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

Simon Blaser,1,3 Cornelia Heusser,1 Hanspeter Diem,4 Andreas von Felten,5 Morgan Gueuning,1 Michael Andreou,6 Neil Boonham,7,8 Jennifer Tomlinson,7 Pie Müller,2,3 Jürg Utzinger,2,3 Jürg E. Frey,1 Beatrice Frey,1 Andreas Bühlmann1

1 Agroscope, Wädenswil, Switzerland; 2 Swiss Tropical and Public Health Institute, Basel, Switzerland; 3 University of Basel, Basel, Switzerland; 4 Federal Office for Agriculture, Zurich Airport, Switzerland; 5 Federal Office for Agriculture, Bern, Switzerland; 6 OptiGene Limited, Horsham, United Kingdom; 7 The Food and Environment Research Agency, York, United Kingdom; 8 Newcastle University, Newcastle upon Tyne, United Kingdom

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

References

Armstrong KF, Ball SL, 2005. DNA barcodes for biosecurity: invasive species identification. Philos Trans Soc B Biol Sci 360:1813-23.
Bacon SJ, Bacher S, Aebi A, 2012. Gaps in border controls are related to quarantine alien insect invasions in Europe. PLoS One 7:e47689.
Badii KB, Billah MK, Afreh-Nuamah K, Obeng-Ofori D, Nyarko G, 2015. Review of the pest status, economic impact and management of fruit-infesting flies (Diptera: Tephritidae) in Africa. Afr J Agric Res 10:1488-98.
Blaser S, Diem H, von Felten A, Gueuing M, Androue M, Boonham N, Tomlinson J, Müller P, Utzinger J, Frey JE, Bühlmann A, 2018. From laboratory to point of entry: development and implementation of a LAMP-based genetic identification system to prevent introduction of quarantine insect species. Pest Manag Sci 74:1504-12.
De Barros EC, Ventura HV, Gontijo PC, Pereira RR, Picanço MC, 2015. Ecotoxicological study of insecticide effects on arthropods in common bean. J Insect Sci 15:14.
Floyd R, Lima J, deWaard J, Humble L, Hanner R, 2010. Common goals: policy implications of DNA barcoding as a protocol for identification of arthropod pests. Biol Invasions 12:2947-54.
Holt J, Leach AW, MacLeod A, Tomlinson D, Christodoulou M, Mumford JD, 2017. A quantitative model for trade pathway analysis of plant pest entry and transfer to a host in European Union territory. EPPO Bulletin 47:220-6.
Horton DR, Lewis TM, Dobbs TT, 2013. Interceptions of Anthocoridae, Lasiochilidae, and Lycocoridinae at the Miami plant inspection station (Hemiptera: Heteroptera). Fla Entomol 96:482-97.
Kogovšek P, Hodgetts J, Hall J, Prezelj N, Nikolić P, Mehle N, Lenarčič R, Rotter A, Dickinson M, Boonham N, Dermastia M, Ravnikar M, 2015. LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. Plant Pathol 64:286-96.
Krieger GR, Bouchard MA, de Sa IM, Paris I, Balge Z, Williams D, Singer BH, Winkler MS, Utzinger J, 2012. Enhancing impact: visualization of an integrated impact assessment strategy. Geospat Health 6:303-6.
Kwasi W, 2008. Assessment of fruit fly damage and implications for the dissemination of management practices for mango production in the Upper West region of Ghana. J Dev Sustain Agric 3:117-34.
Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA, 2000. Biotic invasions: causes, epidemiology,
global consequences, and control. Ecol Appl 10:689-710.
McCullough DG, Work TT, Cavey JF, Liebhold AM, Marshall D, 2006. Interceptions of nonindigenous plant pests at US ports of entry and border crossings over a 17-year period. Biol Invasions 8:611-30.
Poland TM, Rassati D, 2018. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J Pest Sci. Available from: https://doi.org/10.1007/s10340-018-1004-y
Sarwar M, 2015. How to manage fruit fly (family Tephritidae) pest damage on different plant host species by take up physical control measures. Int J Anim Biol 1:124-9.
Schutze MK, Aketarawong N, Amornsak W, Armstrong KF, Augustinos AA, Barr N, Bo W, Bourtzis K, Boykin LM, Cáceres C, Cameron SL, Chapman TA, Chinvinijkul S, Chomič A, De Meyer M, Drosopoulou E, Englezou A, Ekesi S, Gariou Papalexiou A, Geib SM, Hailstones D, Hasanuzzaman M, Haymer D, Hee AK, Hendrichs J, Jessup A, Ji Q, Khamis FM, Krosch MN, Leblanc L, Mahmood K, Malaracida AR, Mavragani Tspinoud P, Mwatalawa M, Nishida R, Ono H, Reyes J, Rubinoff D, San Jose M, Shelly TE, Srikachar S, Tan KH, Thanaphum S, Haq I, Vijaysegaran S, Wee SL, Yesmin F, Zacharopoulou A, Clarke AR, 2015. Synonymization of key pest species within the Bactrocera dorsalis species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. Syst Entomol 40:456-71.
Shi W, Kerdelhué C, Ye H, 2010. Population genetic structure of the oriental fruit fly, Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) from Yunnan province (China) and nearby sites across the border. Genetica 138:377-85.
Suckling DM, Kean JM, Stringer LD, Cáceres-Barrios C, Hendrichs J, Reyes-Flores J, Dominiak BC, 2014. Eradication of tephritid fruit fly pest populations: outcomes and prospects. Pest Manag Sci 72:456-65.
Theron CD, Manrakhan A, Weldon CW, 2017. Host use of the oriental fruit fly, Bactrocera dorsalis (Hendel) (Diptera: Tephritidae), in South Africa. J Appl Entomol 141:810-6.
Vargas RI, Piñero JC, Leblanc L, 2015. An overview of pest species of Bactrocera fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the pacific region. Insects 6:297-318.
Venette RC, Kriticos DJ, Magarey RD, Koch FH, Baker RHA, Worner SP, Gómez-Raboteaux NN, McKenney DW, Dobesberger EJ, Yemshanov D, De Barro PJ, Hutchison WD, Fowler G, Kalaris TM, Pedlar J, 2010. Pest risk maps for invasive alien species: a roadmap for improvement. BioScience 60:349-62.