

Identification of the Meam1 Cryptic Species of Bemisia tabaci (Hemiptera: Aleyrodidae) by Loop-Mediated Isothermal Amplification §

Authors: Dickey, Aaron M., Osborne, Lance S., Shatters, Robert G., and Mckenzie, Cindy L.

Source: Florida Entomologist, 96(3): 756-764

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0308

The BioOne Digital Library (<u>https://bioone.org/</u>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<u>https://bioone.org/subscribe</u>), the BioOne Complete Archive (<u>https://bioone.org/archive</u>), and the BioOne eBooks program offerings ESA eBook Collection (<u>https://bioone.org/esa-ebooks</u>) and CSIRO Publishing BioSelect Collection (<u>https://bioone.org/csiro-ebooks</u>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

IDENTIFICATION OF THE MEAM1 CRYPTIC SPECIES OF BEMISIA TABACI (HEMIPTERA: ALEYRODIDAE) BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION[§]

AARON M. DICKEY^{1,3}, LANCE S. OSBORNE², ROBERT G. SHATTERS, JR.¹ AND CINDY L. MCKENZIE^{1*} ¹USDA-ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL 34945, USA

²Mid-Florida Research & Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703, USA

³Current Address: Mid-Florida Research & Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703, USA

*Corresponding author; E-mail: Cindy.McKenzie@ars.usda.gov

[§]Summarized from a presentation and discussions at the "Native or Invasive - Florida Harbors Everyone" Symposium at the Annual Meeting of the Florida Entomological Society, 24 July 2012, Jupiter, Florida.

Supplementary material for this article in Florida Entomologist 96(3) (2013) is online at http://purl.fcla.edu/fcla/entomologist/browse

ABSTRACT

There are 2 major invasive cryptic species within the Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) cryptic species complex in Florida, called MEAM1 or biotype B, and MED or biotype Q. We used loop-mediated isothermal amplification of DNA to detect these groups. Primer sets developed in-house and those previously published were compared for specificity to the target species by measuring time-to-amplification of non-target and target DNA templates using real-time PCR. All these primer sets were designed using the mitochondrial cytochrome oxidase I gene. Our findings indicate that primer sets designed for MEAM1 were more specific than those designed for MED across published studies and in-house designed primers. The optimal primer set for MEAM1 detection, in conjunction with the magnesium ion color indicator hydroxynaphthol blue, provided visual confirmation of target whitefly DNA amplification in 45 min. This assay was highly specific and did not amplify DNA from 8 additional sweetpotato whitefly cryptic species nor from 10 non-Bemisia whitefly species found in Florida. The assay amplified non-target DNA from 1 sweetpotato whitefly cryptic species not present in Florida and shows potential to amplify MED DNA rarely. While additional genes should be used to design more specific primers, particularly for MED, this MEAM1 assay shows promise as the foundation of a field-based tool that could quickly identify the most commonly encountered Florida whitefly species.

Key Words: biotype B, biotype Q, DNA, LAMP, molecular identification, $\mathit{Trialeuroides}$ vaporariorum

RESUMEN

Hay 2 especies invasoras crípticas principales dentro de las especies crípticas del complejo de Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) en Florida, llamadas MEAM1 o biotipo B y MED o biotipo Q. Utilizamos la amplificación isotérmica de ADN mediada por lazo para detectar estos grupos. Se comparó la especificidad de los juegos de cebadores (primers) desarrollados en casa y de publicados anteriormente hacia el grupo de enfoque mediendo el tiempo de amplificación de patrones de ADN de grupos del enfoque y no enfoque utilizando PCR en tiempo real. Todos estos juegos de cebadores fueron diseñados utilizando el gen de citocromo oxidasa I mitocondrial. Nuestros resultados indican que los juegos de cebadores diseñados para MEAM1 fueron más específicos que los diseñados para MED a través de estudios publicados y de los cebadores diseñados en casa. El juego de cebadores óptimo para la detección de MEAM1, junto con hidroxinaftol azul indicador de color de iones de magnesio, proveyeron la confirmación visual de la amplificación de ADN de la mosca blanca en 45 min. Este ensayo fue muy específico y no amplificó el ADN de 8 especies adicionales crípticas de mosca blanca del camote o de 10 especies diferentes de Bemisia que se encuentran en la Florida. El ensayo amplificó el ADN de no-enfoque en una especie críptica de la mosca blanca del camote que no está presente en la Florida y tiene potencial para amplificar el ADN de MED raramente. Mientras que los genes adicionales deben ser utilizados para diseñar cebadores más específicos, en particular para el MED, este ensayo para el MEAM1 muestra ser prometedor como una herramienta básica para el campo que podría identificar rápidamente las especies de mosca blanca más comúnmente encontradas en la Florida.

