un método preciso para diferenciar los adultos de estas 8 especies de palomillas de alas claras encontradas frecuentemente en trampas cebadas con señuelos de feromonas comercialmente disponsibles.

The Sesiidae are a well defined lepidopteran family with over 1000 described species worldwide (Eichlin & Duckworth 1988). In North America north of Mexico 123 species in 20 genera are represented (Eichlin & Duckworth 1988). Geographic distribution and abundance at all taxonomic levels are poorly known. The Sesiidae are quite inconspicuous and difficult to collect due to their mimicry of wasps and diurnal flight. This has resulted in very poor representation in collections. Sesiid species in the genera *Paranthrene* (Hübner) and *Synanthedon* (Hübner) cause economic loss to commercial nurseries and timber producers in the United States (Solomon et al. 1982). If not controlled, *Synanthedon exitiosa* (Say) and *S. pictipes* (Grote & Robinson) can destroy entire orchards of fruit trees (Nielson 1978). *Vitacea polistiformis* (Engelhardt) is a common pest in vineyards (Johnson et al. 1981); *Melittia satyriniformis* (Hübner) is a pest of squash (Klun et al. 1990), and *Pennisetia marginata* (Harris) will damage blackberry and raspberry plants (Raine 1962).

Pheromone wing traps are a popular method of monitoring for the adult moths in areas at risk for sesiid damage. These traps are economical and easy to use. The insect becomes entangled in sticky glue on the trap bottom and can survive several days, losing scales and limbs while trying to break free. Unless these traps are checked on a daily basis, moths trapped on the sticky bottoms can become impossible to identify to species. Sesiids are often cross-attracted to pheromones and different species are commonly found in traps baited with species-specific pheromone lures (McKern 2005). Even when specimens are properly preserved, species can be difficult to differentiate morphologically. A molecular diagnostics protocol could be very helpful in distinguishing sesiids that are target pests from non-pest species.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) is a technique that is inexpensive, simple, reliable, repeatable, and can be used on the insect during any developmental stage, including eggs, larvae, pupae and adults (Taylor & Szalanski 1999). The mitochondrial region has proven useful in other molecular diagnostic protocols. Brown et al. (1999) used the cytochrome oxidase region of mtDNA to differentiate 6 moth species in the genus *Wiseana* (Viette) (Lepidoptera: Hepalidae) and Lewter et al. (2006) developed molecular diagnostics using mtDNA to distinguish 7 noctuid species commonly found in *Spodoptera frugiperda* (J.E. Smith) pheromone traps. The purpose of this study was to identify PCR-RFLP diagnostic characters with mtDNA marker for 8 species of Sesiidae commonly found in wing traps baited with commercially available pheromone lures.

Materials and Methods

Samples

Sesiids were collected in 2004 with Trécé Pherocon IC wing traps (Trécé, Inc., Adair, OK) baited with commercially available pheromone lures. Moths were collected from 4 counties in Arkansas as follows: blackberry planting and vineyard in Faulkner County; vineyard in Madison County, peach and apple orchard in Carroll County; and forest, squash planting, and apple orchard in Washington County (Table 1). Two *P. marginata* larvae were collected from blackberry crowns for analysis. All other samples consisted of adults. Samples were identified morphologically with the key "The Moths of America North of Mexico" (Eichlin & Duckworth 1988) and placed separately in 1.5-mL Eppendorf tubes and stored at -20°C until processing. Voucher specimens were deposited in the University of Arkansas Arthropod Museum Fayetteville, AR.

DNA Extraction, Amplification, and Purification

DNA was extracted from the thoraces of individual adult specimens or the head capsules of larval specimens by using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN).

Table 1. Sample collection data, and samples subjected to DNA sequencing and PCR-RFLP.

