



Dispersal of harmful fruit fly pests by international trade and a loop-mediated isothermal amplification assay to prevent their introduction

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Abstract

Global trade of plant products represents a major driving force for the spread of invasive insect pests. This visualisation illustrates

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the problem of unintended dispersal of economically harmful fruit fly pests (Diptera: Tephritidae) using geospatial tools and a time series of interception data from the Swiss import control system. Furthermore, it reports the development of a molecular diagnostic assay for rapid identification of these pests at points of entry (POEs) such as sea- and airports as a prevention measure. The assay reliably differentiates between target and non-target species within one hour and has been successfully evaluated for on-site use at a Swiss POE.

Video link

<https://www.youtube.com/watch?v=d4Bv1E0pUoc>

Background

Introduction and dispersal of invasive insect pests into regions outside their native ranges can lead to substantial economic damage for local agriculture (Bacon *et al.*, 2012; Horton *et al.*, 2013). One of the primary means for the movement of non-indigenous insects is their inadvertent spread through global trade (Bacon *et al.*, 2012; Horton *et al.*, 2013). Invasive insects are vectored along major trading routes, as contaminants of a variety of import products, such as forest and agricultural goods, ornamental plants, nursery stocks, and also within packaging material (Horton *et al.*, 2013; Blaser *et al.*, 2018). Moreover, insect pests are carried along as stowaways of transport vehicles, such as aeroplanes, cargo trains, ships and trucks (Horton *et al.*, 2013; Blaser *et al.*, 2018). Additionally, the global spread of invasive pests is further facilitated through international tourism and environmental effects due to changes in climate and land use (Armstrong and Ball, 2005).

Fruit fly species of the family Tephritidae are among the economically most harmful invasive insect pests (Vargas *et al.*, 2015). Due to their ability to feed on a wide range of fruits and fleshy vegetables and their high reproductive capacity, numerous species of this family have the potential to cause serious crop losses, and hence, constitute a worldwide threat for fruit and vegetable producers and traders (Vargas *et al.*, 2015). *Bactrocera dorsalis*, the oriental fruit fly, is a prominent example of a highly invasive and destructive fruit fly pest (Theron *et al.*, 2017). First recorded in Taiwan in 1912, the species dispersed throughout Southeast Asia, the Pacific region, and sub-Saharan Africa (Shi *et al.*, 2010; Vargas *et al.*, 2015; Theron *et al.*, 2017). Several transient intro-

duction events were also reported from North America (Vargas *et al.*, 2015). It was shown that *B. dorsalis* is not a single species, but rather forms a species complex, consisting of nearly 100 morphologically similar species (Kwasi, 2008; Schutze *et al.*, 2015). Members of this complex have a host plant range including more than 250 species and varieties, among them commercially grown fruits (e.g. banana, guava and mango) traded on the global market (Shi *et al.*, 2010; Vargas *et al.*, 2015).

The economic impact of a fruit fly invasion on local horticulture can be exemplified using data from Ghana. After introduction and establishment of *Bactrocera invadens*, a member of the *B. dorsalis* complex, direct yield losses for fruit producers were estimated in excess of 40% (Kwasi, 2008; Badii *et al.*, 2015). Additional indirect losses resulted from quarantine regulations imposed by importing countries such as import bans and costly monitoring and elimination programmes (Kwasi, 2008; Badii *et al.*, 2015).

Biotic invasions are often initiated by a small number of individuals (Mack *et al.*, 2000). While containment at that stage is comparatively simple, it is highly challenging and costly to contain successfully established communities (Mack *et al.*, 2000). When analysing the entries ($n = 211$) of fruit fly elimination programmes recorded in the global eradication database (b3.net.nz/gerda) in 2014, the average costs per elimination were calculated to be about US\$ 12 million (Suckling *et al.*, 2014). However, the elimination of an invasive insect pest from a given area is challenging (Badii *et al.*, 2015). Depending on the method used, elimination efforts can affect the environment and human health, especially when insecticides are being employed (Badii *et al.*, 2015). Consequences of insecticide applications include chemical residues in crops, health problems of farmers and other community members due to insecticide exposure, contamination of water and soil, and decreases of frequency, relative abundance and diversity of native arthropod populations (De Barros *et al.*, 2015; Sarwar, 2015).

