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

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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, *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 PRIMERS.

Target Species	Primer Set	Primer Type	Sequence	Source
MEAM1	*J_B*	FIP	ACTTTATTCCACCCAAAGTAGCAAGAATTATTGCTGTTCCCACAG	Adachi et al. 2010
		BIP	TAAGGCCTCTTGGCCTTTGAAATTCCAGTTAACCCACCTA	
		F3	GATACTCGAGCTTATTTCACTT	
		B3	CACATCTACAGAAGAATTACCAA	
		LF	CCAACTAAAAATTTTAATTC	
		LB	ACAGGATTTTTATTTTTATT	
	T_B	FIP	CCAAAGTAGCAAGCCAACTAAAAATACTCGAGCTTATTTCACTTCAG	Hseieh et al. 2012
		BIP	TAAGGCCTCTTGGCCTTTGAGAATAATTCCAGTTAACCCACC	
		F3	ATATTCACAGTTGGAATAGATGT	
		B3	TGTCATGCAGACACACATC	
		LF	GTGGGAACAGCAATAATTATAGTGG	
		LB	ACAGGATTTTTATTTTTTATTTA	
	M_B1	FIP	GAATACCAATAGTCAATATAGCGTGAGGCTGGAAAATTAGAGG	In- House
		BIP	TCATCATATATTCACAGTTGGATAATTATAGTGGCTGAAGTGA	
		F3	GATTTGGAATTGTTTCTCATCT	
		B3	TTTAATTCCTGTGGGAACAG	
		LF	ATAAATTATACCCAACCTTCCAAATA	
		LB	AATAGATGTAGATACTCGAGCTTAT	
	*M_B2**	FIP	AGAATACCAATAGTCAATATAGCGTGAGGCTGGAAAATTAGAGG	In- House
		BIP	GTCATCATATATTCACAGTTGGATAATTATAGTGGCTGAAGTGA	
		F3	GGATTTGGAATTGTTTCTCATCT	
		B3	TTTTAATTCCTGTGGGAACAG	
		LF	ATAAATTATACCCAACCTTCCAAATA	
		LB	AATAGATGTAGATACTCGAGCTTAT	
MED	*J_Q*	FIP	AGCATAAATTATCCCCAACCTTCCTTTCTCATTTAATTAGCAGCGAG	Adachi et al. 2010
		BIP	TCTTAGGGTTTATTGTTTGAGGACAGCTGAAGTGAAATAAGCTCGA	
		F3	CTTATTTTACCAGGGTTTGGAA	
		B3	TCCTGTAGGAACGGCAAT	

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*

TABLE 1. (CONTINUED) LAMP PRIMERS.

Target Species	Primer Set	Primer Type	Sequence	Source
	T_Q	FIP	ACCCAAAGTAGCAAGCCAACTAAACAGCTACTATGATTATTGCCGT	Hseieh et al. 2012
		BIP	GTCCAATAAATTCAGGCCCCTTGGAATAATTCCAGTTAATCCACCTA	
		F3	GATACTCGAGCTTATTTCACTT	
		B3	ACATCTACAGAAGAGTTACCAA	
	M_Q1	FIP	GTCCTCAAACAATAAACCCTAAGATACATTAGAGGTATTTGGAAGGTTG	In- House
		BIP	TTACAGTTGGAATAGATGTAGATACTCCCTGTAGGAACGGCAATA	
		F3	TTAATTAGCAGCGAGGCT	
		B3	CCACCCAAAGTAGCAAGC	
	*M_Q2**	FIP	GTCCTCAAACAATAAACCCTAAGATACAGGCTGGAAAATTAGAGGT	In- House
		BIP	TTACAGTTGGAATAGATGTAGATACTCCCTGTAGGAACGGCAATA	
		F3	GGGTTTGGAATTGTTTCTCAT	
		B3	CCACCCAAAGTAGCAAGC	
		LF	AGCATAAATTATGCCCAACCTTCC	
		LB	AGCTTATTTCACTTCAGCTACTATG	

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*

TABLE 2. WHITEFLY SAMPLES SCREENED WITH LAMP ASSAY *M_B1*.

Source	Morphological Species	Cryptic species within *B. tabaci*
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

*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 web-based 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 $(NH_4)_2SO_4$, 8mM $MgSO_4$, 5.6 mM dNTPs, 0.1% Tween 20, 8U of Large Fragment *Bst* DNA Polymerase (Loopamp DNA amplification kit, Eiken Chemical Co. Ltd., Japan), 1.6µM each inner primer, 0.2 µM each outer primer (Life Technologies, Carlsbad, California), and 15 ng genomic DNA in a 25 µL 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_Q1*, 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_B1*, was left unmodified. Two additional primer sets, *M_B2* and *M_Q2*, were designed around the same species-specific mutations as *M_B1* (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 $(NH_4)_2SO_4$, 8 mM $MgSO_4$, 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 non-target 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

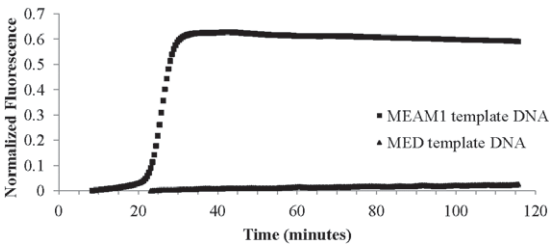

Fig. 1. Real-time loop-mediated amplification of target DNA in 28 min with the *M_B1* LAMP kit.

house, but they still had less than ½ of the time-delays 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.

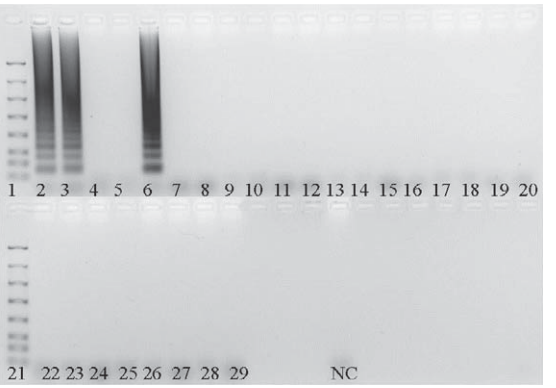

Fig. 2. LAMP products from *B. tabaci* group MEAM1 (lanes 2 and 6) and from the non-invasive *B. tabaci* group Asia II-1 (lane 3) amplified using the *M_B1* LAMP assay. The LAMP assay was negative for all other DNA templates including 10 invasive whitefly species in Florida (lanes 5, 9, 13, 16, 17, 20, 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 non-specificity, 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 time-to-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 quarantine 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.

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