

Development of an on-site diagnostic LAMP assay for rapid differentiation of the invasive pest *Phthorimaea absoluta* (Meyrick) using insect tissues

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Abstract

BACKGROUND: The tomato leafminer, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a destructive invasive pest that originated in South America and has spread within China since 2017. A rapid method for on-site identification of *P. absoluta* is urgently needed for interception of this pest across China.

RESULTS: We developed a loop-mediated isothermal amplification (LAMP) technique to differentiate *P. absoluta* from *Liriomyza sativae*, *Chromatomyia horticola*, and *Phthorimaea operculella* using extracted genomic DNA, which was then refined to create an on-site LAMP diagnostic method that can be performed under field conditions without the need for laboratory equipment.

CONCLUSION: In the present research, we developed an on-site diagnostic method for rapid differentiation of *P. absoluta* from other insects with similar morphology or damage characteristics in China.

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Keywords: loop mediated isothermal amplification (LAMP); *Phthorimaea absoluta*; invasive species; on-site diagnostics

1 INTRODUCTION

The tomato leafminer, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a destructive insect pest native to South America.¹ It mainly damages tomato, but can also harm other Solanaceae crops, including potatoes, eggplants, and chili peppers.² It has spread globally, mainly through agricultural trade activities (especially of tomatoes and fruits), and it was reported in 105 countries as of February 2021: ten in South America, three in North America, 31 in Europe, 35 in Africa, and 26 in Asia.³ The damage caused to tomato and vegetable crops is mainly through larval feeding of aboveground tissues at all developmental stages. If not prevented, such damage can result in 80–100% yield losses in tomato, with significant economic consequences. In the Netherlands, for example, *P. absoluta* causes direct economic losses to tomato production of approximately \$6.2–\$155 million annually. The chemical control of *P. absoluta* increases 13–15 fold from one season to the next, resulting in control costs of up to \$4.96 million.^{4,5}

Phthorimaea absoluta was first discovered in China in Ili, Xinjiang in 2017 and had spread to 13 provinces by March 2022.⁶ It is imperative that its further spread in China is curtailed to prevent potentially devastating damage in vegetable-producing provinces such as Shandong,⁷ which has the largest vegetable glasshouse production system and is the largest export center for vegetables (including fresh market vegetables and cherry tomatoes) in China.

The invasion of an alien species typically occurs in four phases: introduction, establishment, spread, and outbreak. Established populations are difficult to eradicate. Curbing the spread of an invasive species therefore relies on early surveillance and monitoring, which enable rapid decision-making and formulation of appropriate prevention and control strategies. Rapid early detection and accurate identification of non-indigenous species are

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crucial for preventing their establishment and expansion.^{8–10} Unfortunately, it is difficult to identify *P. absoluta* accurately on the basis of morphological or feeding characteristics in the field.¹¹ Most field samples are not fresh and are collected on trap boards or in traps, resulting in poor-quality samples or mixtures of morphologically similar pests. Its damage pattern is similar to that of other common leafminer pests (*Liriomyza sativae* and *Chromatomyia horticola*), and also has similar morphological characteristics to those of *Phthorimaea operculella*, making their field identification, especially of larvae, difficult.^{12–14} Morphological confirmation of *P. absoluta* involves making a slide mount of the genitalia, which requires two or more hours of preparation by a trained taxonomist.¹⁵ To date, a number of molecular identification methods have been developed in the laboratory,^{11,12,14,16,17} but most cannot be used for field identification. Loop-mediated isothermal amplification (LAMP) is a rapid, efficient, and specific detection technique, that involves DNA amplification at isothermal temperatures and requires only a water bath or metal heating apparatus for the reaction. The simple and rapid LAMP process is amenable to field use¹⁸ and has been applied to many insect pests.^{8,19–27}

The aim of the present research was to develop an on-site diagnostic method for rapid differentiation of *P. absoluta* from morphologically similar insects or species with similar damage characteristics in China. We first developed a LAMP method to differentiate *P. absoluta* from *Liriomyza sativae*, *C. horticola*, and *P. operculella* using extracted genomic DNA. We then optimized this diagnostic method for field deployment using only insect tissue and a temperature-controlled, insulated cup. This study thus describes an on-site LAMP diagnostic method that can be performed under field conditions without specialized laboratory equipment.