Palabras Clave: biotipo B, biotipo Q, ADN, LAMP, identificación molecular, Trialeurodes vaporariorum

The sweetpotato whitefly, Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae), is a cryptic species complex representing at least 24 morphologically indistinguishable but reproductively isolated groups (Dinsdale et al. 2010; De Barro et al. 2011). Of these, the groups originating in the Middle-East Asia Minor (MEAM1), and Mediterranean (MED) regions, are globally invasive pests of hundreds of crop plant species (Oliveira et al. 2001), vectoring over 100 different plant viruses (Jones 2003). MEAM1 and MED have been referred to extensively in the literature as biotype B and biotype Q respectively (De Barro et al. 2011). Additionally MEAM1 causes nonviral disorders (Yokomi et al. 1990; Brown et al. 1995) and shows flexible reproductive behavior in the presence of MED, rendering it a superior competitor in direct interactions (Crowder et al. 2010). Despite this, MED is a more competent vector of at least one damaging virus (Pan et al. 2012) and shows dramatically elevated levels of resistance to neonicotinoid insecticides (Dennehy et al. 2010; Wang et al. 2010). This last biological difference has prompted different management recommendations for MED (Horowitz et al. 2005; Bethke et al. 2011) to prevent insecticide resistance from developing. But implementing these recommendations or making any decisions about plant quarantine during shipment inspections requires correct identification of the cryptic B. tabaci species.

Distinguishing among *Bemisia tabaci* cryptic species has been accomplished using a variety of genetic markers (Gawel & Bartlett 1993; Wool et al. 1993; Cervera et al. 2000; De Barro 2005) with particular attention recently to sequencing a portion of the mitochondrial cytochrome oxidase I (mt COI) gene (Boykin et al. 2007; Dinsdale et al. 2010; De Barro et al. 2011). However traditional molecular-based methods (e.g., polymerase chain reaction) for species discrimination require expertise in laboratory techniques and access to expensive laboratory equipment (e.g., thermocyclers). Additionally, the time required to send specimens to a biotyping lab and wait for a response is prohibitive to stakeholders who need to make decisions about pesticide applications or quarantined shipments in a matter of hours rather than days or weeks. Furthermore, Florida has a large number of recently invaded whitefly species (see Hodges 2007; Stocks 2012; Stocks & Hodges 2012), which further exacerbates the whitefly identification burden of plant quarantine inspectors. Therefore access to rapid, low cost, and accurate field applicable methods to differentiate these cryptic species would be highly beneficial to various stakeholders.

Loop-mediated isothermal amplification of DNA (LAMP) is a method to rapidly amplify a target DNA sequence using 4 to 6 specially designed primers (Notomi et al. 2000; Nagamine et al. 2002). This method overcomes 2 critical barriers to implementing DNA-based diagnostics in the field: temperature cycling to amplify DNA (Notomi et al. 2000) and high temperature DNA denaturing (Walker et al. 1992; Nagamine et al. 2002). Because of these features of LAMP, several recent applications of the technique have been demonstrated outside the traditional molecular biology laboratory (Boehme et al. 2007; Hatano et al. 2011). Recently, 2 research groups (Adachi et al. 2010; Hsieh et al. 2012) have reported success carrying out LAMP assays to distinguish between MEAM1 and MED cryptic species. Here we report advances in the use of LAMP to identify MEAM1 whiteflies, compare in-house designed primers to those previously published, and suggest avenues for future research to make a field-capable LAMP identification kit for both MEAM1 and MED.

METHODS

Samples

MED whiteflies from a colony maintained by Dr. Lance Osborne (Mid-Florida Research and Education Center, Apopka, Florida) on poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch; Malpighiales: Euphorbiaceae) MEAM1 whiteflies from a colony maintained by Dr. Cindy McKenzie on cotton (Gossypium sp.; Malvales: Malvaceae), and the greenhouse whitefly, Trialeuroides vaporariorum Westwood (Hemiptera: Aleyrodidae), provided by Dr. Scott Ludwig (Nichino America, Inc., Arp, Texas) were used as the source of genomic DNA extracted using a DNEasy blood and tissue kit (Qiagen, Valencia, California). This DNA was standardized to 7.5 ng/µL and used in initial in-house-designed primer screening and comparison among published primers (Table 1).

DNA was also extracted from 10 whitefly species invasive in the U.S. and additional cryptic species within *Bemisia tabaci* (Table 2) by boiling each individual insect in 100 µL lysis buffer