Sample ID	County, AR	Manufacturer, lure	No. sequenced	No. PCR-RFLP
1. *Paranthrene simulans*	Washington	Scentry, BC	3	7
	Carroll		2	3
	Faulkner		2	5
	Madison		1	1
2. *Vitacea polistiformis*	Faulkner	Trécé, GRB	2	3
3. *Melittia satyriniformis*	Washington		2	8
	Faulkner		2	2
	Madison		2	1
4. *Pennisetia marginata*	Faulkner	APTIV, RCB	4	8
5. *Synanthedon rileyana*	Washington	APTIV, RCW	2	3
	Carroll		2	3
	Faulkner		1	4
6. *Synanthedon exitiosa*	Faulkner	Trécé, GPTB	2	10
7. *Synanthedon pictipes*	Washington	Trécé, LPTB	2	10
	Carroll		2	2
8. *Synanthedon scitula*	Washington	Trécé, DWB	4	7
	Carroll		2	3
	Faulkner		1	1

Abbreviations: BC: Oak, Rhododendron, Ash, Banded Ash, Lilac borer; GRB: Grape Root Borer; SVB: Squash Vine Borer; RCB: Raspberry Crown Borer; RCW: Raspberry Clearwing; GPTB: Greater Peach Tree Borer; LPTB: Lesser Peach Tree Borer; DWB: Dogwood Borer.

Manufacturer Locations: Scentry (Billings, Montana); Trécé (Adair, Oklahoma); APTIV (Portland, Oregon).

Extracted DNA was resuspended in 50 µL of Tris: EDTA and stored at -20°C.

DNA PCR was conducted with primers C1-J-2797 (5'-CCTCGACGTTATTCAGATT ACC-3') (Simon et al. 1994) and C2-N-3400 (5'-TCAATATCATTGATGACCAAT-3') (Taylor et al. 1997). These primers amplify approximately 606 bp of the mtDNA cytochrome oxidase I gene (COI), tRNA-leu and cytochrome oxidase II gene (COII). PCR reactions were conducted with 2 µL of the extracted DNA. The thermal cycler profile for the mtDNA COII gene consisted of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 45 s per Szalanski et al. (2000).

Amplified DNA from individual sesiids was purified and concentrated with minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega) (Table 1). Samples were sent to the University of Arkansas Medical School Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. DNA sequences were aligned with Clustal W (Thompson et al. 1994) and consensus sequences obtained with BioEdit 5.89 (Hall 1999). Sequence data were deposited in GenBank with accession numbers DQ205539-DQ205573.

Digests

Restriction sites were predicted from the DNA sequence data with BioEdit 5.89 (Hall 1999). Amplified DNA from unknown specimens of each species (*P. simulans*, *V. polistiformis*, *M. satyriniformis*, *P. marginata*, *S. rileyana*, *S. pictipes*, and *S. scitula*) was digested according to manufacturer's (New England Biolabs, Ipswich, MA) recommendations following Cherry et al. (1997) with the restriction enzymes *Dra* I and *Hinf* I (Table 2). Fragments were separated by 2% agarose gel electrophoresis per Taylor et al. (1996). Gels were photographed with a UVP BioDoc-it documentation system (Upland, CA).

Results and Discussion

DNA sequencing of the mtDNA amplicon from 38 sesiids resulted in an average amplicon size of 606 bp. Interspecific genetic variation ranged between 7.1-19.5% among the 8 species of Sesiidae. Two restriction enzymes, which did not have any intraspecific variation, were selected for PCR-RFLP. The *Dra* I digest had 6 restriction patterns among the 8 sesiid moths (Table 2 and Fig. 1), and the *Hinf* I digest produced four restriction patterns (Table 2 and Fig. 2). From the 81 moths subjected to PCR-RFLP no intraspecific variation was observed for the 2 restriction enzymes. By combining the restriction patterns from the 2 digests, the 8 sesiid species in this study can be readily differentiated. This is the first time PCR-RFLP has been utilized to distinguish sesiid pests commonly found in pheromone traps.