Against this background, inspections of plant imports at points of entry (POEs), such as sea- and airports, are a crucially important and cost-effective control measure, as they prevent introduction of invasive, non-native pests (McCullough *et al.*, 2006; Bacon *et al.*, 2012; Poland and Rassati, 2018). Pest interception records from such inspections collected over time provide important information about the extent of human-mediated movement of plant pests by global trade and can inform about high risk invasion pathways of harmful pest species (McCullough *et al.*, 2006; Holt *et al.*, 2017). Beside information about pest abundance and origin, interception data have the power to inform about types of shipment associated with pest migration. Such information can be utilized by regulatory agencies to develop risk management measures mitigating the likelihood of pest introduction events (McCullough *et al.*, 2006). Risk management measures can comprise refinements of inspection programmes as well as adoptions of international regulations and trade policies (McCullough *et al.*, 2006). Evidence for pathway-associated pest movement can furthermore initiate in-depth pest risk analyses, including evaluations about the potential of a pest to establish outside its native range and estimations of accompanying economic and social impacts (Venette *et al.*, 2010; Holt *et al.*, 2017). Moreover, long-term interception data can reflect effects of novel trade policies, changes in market demand, efforts by exporters, and revisions of national regulations (McCullough *et al.*, 2006).

Here, we use the format of a short video to communicate the issue of unintended spread of plant pests. We focus on the movement of harmful fruit flies, using a 7-year time series of intercep-

tion data from Switzerland. As emphasised by Krieger and colleagues, a video-based approach has the potential to facilitate communication of complex geospatial correlations in an easy and understandable format that is readily accessible by different stakeholders (Krieger *et al.*, 2012).

Inspecting Swiss plant imports between 2011 and 2017 revealed that there were 435 (0.6%) out of a total of 71,980 shipments that contained harmful insect pests. Among these, fruit flies of the family Tephritidae represented the most frequently intercepted taxonomic unit ($n = 139$, 32.0%) of all insect pest interceptions. The orders Hemiptera and Thysanoptera accounted for 106 (24.4%) and 105 (24.1%), respectively, while 67 (15.4%) of the intercepted insects were leaf-mining flies of the family Agromyzidae. The smallest contributions originated from interceptions of the orders Lepidoptera ($n = 15$, 3.5%) and Coleoptera ($n = 3$, 0.7%). Harmful fruit flies were intercepted on shipments originating from 19 different countries. The most common country of origin was Sri Lanka (23.7%), followed by Thailand (18.0%). India and Vietnam, each accounting for an additional 13.7% of the total fruit fly interceptions. The most common plant shipments associated with fruit fly interceptions were guava fruits (*Psidium guajava*, 27.5%), mango fruits (*Mangifera indica*, 26.1%), java apples (*Syzygium samarangense*, 16.0%) and peppers (*Capsicum* sp., 13.8%).

In the Swiss import control process, plant health inspections are based on visual examinations of incoming plant shipments suspected to harbour pest species (Blaser *et al.*, 2018). Suspicious insects such as fruit flies are often encountered in the larval development stage, for which comprehensive morphological keys are missing, thus rendering morphological differentiation between harmful and non-harmful species challenging (Armstrong and Ball, 2005; Blaser *et al.*, 2018). In order to ensure a reliable identification, the intercepted specimens are therefore sent to a reference laboratory where they are analysed by DNA-barcoding, an elaborate molecular identification method based on sequencing of a signature DNA-sequence, which is then queried against a reference database of sequences from previously identified specimens (Floyd *et al.*, 2010; Blaser *et al.*, 2018). The shipment of the specimens to the laboratory as well as their subsequent analysis requires two to three working days. In the meantime, the plant imports suspected to harbour pest species are held back at the POE (Blaser *et al.*, 2018). To circumvent such import delays, we developed a molecular on-site assay for the rapid identification of harmful fruit flies based on the loop-mediated isothermal amplification (LAMP) technology. The novel assay can be performed directly at POEs and results are available within only one hour. LAMP is a highly specific and robust identification method for species with previously known DNA or RNA sequences and suitable for on-site application because it can be performed in a laboratory-free environment after minimal training (Kogovšek *et al.*, 2015).