TABLE 1. LAMP PRIV	MERS.			
Target Species	Primer Set	Primer Type	Sequence	Source
MEAM1	J_{-B}	FIP	ACTTTATTCCACCCAAAGTAGCAAGAATTATTGCTGTTCCCCACAG	Adachi et al. 2010
		BIP	TAAGGCCTCTTGGCCTTTGAAATTCCAGTTAACCCAACCTA	
		F3	GATACTCGAGCTTATTTCACTT	
		B3	CACATCTACAGAAGAATTACCAA	
		LF	CCAACTAAAATTTTTAATTC	
		LB	ACAGGATTTTTTTTTTTTTTT	
	T_B	FIP	CCAAAGTAGCAAGCCAACTAAAAATACTCGAGCTTATTTCACTTCAG	Hseieh et al. 2012
		BIP	TAAGGCCTCTTGGCCTTTGAGAATAATTCCAGTTAACCCACC	
		F3	ATATTCACAGTTGGAATAGATGT	
		B3	TGTCATGCAGACACATC	
		LF	GTGGGAACAGCAATAATTATAGTGG	
		LB	ACAGGATTTTTTTTTTTTTTTAT	
	M_BI	FIP	GAATACCAATAGTCAATATAGCGTGAGGCTGGAAAATTAGAGG	In- House
		BIP	TCATCATATATTCACAGTTGGATAATTATAGTGGCTGAAGTGA	
		F3	GATTTGGAATTGTTTCTCATCT	
		B3	TTTAATTCCTGTGGGAACAG	
		LF	ATAATTATACCCAACCTTCCAAATA	
		LB	AATAGATGTAGATACTCGAGCTTAT	
	M_B2^*	FIP	AGAATACCAATAGTCAATATAGCGTGAGGCTGGAAAATTAGAGG	In- House
		BIP	GTCATCATATATTCACAGTTGGATAATTATAGTGGCTGAAGTGA	
		F3	GGATTTGGAATTGTTTCTCATCT	
		B3	TTTTAATTCCTGTGGGAACAG	
		LF	ATAAATTATACCCAACCTTCCAAATA	
		LB	AATAGATGTAGATACTCGAGCTTAT	
MED	g d	FIP	AGCATAAATTATCCCCCAACCTTTCCTCATTTAATTAGCAGCGAG	Adachi et al. 2010
		BIP	TCTTAGGGTTTTATTGTTTGAGGACAGCTGAAGTGAAATAAGCTCGA	
		F3	CTTATTTTACCAGGGTTTTGGAA	
		B3	TCCTGTAGGAACGGCAAT	
FID Formand Innow	Duimon			

FIP-Forward Inner Primer BIP-Backward Inner Primer LF-Forward Loop Primer LB-Backward Loop Primer F3-Forward Outer Primer B3-Backward Outer Primer *Sequence modified from initial primer screen *Primer set designed around the same mutations as primer set MB_{-1}

Downloaded From: https://complete.bioone.org/journals/Florida-Entomologist on 06 Jun 2025 Terms of Use: https://complete.bioone.org/terms-of-use

Target Species	Primer Set	Primer Type	Sequence	Source
	TQ	FIP	ACCCAAAGTAGCAAGCCAACTAAACAGCTACTATGATTATTGCCGT	Hseieh et al. 2012
		BIP	GTCCAATAAATTCAGGCCCCTTGGAATAATTCCAGTTAATCCACCTA	
		F3	GATACTCGAGCTTATTTCACTT	
		B3	ACATCTACAGGAGGTTACCAA	
	$M_{-}QI$	FIP	GTCCTCAAACAATAAACCCTAAGATACATTAGAGGTATTTTGGAAGGTTG	In- House
		BIP	TTACAGTTGGAATAGATGGATAGTAGCTCCTGTAGGAACGGCAATA	
		F3	TTAATTAGCAGCGAGGCT	
		B3	CCACCCAAAGTAGCAAGC	
	$MQ2^*$	FIP	GTCCTCAAACAATAAACCCTAAGATACAGGCTGGAAAATTAGAGGT	In- House
		BIP	TTACAGTTGGAATAGATGTAGATACTCCCTGTAGGAACGGCAATA	
		F3	GGGTTTTGGAATTGTTTGTCAT	
		B3	CCACCCAAAGTAGCAAGC	
		LF	AGCATAAATTATGCCCAACCTTCC	
		LB	AGCTTATTTCACTTCAGCTACTATG	
FIP-Forward Inne	r Primer			

FIT-Forward Inner Frimer BIP-Backward Inner Frimer LF-Forward Loop Primer LB-Backward Outer Primer F3-Forward Outer Primer B3-Backward Outer Primer *Sequence modified from initial primer screen *Primer set designed around the same mutations as primer set MB_{-I}

TABLE 1. (CONTINUED) LAMP PRIMERS.