2 MATERIALS AND METHODS

2.1 Sample collection and DNA extraction

Samples of *P. absoluta* were collected from Midu, Yunnan Province and Tianjin, China. *Liriomyza sativae*, *C. horticola*, and *P. operculella* were collected from Laiyang, Jinan, Anqiu of Shandong Province, China.²⁸ All sampled insects were soaked in 100% alcohol and stored at -20°C for subsequent analysis. Genomic DNA was extracted from the samples using the TIANamp Genomic DNA Kit (Tiangen) according to the manufacturer's instructions. The extracted DNA was either used immediately for LAMP assays or stored at -20°C for further experiments.

2.2 LAMP primer design

Mitochondrial cytochrome oxidase subunit I (COI) sequences of *P. absoluta* (target) and other common leafminer pests (non-targets) were downloaded from the National Center for Biotechnology Information (NCBI) database: *P. absoluta* (MN066591.1), *P. operculella* (MF121882.1), *Liriomyza sativae* (KF962594.1), *C. horticola* (KC136060.1), *Liriomyza chinensis* (LC577649.1), and *Lyonetia clerkella* (MZ610728.1). The sequences were aligned using CLUSTAL W (<https://www.genome.jp/tools-bin/clustalw>), and specific points were marked with ESPript3.0 software (<https://esprict.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>). Specific primers for *P. absoluta* COI (MN066591.1) were designed using PrimerExplorer V5 (<http://primerexplorer.jp/lampv5e/index.html>), and nine final primer sets were selected from more than 200 potential sets after exclusion of highly repetitive primers. The primers were synthesized by Beijing Qingke Biotech, Beijing,

Table 1. Specific loop-mediated isothermal amplification (LAMP) primer sets used for *Phthorimaea absoluta*

Primer	Sequence (5'–3')
F3-562	AGAATCGTAGAAATGGAGCA
B3-562	AGCAGTAATACCAACAGCT
FIP-562	CTGAACCTACCTCCATGAGCAAGGTACTGGTGAACGTCT
BIP-562	CTGGTATTTTCATCGATTTTAGAGC ATCAAATGAAAGTCCATTAACCTCG

China, for subsequent experimental verification. The information of the selected *P. absoluta*-specific primers is listed in Table 1.

2.3 Reaction system and conditions

The 25- μL polymerase chain reaction (PCR) system included 12.5 μL 2 \times LAMP master mix (Shenggong Biotechnology, Shanghai, China), 0.5 μL each F3/B3 primers (10 μM), 4 μL each FIP/BIP primers (10 μM), 0.5 μL DNA polymerase (Shenggong Biotechnology, Shanghai, China), 1 μL template DNA, and sterilized double-distilled water (ddH₂O; Shenggong Biotechnology) to 25 μL . A movable diaphragm was placed at the upper end of the reaction tube and was not in contact with the reaction system, thus preventing the nucleic acid dye from mixing with the reaction system in advance, which often causes inaccurate results. Prior to amplification, a 0.5–1 μL drop of SYBR Green I (Solarbio) nucleic acid dye was placed in the cap of the reaction tube. The tube was placed in a constant-temperature water bath at 63°C for 45 min for the reaction and at 80°C for 10 min to inactivate the enzyme. After amplification, the reaction tube was briefly centrifuged or the SYBR Green I dye briefly shaken into the bottom of the tube and mixed with the reaction solution.

2.4 Detection of LAMP products

The LAMP products were separated by 1% agarose gel electrophoresis, and positive samples exhibited clear ladder-like bands on the gel.²³ Colorimetric determination of positive LAMP reactions was performed by observing the change in SYBR Green I from colorless to green.