This technique is cost efficient and useful for identification of degraded or badly damaged adult specimens and hard to identify larvae. Each reaction costs <$1.00 per sample and the use of a small amplicon facilitates the use of specimens that are slightly degraded (Taylor et al. 1996). Other advantages to the PCR-RFLP technique include reliability and time efficiency. Because the restriction patterns are based on specific DNA sequences, there are no false positives obtained (Roehrdanz 1997) and the whole procedure can be performed in under 8 h.

An important advantage in using the COI, COII mtDNA region for this PCR-RFLP technique is that most fragments created by the digests are relatively large and clearly separated with 2% agarose gel during electrophoresis, which eliminates the need for polyacrylamide-gel electrophoresis or high resolution agarose gels. This increases cost efficiency when dealing with large numbers of samples.

The results described here demonstrate that PCR-RFLP of mtDNA provides a simple and reli-

Table 2. Restriction sites, fragments, and patterns for sesiid PCR-RFLP using restriction enzymes *Hinf* I and *Dra* I.

	Hinf I			*Dra* I		
Species	Restriction site	Fragments	Pattern	Restriction site	Fragments	Pattern
Paranthrene simulans	—[a]	606	A	256, 312, 582	256, 270	A
Vitacea polistiformis	203	203, 403	B	252, 309	252, 297	A
Melittia satyriniformis	102	102, 504	C	139, 253, 309	139, 114, 297	B
Pennisetia marginata	102	102, 504	C	253, 558, 579	253, 305	A
Synanthedon rileyana	15, 102, 270	87, 168, 336	D	252, 306	252, 300	A
S. exitiosa	15, 102	87, 504	C	252	252, 354	C
S. pictipes	15, 102	87, 504	C	139, 252	139, 113, 354	D
S. scitula	15, 102	87, 504	C	—	606	E

[a]— = "no cut".