Downloaded From: https://complete.bioone.org/journals/Florida-Entomologist on 06 Jun 2025 Terms of Use: https://complete.bioone.org/terms-of-use

Source	Morphological Species Cryptic species within <i>B. tabaci</i>		
P. Bruno	Bemisia tabaci	New World (Mexico)	
A. Bellotti	Bemisia tabaci	New World (Columbia)	
R. Ellison	Bemisia tabaci	Mediterranean (Florida)	
R. Oetting	Bemisia tabaci	Mediterranean (Georgia)	
R. Oetting	Bemisia tabaci	Mediterranean (Georgia)	
A. Hanafi	Bemisia tabaci	Mediterranean (Morocco)	
R. Ellison	Bemisia tabaci	Middle East-Asia Minor 1 (Florida)*	
K. Kijima	Bemisia tabaci	Middle East-Asia Minor 1 (Japan)	
H. Liansheng	Bemisia tabaci	Asia I	
J. Colvin	Bemisia tabaci	Asia I	
S. Liu	Bemisia tabaci	Asia II-1	
J. Colvin	Bemisia tabaci	Asia II-5	
K. Kijima	Bemisia tabaci	Asia II-6	
H. Liansheng	Bemisia tabaci	Asia II-7	
H. Delatte	Bemisia tabaci	Indian Ocean	
J. Colvin	Bemisia tabaci	Australia	
C. Williams	Bemisia tabaci	? (Malaysia)	
I. Stocks	Aleurodicus dugesii	n/a	
I. Stocks	Aleurodicus rugioperculatus	n/a	
L. Osborne	Dialurodes sp.	n/a	
I. Stocks	Metaleurodicus cardini	n/a	
I. Stocks	Paraleyrodes bondari	n/a	
P. Avery	Singhiella simplex	n/a	
J. Prokop	Tetraleurodes acaciae	n/a	
C. McKenzie	Trialeurodes abutilonea	n/a	
S. Ludwig	Trialeurodes vaporarorium	n/a	
L. Osborne	Trialeurodes variabilis	n/a	

TABLE 2. WHITEFLY SAMPLES SCREENED WITH LAMP ASSAY M_B1 .

*Denotes positive control reference sample for independent assessment of color change (see text)

(50mM KCl, 50mM Tris-HCl pH 8.4, 0.45% Tween 20, 0.45% NP40) for 5 min and then diluting 1/10 in water. These samples were used for further specificity testing with the M_B1 LAMP kit.

Primer Design and Initial Screen

Primer sets were designed to be specific to MEAM1 and MED consensus mt COI gene sequences using slight modifications from the default settings for AT rich templates in the webbased application Primer Explorer v4 http:// primerexplorer.jp/e/intro/index.html with polymorphisms coded as allowable mutations. Four primer sets were selected based on the presence of species specific residues occuring in the terminal 5 bases of the 5' end of the forward (F) target regions and the 3' end of the backward (B) target regions. No loop primers were initially designed. LAMP reactions for screening primer sets contained 1.6 M Betaine, 20 mM Tris-HCL (pH 8.8), 10 mM KCl, 10 mM (NH₄) SO₄, 8mM MgSO₄, 5.6 mM dNTPs, 0.1% Tween 20, 8U of Large Fragment Bst DNA Polymerase (Loopamp DNA amplification kit, Eiken Chemical Co. Ltd., Japan), 1.6u

M each inner primer, 0.2 μ M each outer primer (Life Technologies, Carlsbad, California), and 15 ng genomic DNA in a 25 μ L final volume. LAMP reactions were incubated in a thermocycler for 1 h at 58 °C and the polymerase was then inactivated at 95 °C for 2 min. Each primer set/DNA template combination was conducted in triplicate and amplification of DNA was validated via gel electrophoresis.

Comparison to Previously Published Primer Sets

One MED primer set from initial testing of $M_Q 1$, that was not specific to its target was modified in an attempt to make it specific by moving species-specific mutations and an additional nucleotide internal to the 5' terminus of the forward (F) target regions and the 3' of the backward (B) target regions (Table 1). One primer set specific to MEAM1, $M_B 1$, was left unmodified. Two additional primer sets, $M_B 2$ and $M_Q 2$, were designed around the same species-specific mutations as $M_B 1$ (Table 1). Loop primers were also designed where possible for all sets to make the reactions faster (Nagamine et al. 2002). These

were compared to 4 previously published primer sets for MEAM1 and MED (Adachi et al. 2010; Hsieh et al. 2012). The 8 primer sets (Table 1) were used in LAMP reactions containing 20 mM Tris-HCL (pH 8.8), 10 mM KCl, 10 mM (NH.) SO., 8 mM MgSO₄, 0.1% Triton X-100 (1 X ThermoPol buffer, New England Biolabs, Ipswich, Massachusetts), 0.8 M Betaine, 5.6 mM dNTPs, 0.1% Tween 20, 8 U of Large Fragment Bst DNA Polymerase (New England Biolabs), 0.8 µM each inner primer, 0.4 µM each loop primer (if present in set), 0.2 µM each outer primer (Life Technologies), 1 µL EvaGreen (Bioline, Tauton, Massachusetts) and 7.5 ng genomic DNA in a 25 µL final volume. LAMP reactions were incubated at 60 °C in a rotorgene 6000 (Corbett, Valencia, California) for 116 min with real-time fluoresecence data taken at ~27 sec intervals. The HRM channel was used for florescence detection. The time-to-amplification was taken as the measurement cycle where 80% of maximum sample fluorescence was obtained and then converted to min.