2.5 Specificity and sensitivity of the LAMP assay

Specificity of the LAMP primers was tested using DNA extracted from adult and larval *P. absoluta* collected in several geographic regions and from adult insects of *Liriomyza sativae*, *C. horticola*, and *P. operculella* (non-target samples). The sterile ddH₂O was used as the non-template control. Meanwhile, the results were confirmed using the species-specific COI primers of *P. absoluta*.¹²

Sensitivity of the primers was tested using various concentrations of *P. absoluta* template DNA. The initial concentration of the extracted *P. absoluta* DNA was 83.05 ng/ μL , and this was used as the template stock solution. Furthermore, 1 μL aliquots of the stock solution were used to prepare a ten-fold dilution series (10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) by sequential addition of 9 μL ddH₂O. The different concentrations of template DNA were used for LAMP reactions, with sterile ddH₂O as the blank control. After the reaction, 1 μL of SYBR Green I was added. Positive reactions showed visible green fluorescence, whereas negative reactions appeared orange.

2.6 LAMP assay using insect tissue and an insulated cup

Confirmation of the presence of *P. absoluta* at monitoring sites such as large glasshouses and green spaces, require use of tissues such as abdomen, legs, and so forth. from mixed samples of pests collected from traps. We therefore assessed the performance of the LAMP assay with adult or larval tissues (about one half of a leg, one half of an abdomen, or one third to one half of a larva) of *P. absoluta* placed directly into the LAMP reaction mixture, replacing template DNA. For ease of field use, constant temperatures were maintained by replacing the laboratory water bath with a portable, insulated, temperature-controlled hot-water cup as shown later (USB smart cup, model TXJ-U1 with adjustable temperature; Tan Xun Jia Co, Zhongshan, Guangdong, China). The cup was constructed with a double-layer vacuum to provide 24-h insulation, and the temperature of the water in the cup was controlled through a USB-C interface using the manufacturer's application installed on a cellular phone. Individual reaction tubes were

placed in a circular holder within the cup. All other aspects of the assay remained the same, and the entire process took approximately 1 h.

In field applications, insect tissues may not be processed immediately after field collection. We therefore assessed the effects of sample storage duration on the results obtained with the LAMP assay. Insect samples were stored under indoor conditions ($27 \pm 2^\circ\text{C}$, 16 h:8 h light/dark photoperiod, $60 \pm 10\%$ relative humidity) for 1, 2, or 3 weeks, then used for LAMP assays as described earlier.

3 RESULTS

3.1 Specificity of the LAMP assay using genomic DNA

The COI gene was used to design nine sets of specific LAMP primers, using the *P. absoluta* sequence as the target and those of *Liriomyza sativae*, *Liriomyza chinensis*, and *P. operculella* as negative controls. All tested *P. absoluta* individuals produced amplified products in the LAMP assay, with amplification occurring within 1 h. None of the other tested species, including closely related leafminer species, produced any amplification within 1 h. Agarose gel electrophoresis revealed that the products of positive LAMP reactions showed a typical ladder-like appearance (Fig. 1), and no amplification was observed in the negative control reactions without the DNA template. All samples were tested in at least three independent runs, which produced comparable results. Among the nine validated primers, only one set had a 100% amplification for *P. absoluta* DNA and had no amplification for *Liriomyza sativae*, *C. horticola*, and *P. operculella* samples, which were used as negative controls. The results were confirmed by the species-specific COI primers,¹² which was consistent with the LAMP method in this experiment (Fig. 2). We therefore used this specific LAMP primer set for *P. absoluta* in all subsequent work.

3.2 Sensitivity of the LAMP assay using genomic DNA

To assess its sensitivity, the LAMP assay was next performed using a ten-fold dilution series of *P. absoluta* template DNA (Fig. 3). Reaction solutions with DNA concentrations of 0.83 ng/ μL or higher

