Taqman real-time quantitative PCR for identification of western flower thrip (*Frankliniella occidentalis*) for plant quarantine

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Western flower thrip (*Frankliniella occidentalis*) is a major global pest of agricultural products. It directly damages crops through feeding, oviposition activity or transmission of several plant viruses. We describe a Taqman real-time quantitative PCR detection system, which can rapidly identify *F. occidentalis* from thrips larvae to complement the traditional morphological identification. The data showed that our detection system targeted on the ribosomal RNA gene regions of *F. occidentalis* has high sensitivity and specificity. The rapid method can be used for on-site testing of samples at ports-of-entry in the future.

Keywords: *Frankliniella occidentalis*; real-time quantitative PCR; plant quarantine

1. INTRODUCTION

Western flower thrip (*Frankliniella occidentalis*) is a polyphagous pest with a wide host range, and is one of the most important vectors for plant viruses such as Tomato Spotted Wilt *Tospovirus* (TSWV), Tobacco Streak *Ilarvirus* (TSV) and *Impatiens Necrotic Spot Tospovirus* (INSV; Whitfield *et al.* 2005; Morse & Hoddle 2006). In Europe, it infects many glasshouse crops (Smith *et al.* 1997). This pest is a small insect originating from the west coast of North America, and spread through Europe in the 1980s as a consequence of international trade in plants (Kirk & Terry 2003). It is now regarded as a significant global pest of horticultural crops (Smith *et al.* 1997; Pappu *et al.* 2009). For this reason, it has become a species of major quarantine concern, identified as an EPPO A2 quarantine pest. It is also an important quarantine pest in Taiwan and has been detected in many imported plant materials. According to inspection records from 2006 to 2008, *F. occidentalis* was intercepted on 25 different kinds of cut flowers imported from eight countries to Taiwan. This pest was also intercepted

on 41 different kinds of imported vegetables and fruits from 10 countries.

It was estimated that *F. occidentalis* has caused a 20 per cent yield loss of glasshouse cucumber in British Columbia, Canada. The pest has also been associated with outbreaks of TSWV in tomatoes in Ontario, Canada (Smith *et al.* 1997). The pest may produce more generations (19–21) per year in Taiwan than in the United States (typically 12–15 generations) per year. The economic losses caused by *F. occidentalis* could be immense in Taiwan.

The genus *Frankliniella* can be differentiated from other genera of thrips by some physical characteristics. However, species of *Frankliniella* are variable and difficult to differentiate (Smith *et al.* 1997). In addition, it is almost impossible to identify immature thrips at the species level by morphology. Molecular detection methods can be used to overcome the limitations of morphological analysis. DNA-based analysis can be applied to identify different thrips at all life stages. Several polymerase chain reaction–restriction fragment-length polymorphism (PCR–RFLP) protocols have been developed to separate selected groups of thrip species, including *F. occidentalis* (Brunner *et al.* 2002; Toda & Komazaki 2002). A real-time PCR-based method was developed for identification of the melon thrips (*Thrips palmi*; Walsh *et al.* 2005), but this study did not include *F. occidentalis*. The objective of this study was to develop a rapid and sensitive method to identify *F. occidentalis* to replace/confirm traditional morphological identification.

2. MATERIAL AND METHODS

(a) Thrip samples collection

Thrip adult and larvae samples were collected on imported plants by the Bureau of Animal and Plant Health Inspection and Quarantine at Tao-Yuan International Airport in Taiwan. Samples were stored in screw thread vials (7 ml) containing 70 per cent ethanol at 4°C for use.

(b) Total genomic DNA extraction

Total genomic DNA was extracted from whole thrip adults and larvae using the EasyPure genomic DNA spin kit (Biomart), according to the manufacturer's instructions. Briefly, samples were lysed with 200 µl ml⁻¹ of proteinase K, and then incubated in 200 µl of buffer. Genomic DNA was precipitated in a column with 200 µl of ethanol, followed by two washing steps. Finally, the genomic DNA was eluted in 30 µl of diethyl pyrocarbonate (DEPC) treated water.

(c) Primers and probes designed for Taqman real-time quantitative PCR (qPCR) detection system

The ribosomal RNA gene sequences of the thrips were downloaded from GenBank to design primers. The thrips included *F. occidentalis*, *Frankliniella intonsa* and *Frankliniella schultzei* (GenBank accession number GQ343254, GQ343258, GQ343259). We designed two primer and probe sets targeted on 5' and 3' regions of the ribosomal RNA gene specifically for *F. occidentalis* (table 1).

(d) Taqman real-time quantitative PCR (qPCR)

Taqman real-time quantitative PCR was performed with LightCycler[®] 480 machine and LightCycler[®] 480 Probes Master kit (Roche). Reactions were performed in a final total volume of 10 µl containing 1 × LightCycler 480 Probes Master, 0.5 µM forward primer, 0.5 µM reverse primer, 0.2 µM Taqman probes and 2.5 µl of the DNA template. Amplification conditions were as follows: denaturation at 95°C for 10 min, 50 cycles of amplification (95°C for 10 s, 60°C for 30 s) and cooling at 40°C for 10 s. All samples were tested by qPCR for both 5' and 3' regions of the ribosomal RNA gene for *F. occidentalis*.