Multiple Species MEAM1 Specificity Testing Using the M_B1 LAMP Kit

Samples representing 10 whitefly species invasive in the U.S. and additional cryptic species within Bemisia tabaci species complex (Table 2) were screened for amplification with the M_B1 LAMP primer set. LAMP chemistry was as described in the primer set comparison experiment, however, 240 mM Hydroxynaphthol Blue (HNB) magnesium indicator dye replaced the EvaGreen fluorescent dye and 1 µL of template DNA was used. LAMP reactions were incubated at 60 °C for 45 min and then the polymerase was inactivated at 95 °C for 2 min. Successful LAMP reactions with HNB dye result in a color change from purple to blue (Goto et al. 2009). Samples were then assessed by 3 independent reviewers. Sample callers (classifiers) were asked if they could see a color difference between the MEAM1 sample from Florida (blue, positive control) and a water control (purple, negative control) and then asked to call the remaining samples as either blue or purple. Subsequently, the samples were verified with gel electrophoresis.

RESULTS AND DISCUSSION

Primer Design and Initial Screen

Initial screening indicated amplification of both species' DNA when using both MED primer sets and one MEAM1 primer set. The primer set M_B1 (minus loop primers) was found to be reliably specific, amplifying MEAM1 DNA but not MED DNA. None of the primer sets amplified the DNA from the greenhouse whitefly (see the supplementary document of Florida Entomologist 96(3) (2013) online at http://purl.fcla.edu/fcla/entomologist/browse).

Comparison of In-House Designed Primer Sets to Previously Published Primer Sets

Table 3 illustrates that each of the LAMP primer sets designed to amplify MEAM1 or MED DNA (respectively) amplified its respective target template DNA earlier than non-target template DNA. The difference between time-to-amplification of target and non-target DNA templates was larger for sets designed to amplify MEAM1 DNA at 58 min or more than those designed to amplify MED DNA at 28 min or less. The primer set M_{-} B1, with loop primers, amplified target MEAM1 DNA in 28 min and had not amplified any nontarget MED DNA after 116 min (Fig. 1). MED primer sets previously published labeled J_Q and T_Q respectively (Adachi et al. 2010; Hsieh et al. 2012) had a time-delay between the amplification of target and non-target templates that was ~4× greater than those of the primer sets designed in-

TABLE 3. Specificity of 8 lamp primer sets inferred by time-to-amplification of target and non-target dna templates using real-time fluorescence data. Primer set M_B1 amplified target MEAM1 DNA in 28 min and had not amplified any non-target MED DNA after 116 min.

		Time to Amplification (min)		
Target species	Primer Set	MEAM1 DNA	MED DNA	Lag Time
MEAM1	J_B	104.6	N/A	N/A
	T_B	38.7	97	58.3
	M_B1	28	N/A	N/A
	M_B2	25.4	90.3	64.9
MED	J_Q	57.4	32.9	24.5
	T_Q	77	49.4	27.6
	M_Q1	40.9	34.7	6.2
	M_Q2	24	17.4	6.6

Downloaded From: https://complete.bioone.org/journals/Florida-Entomologist on 06 Jun 2025 Terms of Use: https://complete.bioone.org/terms-of-use



Fig. 1. Real-time loop-mediated amplification of target DNA in 28 min with the M_B1 LAMP kit.

house, but they still had less than ½ of the timedelays found for MEAM1 primer sets. The ability of most primer sets to amplify non-target DNA, often with 30 min or less of additional incubation time, suggests that these primer sets come with some risk for non-target amplification as was shown in both previous studies of LAMP in *Bemisia* (Adachi et al. 2010; Hsieh et al. 2012), particularly for primer sets designed for MED. In general, the MEAM1 primer sets from all research groups appear promising for the potential in-field detection applications.

Multiple Species MEAM1 Specificity Testing Using the M_B1 LAMP KIT

In the specificity screen, the 2 MEAM1 samples produced positive LAMP reactions indicated by color change from purple to blue (not shown, but see an example color change in the supplementary document of Florida Entomologist 96(3) (2013) online at http://purl.fcla.edu/fcla/entomologist/browse) and expected ladder of LAMP DNA products visible on the agarose gel (Fig. 2, lanes 2 and 6). One of the MEAM1 samples was used as a positive control to train sample callers, and the color change from purple to blue in the second MEAM1 sample was corroborated independently by all 3 sample callers. The Asia II-1 sample (Table 2) was called as positive by 2 of 3 sample callers, and also had the expected gel banding pattern (Fig. 2, lane 3). The samples of the remaining whitefly species were negative for both a color change and lacked LAMP products on an electrophoresis gel (Fig. 2; lanes 4-5, 7-20, 22-29). The 3 independent sample callers were in agreement for 96% (25 of 26) non-reference samples.