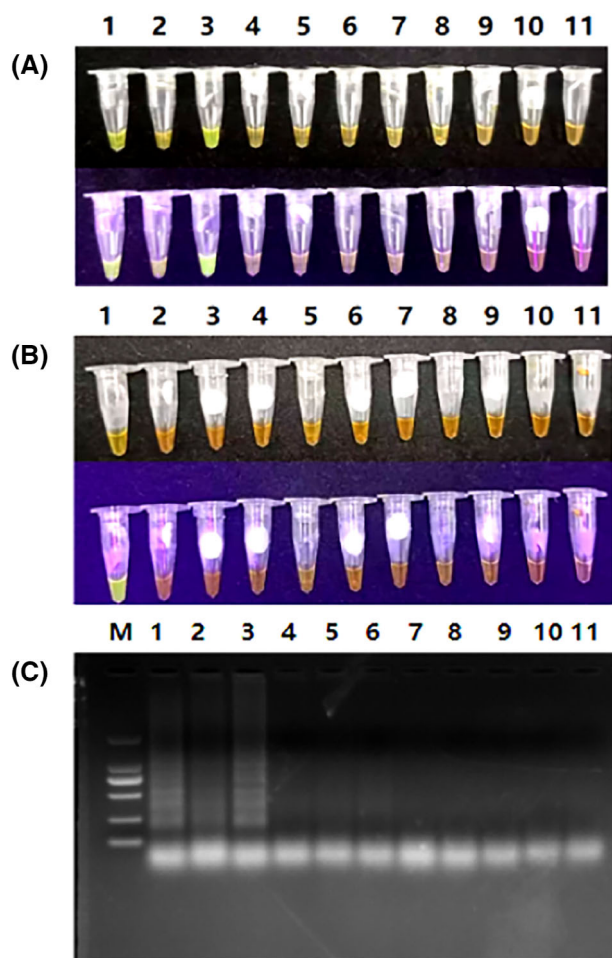


Figure 1. *Phthorimaea absoluta* specificity testing loop-mediated isothermal amplification (LAMP). The LAMP assay was performed with extracted DNA from *P. absoluta* and other pest species. (A) *Phthorimaea absoluta* (1–3), *Liriomyza sativae* (4–6), *Chromatomyia horticola* (7–9), and sterile double-distilled water (ddH₂O) (10, 11). (B) *Phthorimaea absoluta* (1), *Liriomyza sativae* (2–4), *C. horticola* (5–7), *Phthorimaea operculella* (8–10), and sterile ddH₂O (11). (C) DL2000 DNA Marker (TaKaRa, Shiga, Japan) (M), *P. absoluta* (1–3), *C. horticola* (4–6), *Liriomyza sativae* (7–9), and sterile ddH₂O (10, 11). Explanatory note: In (A, B), the top image was obtained under normal indoor lighting, and the lower image was obtained under an ultraviolet light.

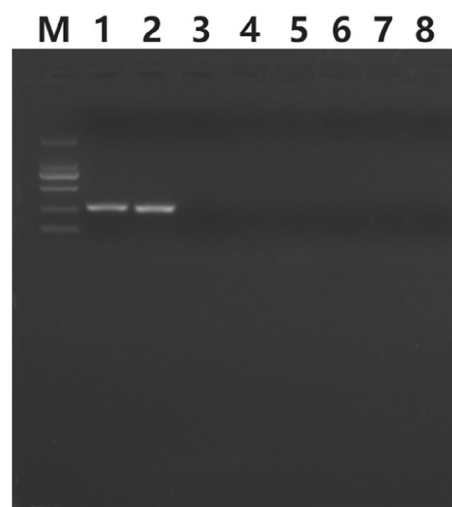


Figure 2. *Phthorimaea absoluta* specificity testing (polymerase chain reaction). DL2000 DNA Marker (TaKaRa, Shiga, Japan) (M), *P. absoluta* (1, 2), *Chromatomyia horticola* (3, 4), *Liriomyza sativae* (5, 6), and *Phthorimaea operculella* (7, 8).

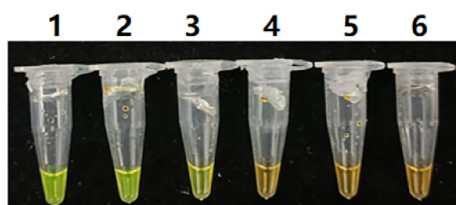


Figure 3. Sensitivity evaluation. The loop-mediated isothermal amplification (LAMP) reaction was performed with a dilution series of *Phthorimaea absoluta* DNA [1, *P. absoluta* DNA concentration 83.05 ng/μL; 2, 83.05 ng/μL × 10⁻¹; 3, 83.05 ng/μL × 10⁻²; 4, 83.05 ng/μL × 10⁻³; 5, 83.05 ng/μL × 10⁻⁴; 6, sterile double-distilled water (ddH₂O)].