Our assay is able to identify regulated fruit flies of the genera *Bactrocera* and *Zeugodacus*, namely *B. latifrons*, members of the *B. dorsalis* complex (*B. cacuminata*, *B. carambolae*, *B. dorsalis*, *B. papayae* and *B. philippinensis*), as well as *Z. cucurbitae*. These pests rank among the most destructive fruit fly species and are frequently intercepted at Swiss borders (Vargas *et al.*, 2015).

The assay is designed in such a way that the primers target a sequence fragment of the mitochondrial gene cytochrome *c* oxidase 1. A detailed protocol of the method has been described elsewhere (Blaser *et al.*, 2018). In brief, insect tissue is boiled for 5 min in an alkaline solution to extract the DNA. Subsequently, the



extraction product is transferred directly into the reaction tube containing all reagents needed for the LAMP reaction without the need of any purification step. The LAMP reaction is pursued at a constant temperature of 65 °C and its analysis can be performed in a battery-driven real-time LAMP device suitable for on-site application.

The fruit fly LAMP assay was initially evaluated for diagnostic accuracy under laboratory conditions with randomly selected fruit fly specimens intercepted during regular border controls and implemented in a second step as a part of the plant health control system at the Zurich Airport, one of the major POEs of Switzerland. For the assay evaluation, all results were rigorously cross-validated using DNA barcoding (Floyd *et al.*, 2010; Blaser *et al.*, 2018).

The results of the evaluation were partially described elsewhere (Blaser *et al.*, 2018). A total of 143 fruit fly specimens originating from 16 different countries were analysed. Among these, 117 specimens were examined in a reference laboratory, whilst the remaining 26 specimens were analysed under on-site conditions at the Swiss POE Zurich Airport. During the evaluation, 78 fruit fly specimens (54.5%) were correctly identified as target species and 64 specimens (44.8%) correctly as non-target species. Only one specimen (0.7%) analysed at the POE was incorrectly identified as a target fruit fly specimen instead of a non-target species. Based on the results of the LAMP assay evaluation, we calculated a test sensitivity (true-positive-rate) of 98.7%, a test specificity (true-negative-rate) of 100% and a test efficiency (percentage of correct test results) of 99.3%.

Outlook

Geospatial maps visualising pest movement are effective tools to sensitise the community for the issue of the unintended spread of harmful invasive organisms along major trading networks. In this visualisation, we used pest interception data from the Swiss import control system to exemplify the problem of hitchhiking fruit flies associated with international trade of fruits and vegetables. We furthermore presented an on-site diagnostic test for rapid and accurate identification at POEs based on LAMP technology. After successful implementation of the LAMP assay for frequently intercepted fruit fly species, future efforts aim at expanding the target range of the LAMP assay to other harmful pest species associated with plant imports.

Overall aim

With this visualisation, we illustrate the problem of unintentional movement of harmful insect pests through global trade of plant products and present a new, rapid molecular on-site diagnostic test to prevent dispersal and introduction of harmful fruit fly pests. The visualisation is of particular interest to policy makers, plant health workers, producers of plant products and other stakeholders involved in the import and export of plant products, as well as to consumers of imported plant products.

Software

All geospatial elements of the visualisation were generated using the open-source software QGIS (version 2.14) based on *Natural Earth* vector maps published in the public domain.

If needed, illustrations were modified with the open-source vector graphics editor Inkscape (version 0.92). The final content visualisation was performed using Microsoft PowerPoint 2013 (Microsoft Corporation, Redmond, WA, USA).

Production of video was implemented using Camtasia Studio (version 9.0.5, TechSmith Corporation, Okemos, MI, USA).

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