The HNB color indicator dye system incorporated to the M_B1 LAMP kit was highly consistent across independent readers with no prior experience. Other researchers have also noted that HNB is comparable (Hill et al. 2008) or preferable (Wastling et al. 2010) to other color indicator methods. This LAMP kit, when coupled with the color indicator dye HNB, amplified MEAM1 DNA targets in 45 min, an improvement over traditional PCR including gel electrophoresis, which generally requires 2-3 h.



Fig. 2. LAMP products from *B. tabaci* group MEAM1 (lanes 2 and 6) and from the non-invasive *B. tabaci* group Asia II-1 (lane 3) amplified using the M_BI LAMP assay. The LAMP assay was negative for all other DNA templates including 10 invasive whitefly species in Florida (lanes 5, 9, 13, 16, 17, 20, 22, 25, 26, and 29), 4 different *B. tabaci* group MED haplotypes (lanes 10, 14, 18, and 23), 2 different *B. tabaci* New World group haplotypes (lanes 12 and 27), and the non-invasive groups within the *B. tabaci* cryptic species complex (lanes 4, 7, 8, 11, 15, 19, 24, and 28). Three MED haplotypes are invasive in Florida and the New World haplotype is native to Florida. See Table 2 for samples screened. Lanes 1 and 21 contain a size standard; NC-negative control.

The M_B1 LAMP kit appears to be highly specific to MEAM1, however the Asia II-1 cryptic species within the *B. tabaci* complex was also amplified. Asia II-1 is native to China and not invasive in the U.S. This sample was called positive by 2 of 3 sample callers; however, 1 of these callers believed the color to be intermediate and therefore ambiguous. The New World cryptic species, which is native to the U.S., was not amplified, nor were any of the other invasive whitefly species established in Florida (Fig. 2).

Specificity and Risk of Non-Target Lamp Amplification with Similar Template Sequences

The small lag time between time-to-amplification of target and non-target DNA templates raises questions about the risk of obtaining a false positive. As a measure of this risk of nonspecificity, researchers often screen a population with LAMP and compare the results to an established method such as PCR (Seki et al. 2005; Bonizzoni et al. 2009; Hsieh et al. 2012). A different approach is to test the same DNA template multiple times with real-time LAMP. We have consistently found that the variance in the timeto-amplification is lower for target DNA than for non-target DNA by several orders of magnitude for M_B1 , M_B2 , and T_Q primer sets (see supplementary document of Florida Entomologist 96(3) (2013) online at http://purl.fcla.edu/fcla/

entomologist/browse). In one experiment, The *M* B1 averaged more than 2 h to amplify MED DNA but amplified one of the replicates of this DNA sample in only 39 min. This high variance in time-to-amplification of non-target (but similar in sequence) DNA may be due in part to the probabilistic nature of sub-optimal binding of the inner primers to the template. It should also be noted that time-to-amplification increases with a decrease in template concentration (Notomi et al. 2000) meaning the risk of obtaining a false negative due to insufficient incubation time increases when using samples of an unknown concentration, and this is likely to be the case in the field (Hsieh et al. 2012). For this reason, LAMP primers designed from other genes more divergent among invasive cryptic species than mt COI, may show greater specificity for both species and lower risk of errors than all currently available based on mt COI. The recently published comparative transcriptome of MEAM and MED (Wang et al. 2011) provides a large number of promising gene targets that could be leveraged to this end.

Progress Towards an In-Field Whitefly Detection Assay

Despite the risk of occasional false positives due to high variance in MED amplification, and some risk of false negatives due to uncertainty about template concentration in the field, a MEAM1-specific identification test based on the M_B1 LAMP primers would be highly beneficial to both the greenhouse industry and plant guarantine inspectors. This test would help growers guard against pesticide resistance developing in newly invasive MED by confirming the presence of the more common MEAM1 in their facility. A confirmation of MEAM1 presence in their facility would greatly expand their repertoire of indicated products for chemical control (Bethke et al. 2011). In the same way, a stand-alone MEAM1 LAMP test would be particularly useful for regulatory and quarantine officials. Whiteflies are often identified based on the immature stage yet this stage is often not present in shipments seized because of plant quarantine concerns. In this case, officials may be forced to destroy the entire shipment if any whitefly is present while a MEAM1 test alone would give these stakeholders the means to screen out the majority of Aleyrodidae detections as a species long established in the U.S. and therefore not of quarantine concern. While it seems wise to pursue different gene targets for LAMP with increasingly greater specificity, a second thrust should be to prepare the existing HNB color indicator M_B1 LAMP assay for field demonstration.