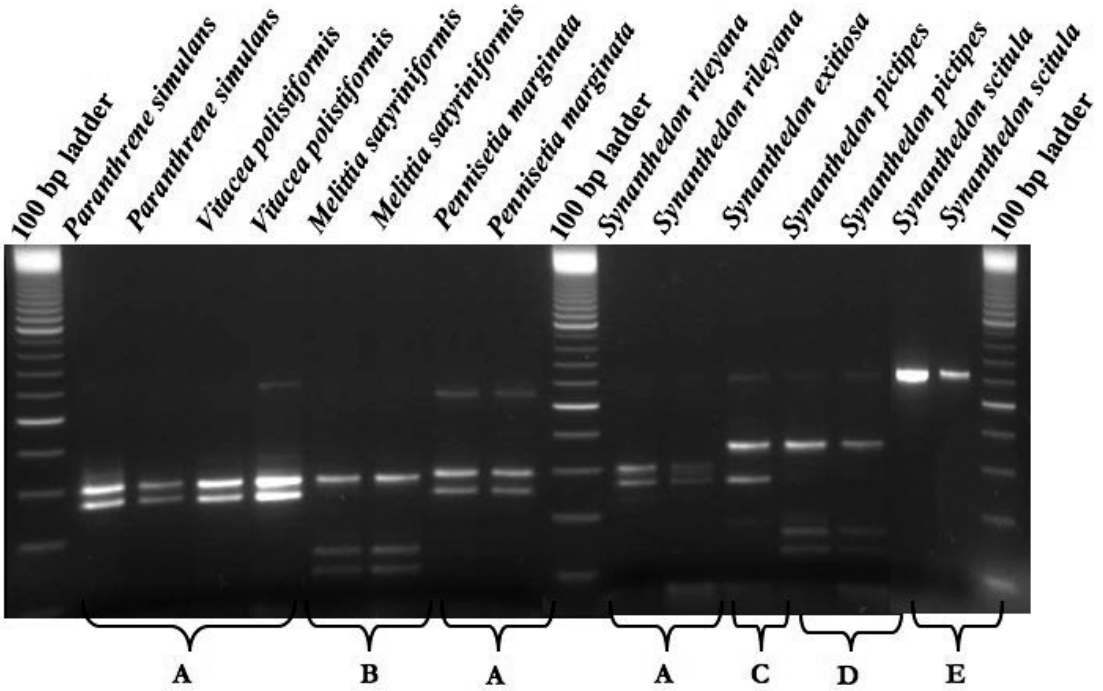

Fig. 1. Agarose gel depicting PCR-RFLP *Dra* I digest patterns of PCR amplified mtDNA for 8 sesiid species.

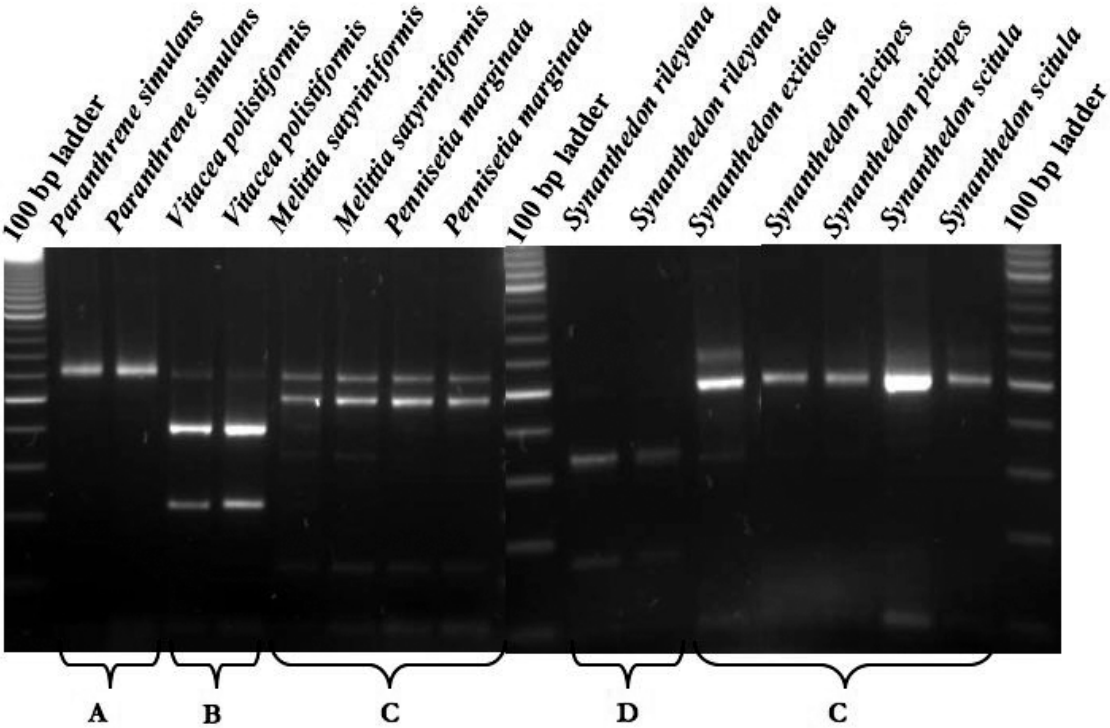

Fig. 2. Agarose gel depicting PCR-RFLP *Hinf* I digest patterns of PCR amplified mtDNA for 8 sesiid species.

able method to distinguish 8 species of Sesiidae. Monitoring and identification of these pests is essential to apply control tactics at crucial points in their life cycle before they have bored too deep within plants for insecticide penetration (McKern et al. in press). The PCR-RFLP procedure can be used by researchers not only for identification of sesiids but also to monitor seasonal and geographical differences. Very little is known about sesiid distribution, abundance, and gene flow, and this procedure will allow us to address these issues in the future.

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