Figure 4. Field apparatus and loop-mediated isothermal amplification (LAMP) results. The reaction tubes are placed in heated water in a commercial insulated cup, whose temperature is controlled by a phone application. Insect tissues are placed directly into the reaction tubes. Tubes shown at the bottom are: *Phthorimaea absoluta* larvae (1, 2), *P. absoluta* adults (3–5), and sterile double-distilled water (ddH₂O) (6).

showed green fluorescence, whereas those with lower DNA concentrations were orange. We therefore concluded that the lowest detection limit of the LAMP assay for *P. absoluta* DNA was approximately 0.83 ng/μL.

3.3 LAMP assay using insect tissues and an insulated cup

Both larvae and adults of *P. absoluta* were successfully identified based on the fluorescent SYBR Green I color which developed when insect tissues were placed directly into the LAMP reaction mixture and the reaction temperatures were controlled in an insulated cup (Fig. 4). This assay is therefore applicable for the identification of *P. absoluta* under field conditions. Tissue storage

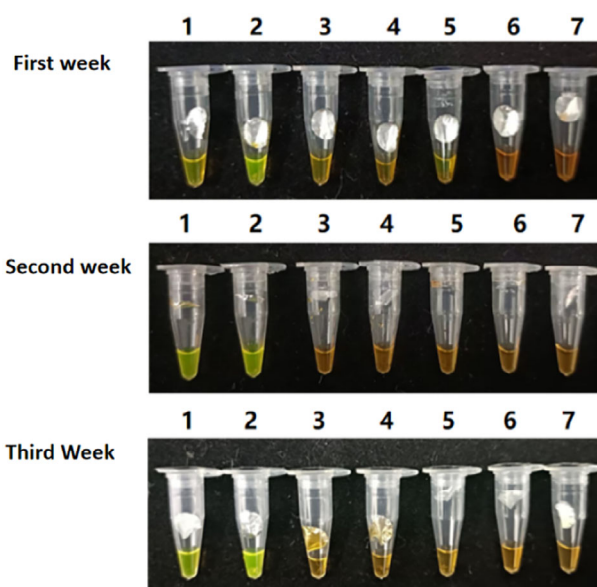


Figure 5. Loop-mediated isothermal amplification (LAMP) assay results after sample storage for 3 weeks under indoor conditions. *Phthorimaea absoluta* DNA (1), larval tissue (2), adult tissue (3–5), and sterile double-distilled water (ddH₂O) (6, 7).

duration of up to 21 days under indoor conditions had no discernable effect on the LAMP results for larval tissues, which were still clearly detected 21 days after storage; by contrast, positive detection of adult tissues declined with storage duration (Fig. 5).

4 DISCUSSION

Phthorimaea absoluta share similar morphological features with other common insect pests such as *P. operculella* (both Gelechiidae) and also similar feeding damage, such as that of *Liriomyza* larvae,^{12,13,29,30} which complicates its accurate identification. To date, many molecular methods for identifying *P. absoluta* have been developed in the laboratory.^{11,12,14,16} These include sequencing of the mitochondrial COI gene,^{16,17,31} real-time PCR,¹¹ and droplet digital PCR,¹⁴ which require various PCR instruments and relatively complex detection procedures using imaging systems or sequencing. They are therefore unsuitable for rapid, large-scale detection during field monitoring or port quarantine testing.

Compared with other molecular diagnostic methods, the LAMP method is more suitable to field application because of its speed and simplicity.¹⁸ It offers several advantages for the identification of *P. absoluta*. First, unlike PCR-based methods that require special equipment and reagents, this method requires only a basic means of temperature control (such as a water bath or dry bath) and a limited number of chemicals. Second, no DNA extraction is required for this assay and can be completed within 1 h. Third, the LAMP assay is cost effective, as one reaction costs less than \$US1 (DNA extraction, \$US0.25; primers, \$US0.07; Bst polymerase, \$US0.5; dNTP, \$US0.04; SYBR, \$US0.04).¹⁹ Fourth, the results can be visualized by simply observing the color of the reaction mixture without the use of specialized equipment.³² Thus, the assays presented here can be used by non-professionals with a limited knowledge of molecular biology.