(e) Specificity of detection system

Seven different thrip species, including *F. schultzei*, *Scirtothrips dorsalis*, *Thrips tabaci*, *Caliothrips fasciopennis*, *Tubulifera*, *F. intonsa*

Figure 1. Taqman qPCR specificity test result. (a) Specific amplification curve of Taqman probe (dark red) targeted on 5' region of the *F. occidentalis* ribosomal RNA gene. (b) Specific amplification curve of Taqman probe (dark red) amplification targeted on 3' region of the *F. occidentalis* ribosomal RNA gene. Other six thrips species including *F. schultzei*, *Scirtothrips dorsalis*, *Thrips tabaci*, *Caliothrips fasciipennis*, *Tubulifera* and *F. intonsa* did not show any positive signal (green lines).

Table 1. Primers and probes used in this study.

name	sequence 5' → 3'	nucleotide number ^a
F1	GTT TCC GTA GGT GAA CCT GC	11 → 30
R1	TGT TTT GGG CCA TCT CCC	396 → 379
F2	GAC CAA ACT CAA AGA CCA GAC TG	1001 → 1023
R2	AAG AAA CGT CAC ACA CCC G	1233 → 1215
western 5' probe	Fam-CCA CTA CGT TCC CGA GTT CGT GAT TGC-Tam	171–197
western 3' probe	Fam-CTT CAC CGC TTC CCC CGT AAA GAG A-Tam	1100–1124

^aThe nucleotide number is in accordance with the following accession number: GQ343254.

and *F. occidentalis* were used to evaluate the specificity of the primer/probe sets.

(f) **Standard curve of detection system**

Taqman qPCR standard curves were established on 10-fold serial dilutions of the thrip genomic DNA from 10 ng μl^{-1} to 0.1 pg μl^{-1} to estimate in tetraplicate the system's linear range, efficiency and detection limitation for both 5' and 3' regions.

3. RESULTS

(a) **Specificity and sensitivity of quantitative PCR detection system**

Only *F. occidentalis* samples showed positive fluorescent signals. The six other thrips species including *F. schultzei*, *S. dorsalis*, *T. tabaci*, *C. fasciipennis*, *Tubulifera* and *F. intonsa*, did not show any positive signal (figure 1a,b). Both 5' and 3' regions primer and probe sets had achieved linear range from 10 ng μl^{-1} to 0.1 pg μl^{-1} , qPCR efficiency up to 95 per cent and the detection limit to 0.1 pg μl^{-1} (figure 2).

(b) **Sample analysis**

We tested 28 thrip larvae from seven groups of inspected plants. Five of the seven imported plant groups carried *F. occidentalis* (table 2). Both 5' and 3' Taqman qPCR obtained similar CT value, showing that they have the similar PCR efficiency and sensitivity (data not shown). *F. occidentalis* positive samples by Taqman qPCR were double checked by direct sequencing the qPCR amplicons.

4. DISCUSSION

Western flower thrips (*F. occidentalis*) are often intercepted on plants or plant products in international trade, and are commonly found on imported perishable plant materials as larvae without the presence of adults. Adult thrips can be identified morphologically at the species level. However, immature stages of thrips usually have not developed the morphological characteristics of adults. For morphological identification at a species level, the immature thrips must be raised to adulthood, resulting in a critical delay of quarantine tests. Rapid identification of *F. occidentalis* is essential for quarantine treatment for infected materials at ports of entry. Only *F. occidentalis* was differentiated by our detection method from six other thrip species. The result showed that our Taqman qPCR detection system was specific for *F. occidentalis* identification. Direct sequencing of the qPCR amplicons confirmed the specificity of our detection system.

The standard curve results showed both two sets of detection systems had high qPCR efficiency of 95 per cent and a low detection limit down to 0.1 pg per reaction. The two sets of Taqman primers and probes could detect the DNA of one thrip larva (figure 2). The power of our rapid, specific, sensitive, reliable and cost-effective detection methods to identify *F. occidentalis* at the larva stage is very helpful for plant quarantine to overcome the limitation of traditional morphological identification. The methods can be used for on-site testing

Figure 2. (i) Amplification and (ii) standard curves of (a) 5' region primer and probe set. (b) 3' region primer and probe set. Template concentrations: (1) 10 ng; (2) 1 ng; (3) 100 pg; (4) 10 pg; (5) 1 pg; (6) 0.1 pg. (a) Correlation coefficient: 0.999; slope: -3.567 ; intercept: 31.548; $Y = -3.567X + 31.548$; PCR efficiency = 95.05%. (b) Correlation coefficient: 0.998; slope: -3.446 ; intercept: 31.229; $Y = -3.4463X + 31.229$; PCR efficiency = 97.55%.

Table 2. Thrips sample list from imported plants and number of positive *F. occidentalis* confirmed by Taqman real-time quantitative detection system.

sample group	imported plant	country of origin	number of larvae	number of <i>F. occidentalis</i> positive individuals
1	strawberry	USA	6	6
2	raspberry	USA	6	6
3	strawberry	USA	6	5
4	<i>Eryngium alpinum</i>	Netherlands	2	2
5	raspberry	USA	3	3
6	thyme	Netherlands	3	0
7	snow pea shoot	Indonesia	2	0

of samples at a port-of-entry in the future, allowing for faster identification to prevent the possible introduction of the pest.

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