ACKNOWLEDGMENTS

We are grateful for all researchers who provided whitefly samples; Keisuke Kijima, Hélène Delatte, and John Colvin provided samples specifically for this study. We thank J. Kent Morgan and Mariangella Bonizzoni for helpful comments on earlier drafts of this manuscript. We also thank John Prokop and Carol Malone for technical support. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

References Cited

- ADACHI, T., UMEZAWA, T., YAMAGUCHI, H., KITAMURA, T., HONDA, K., SHIBAO, M., NASU, Y., AND TANAKA, H. 2010. Identification of *Bemisia tabaci* B and Q biotypes in Osaka Prefecture by DNA sequencing and a LAMP method. Annu. Rep. Kansai Plant Protect. Soc. 52: 103-104.
- BETHKE, J., CANAS, L., CHAMBERLIN, J., CLOYD, R., DOBBS, J., FLETCHER, R., FUJINO, D., GILREIN, D., LINDQUIST, R., LUDWIG, S., MCKENZIE, C., OET-TLING, R., OSBORNE, L., PALMER, C., AND SANDER-SON, J. 2011. Whitefly (*Bemisia tabaci*) management program for ornamental plants. http://www.mrec. ifas.ufl.edu/LSO/bemisia/bemisia.htm.
- BOEHME, C. C., NABETA, P., HENOSTROZA, G., RAQIB, R., RAHIM, Z., GERHARDT, M., SANGA, E., HOELSCHER, M., NOTOMI, T., HASE, T., AND PERKINS, M. D. 2007. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J. Clin. Microbiol. 45: 1936.
- BONIZZONI, M., AFRANE, Y., AND YAN, G. 2009. Loopmediated isothermal amplification (LAMP) for rapid identification of *Anopheles gambiae* and *Anopheles arabiensis* mosquitoes. American J. Trop. Med. Hyg. 81: 1030-1034.
- BOYKIN, L. M., SHATTERS JR, R. G., ROSELL, R. C., MCKENZIE, C. L., BAGNALL, R., DE BARRO, P., AND FROHLICH, D. R. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using bayesian analysis of mitochondrial COI DNA sequences. Mol. Phylogenet. Evol. 44: 1306-1319.
- BROWN, J. K., FROHLICH, D. R., AND ROSELL, R. C. 1995. The Sweetpotato or Silverleaf Whiteflies: Biotypes of Bemisia tabaci or a Species Complex? Annu. Rev. Entomol. 40: 511-534.
- CERVERA, M. T., CABEZAS, J. A., SIMON, B., MARTINEZ-ZAPATER, J. M., BEITIA, F., AND CENIS, J. L. 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. Bull. Entomol. Res. 90: 391-396.
- CROWDER, D. W., SITVARIN, M. I., AND CARRIERE, Y. 2010. Plasticity in mating behaviour drives asymmetric reproductive interference in whiteflies. Animal Behav. 79: 579-587.
- DE BARRO, P. J. 2005. Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. Mol. Ecol. 14: 3695-3718.
- DE BARRO, P. J., LIU, S. S., BOYKIN, L. M., AND DIN-SDALE, A. B. 2011. *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56: 1-19.
- DENNEHY, T. J., DECAIN, B. A., HARPOLD, V. S., ZABO-RAC, M., MORIN, S., FABRICK, J. A., NICHOLS, R. L., BROWN, J. K., BYRNE, F. J., AND XIANCHUN, L. I. 2010. Extraordinary resistance to insecticides re-

veals exotic Q biotype of *Bemisia tabaci* in the New World. J. Econ. Entomol. 103: 2174-2185.