ACKNOWLEDGEMENTS

This research was supported by the National Key R&D Program of China (2021YFD1400200) and the Taishan Scholar Foundation of Shandong Province (tstp20221135).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

- Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA *et al.*, Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J Pest Sci* **83**:197–215 (2010).
- Zhang GF, Wang YS, Gao YH, Liu WX, Zhang R, Fu WJ *et al.*, First report of the south American tomato leafminer, *Tuta absoluta* (Meyrick), in China. *J Integr Agric* **19**:1912–1917 (2020).
- Chang LN, Wang WQ, Yang Y, Yang H, Yang JB, Du GZ *et al.*, Study on the composition of culturable gut bacteria in the larvae of Yunnan population of *Tuta absoluta* and the degradation for macromolecular compounds. *J Environ Entomol* **44**:1240–1251 (2022).
- Potting RPJ, Van der Gaag DJ, Loomans A, Van Der Straten M, Anderson H, MacLeod A *et al.*, *Tuta absoluta*, Tomato Leaf Miner Moth or South American Tomato Moth. Ministry of Agriculture, Nature and Food Quality Plant Protection Service of the Netherlands, Utrecht, the Netherlands (2013).
- Zhang GF, Liu WX, Wan FH, Sheng XQ, Zhang YB and Guo JY, Bioecology, damage and management of the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a worldwide quarantine pest. *J Biosafety* **27**:155–163 (2018).
- Zhang GF, Zhang YB, Sheng YB, Liu WX, Li P, Liu WC *et al.*, Damage of an important and newly invaded agricultural pest, *Phthorimaea absoluta*, and its prevention and management measures. *Plant Protect* **48**:51–58 (2022).
- Zhang GF, Xian XQ, Zhang YB, Liu WX, Liu H, Feng XD *et al.*, Outbreak of the south American tomato leafminer, *Tuta absoluta*, in the Chinese mainland: geographic and potential host range expansion. *Pest Manag Sci* **77**:5475–5488 (2021).
- Ide T, Kanzaki N, Masuya H and Okabe K, Application of the LAMP assay for the detection of the Argentine ant, *Linepithema humile* (hymenoptera: Formicidae), from captures of pan traps. *Appl Entomol Zool* **53**:275–279 (2018).
- Woodell JD, Neiman M and Levri EP, Matching a snail's pace: successful use of environmental DNA techniques to detect early stages of invasion by the destructive New Zealand mud snail. *Biol Invasions* **23**:3263–3274 (2021).
- Yang LF, Yang N, Fu HB and Chu D, Research advances in the application of environmental DNA (eDNA) technique in biological invasions. *Journal of Plant Protect* **50**:1–10 (2023).
- Zink FA, Tembrock LR, Timm AE and Gilligan TM, A real-time PCR assay for rapid identification of *Tuta absoluta* (Lepidoptera: Gelechiidae). *J Econ Entomol* **113**:1479–1485 (2020).
- Zhang GF, Liu WX, Guo JY, Zhang YB and Wan FH, Species-specific COI primers for rapid identification of *Tuta absoluta* (Meyrick), a significant, potential alien species. *J Biosafety* **22**:80–85 (2013).
- Wang WQ, Chang LN, Yang JR, Yang Y, Du GZ XGL *et al.*, Comparison of morphological characteristics and damage symptoms of *Phthorimaea absoluta* and *P. operculella*. *Plant Protect* **48**:245–251 (2022).
- Zink FA, Tembrock LR, Timm AE and Gilligan TM, A droplet digital PCR (ddPCR) assay to detect *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) in bulk trap samples. *J Econ Entomol* **115**:2125–2129 (2022).
- Butterworth V, Dansby H, Zink FA, Tembrock LR, Gilligan TM, Godoy A *et al.*, A DNA extraction method for insects from sticky traps: targeting a low abundance pest, *Phthorimaea absoluta* (Lepidoptera: Gelechiidae), in mixed species communities. *J Econ Entomol* **115**:844–851 (2022).
- Kinyanjui G, Khamis FM, Ombura FLO, Kenya EU, Ekesi S and Mohamed SA, Infestation levels and molecular identification based on mitochondrial COI barcode region of five invasive Gelechiidae pest species in Kenya. *J Econ Entomol* **112**:872–882 (2019).
- Mukwa LF, Mukendi J, Adakate FG, Bugeme DM, Kalonji-Mbuyi A and Ghimire S, First report of the south American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and its damage in the Democratic Republic of Congo. *Bioinvasions Rec* **10**:33–44 (2021).
- Rizzo D, Moricca S, Bracalini M, Benigno A, Bernardo U, Luchi N *et al.*, Rapid detection of *Pityophthorus juglandis* (Blackman) (Coleoptera, Curculionidae) with the loop-mediated isothermal amplification (LAMP) method. *Plants (Basel)* **10**:1048 (2021).
- Hsieh CH, Wang HY, Chen YF and Ko CC, Loop-mediated isothermal amplification for rapid identification of biotypes B and Q of the globally invasive pest *Bemisia tabaci*, and studying population dynamics. *Pest Manag Sci* **68**:1206–1213 (2012).
- Nakajima N, Sakamoto Y and Goka K, Rapid detection of the red fire ant *Solenopsis invicta* (hymenoptera: Formicidae) by loop-mediated isothermal amplification. *Appl Entomol Zool* **54**:319–322 (2019).
- Agarwal A, Rako L, Schutze MK, Starkie ML, Tay WT, Rodoni BC *et al.*, A diagnostic LAMP assay for rapid identification of an invasive plant pest, fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Sci Rep* **12**:1116 (2022).
- Rako L, Agarwal A, Semeraro L, Broadley A, Rodoni BC and Blacket MJ, A LAMP (loop-mediated isothermal amplification) test for rapid identification of Khapra beetle (*Trogoderma granarium*). *Pest Manag Sci* **77**:5509–5521 (2021).
- Kyei-Poku G, Gauthier D and Quan G, Development of a loop-mediated isothermal amplification assay as an early-warning tool for detecting emerald ash borer (Coleoptera: Buprestidae) incursions. *J Econ Entomol* **113**:2480–2494 (2020).
- Nam HY, Kim JH, Lee SH, Heckel DG and Kim J, Development of a LAMP-based molecular species diagnosis method for four major agricultural pests in the genus *Spodoptera* (Lepidoptera: Noctuidae). *Insects* **12**:883 (2021).
- Huang P, Zhang J, Yao JA, Lan YY and Yu DY, Biological characters and loop-mediated isothermal amplification detection of *Planococcus lilacinus* (Hemiptera: Pseudococcidae). *Acta Entomol Sin* **66**:1052–1062 (2023).
- Agarwal P, Cunningham JP, Valenzuela I and Blacket MJ, A diagnostic LAMP assay for the destructive grapevine insect pest, phylloxera (*Daktulosphaira vitifoliae*). *Sci Rep* **10**:21229 (2020).
- Kitano D and Takakura K, Simple and on-site DNA purification for LAMP reaction applicable to non-adult tephritid fruit fly (Diptera: Tephritidae). *J Appl Entomol* **144**:824–829 (2020).
- Yan JJ, Zhang MD and Gao YL, Biology, ecology and integrated management of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Acta Entomol Sin* **62**:1469–1482 (2019).
- Leite GLD, Picanco M, Jham GN and Marquini F, Intensity of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) and *Liriomyza* spp. (Diptera: Agromyzidae) attacks on *Lycopersicon esculentum* mill. leaves. *Cienc Agroec* **28**:42–48 (2004).
- Miranda MMM, Picanco MC, Zanuncio JC, Bacci L and Silva ÉMD, Impact of integrated pest management on the population of leafminers, fruit borers, and natural enemies in tomato. *Cienc Rural* **35**:204–208 (2005).
- Sint D, Sporleder M, Wallinger C, Zegarra O, Oehm J, Dangi N *et al.*, A two-dimensional pooling approach towards efficient detection of parasitoid and pathogen DNA at low infestation rates. *Methods Ecol Evol* **7**:1548–1557 (2016).
- Wang XX, Liu B, Zhang Y, Zhai YF, Ullah F and Li ZH, A rapid LAMP-based colorimetric assay with quick DNA extraction for on-site identification of *Drosophila suzukii* Matsumura. *J Appl Entomol* **145**:922–928 (2021).