- DINSDALE, A., COOK, L., RIGINOS, C., BUCKLEY, Y. M., DE BARRO, P., AND DINSDALE, A. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Ann. Entomol. Soc. Am. 103: 196-208.
- GAWEL, N. J., AND BARTLETT, A. C. 1993. Characterization of differences between whiteflies using RAPD-PCR. Insect Mol. Biol. 2: 33-38.
- GOTO, M., HONDA, E., OGURA, A., NOMOTO, A., AND HANAKI, K. I. 2009. Colorimetric detection of loopmediated isothermal amplification reaction by using hydroxy naphthol blue. BioTechniques 46: 167-172.
- HATANO, B., GOTO, M., FUKUMOTO, H., OBARA, T., MAKI, T., SUZUKI, G., YAMAMOTO, T., HAGISAWA, K., MATSUSHITA, Y., FUJII, T., IMAKIIRE, T., KIKUCHI, Y., TAKAHASHI, R., KANAI, M., TAMURA, K., IZUMI, T., TAKAHASHI, Y., IWAMOTO, Y., MIMURA, S., MUKAI, Y., TAKITA, K., TAKEO, H., KITAMURA, R., SHIMIZU, E., FUKUSHIMA, K., HAKOZAKI, Y., UEHATA, A., SAKAI, M., OHSHIMA, S., SHIROTANI, T., OBA, K., HASEGAWA, H., SATA, T., AND KATANO, H. 2011. Mobile and accurate detection system for infection by the 2009 pandemic influenza A (H1N1) virus with a pocket-warmer reverse-transcriptase loop-mediated isothermal amplification. J. Med. Virol. 83: 568-573.
- HILL, J., BERIWAL, S., CHANDRA, I., PAUL, V. K., KAPIL, A., SINGH, T., WADOWSKY, R. M., SINGH, V., GOYAL, A., JAHNUKAINEN, T., JOHNSON, J. R., TARR, P. I., AND VATS, A. 2008. Loop-mediated isothermal amplification assay for rapid detection of common strains of Escherichia coli. J. Clin. Microbiol. 46: 2800-2804.
- HODGES, G. 2007. Pest Alert: The fig whitefly *Singhiella simplex* (Singh) (Hemiptera: Aleyrodidae): A new exotic whitefly found on ficus species in south Florida. http://www.freshfromflorida.com/pi/pest-alerts/singhiella-simplex.html.
- HOROWITZ, A. R., KONTSEDALOV, S., KHASDAN, V., AND ISHAAYA, I. 2005. Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Arch. Insect Biochem. Physiol. 58: 216-225.
- HSIEH, C. H., WANG, H. Y., CHEN, Y. F., AND KO, C. C. 2012. Loop-mediated isothermal amplification for rapid identification of biotypes B and Q of the globally invasive pest, *Bemisia tabaci*, and studying population dynamics. Pest Mgt. Sci. 68: 1206-1213.
- JONES, D. R. 2003. Plant viruses transmitted by whiteflies. European .J Plant Pathol. 109: 195-219.
- NAGAMINE, K., HASE, T., AND NOTOMI, T. 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers* 1. Mol. Cell Probes 16: 223-229.

- NOTOMI, T., OKAYAMA, H., MASUBUCHI, H., YONEKAWA, T., WATANABE, K., AMINO, N., AND HASE, T. 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28: e63.
- OLIVEIRA, M., HENNEBERRY, T., AND ANDERSON, P. 2001. History, current status, and collaborative research projects for *Bemisia tabaci** 1. Crop Prot. 20: 709-723.
- PAN, H., CHU, D., YAN, W., SU, Q., LIU, B., WANG, S., WU, Q., XIE, W., JIAO, X., LI, R., YANG, N., YANG, X., XU, B., BROWN, J. K., ZHOU, X., AND ZHANG, Y. 2012. Rapid spread of *tomato yellow leaf curl virus* in China is aided differentially by two invasive whiteflies. PLoS ONE 7: e34817.
- SEKI, M., YAMASHITA, Y., TORIGOE, H., TSUDA, H., SATO, S., AND MAENO, M. 2005. Loop-mediated isothermal amplification method targeting the lytA gene for detection of Streptococcus pneumoniae. J. Clin. Microbiol. 43: 1581.
- STOCKS, I. C. 2012. Pest Alert: Bondar's nesting whitefly, *Paraleyrodes bondari*, a whitefly (Hemiptera: Aleyrodidae) new to Florida attacking ficus and other hosts. http://www.freshfromflorida.com/pi/pestalerts/pdf/paraleyrodes-bondari.pdf.
- STOCKS, I. C., AND HODGES, G. 2012. Pest Alert: The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, a new exotic whitefly in south Florida (Hemiptera: Aleyrodidae). http://www.freshfromflorida. com/pi/pest-alerts/pdf/aleurodicus-rugioperculatuspest-alert.pdf.
- WALKER, G. T., FRAISER, M. S., SCHRAM, J. L., LITTLE, M. C., NADEAU, J. G., AND MALINOWSKI, D. P. 1992. Strand displacement amplification - an isothermal, *in vitro* DNA amplification technique. Nucleic Acids Res. 20: 1691-1696.
- WANG, X. W., LUAN, J. B., LI, J. M., SU, Y. L., XIA, J., AND LIU, S. S. 2011. Transcriptome analysis and comparison reveal divergence between two invasive whitefly cryptic species. BMC Genomics 12: 458.
- WANG, Z., YAN, H., YANG, Y., AND WU, Y. 2010. Biotype and insecticide resistance status of the whitefly *Bemisia tabaci* from China. Pest Mgt. Sci. 66: 1360-1366.
- WASTLING, S. L., PICOZZI, K., KAKEMBO, A. S. L., AND WELBURN, S. C. 2010. LAMP for human African trypanosomiasis: A comparative study of detection formats. PLoS Neglected Tropical Diseases 4.
- WOOL, D., GERLING, D., BELLOTTI, A. C., AND MORALES, F. J. 1993. Esterase electrophoretic variation in *Bemisia tabaci* (Genn.)(Hom., Aleyrodidae) among host plants and localities in Israel. J. Appl. Entomol. 115: 185-196.
- YOKOMI, R. K., HOELMER, K. A., AND OSBORNE, L. S. 1990. Relationship between the sweet-potato whitefly and the squash silverleaf disorder. Phytopathology 80: 895-900.